



Iron Disorders Workshop

GENOME RESEARCH INNOVATION

Pathology Research Facility, Faculty of Health Sciences
6th Floor, Room 6042

University of Stellenbosch, Tygerberg 7500

T: 021 9389324 M: 0828799108

Email: maritha.kotze@gmail.com

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GeneTalk Workshop Disorders of Iron Metabolism and Well-being

3rd Applied Genetics Workshop: 27 May 2009 from 9h00-16h00
VENUE: Faculty of Health Sciences, Teaching Block, 4th Floor, Lecture Room
 K4053B, University of Stellenbosch, Tygerberg

PROGRAM

TIME	TOPIC	SPEAKERS
8h30	Registration	
9h00	Introduction: Iron disorders in haematology	Prof Akin Abayomi – Head: Division of Haematology, University of Stellenbosch and NHLS, Tygerberg
9h30	The role of iron regulatory proteins in iron homeostasis, anaemia, and neurodegeneration	Dr Tracey Rouault – Head: Molecular Medicine Program, Section on Human Iron Metabolism, NIH, USA.
10h30	Influence of the iron-carrier protein transferrin on age of onset in patients with Alzheimer's disease	Dr SJ van Rensburg – Division of Chemical Pathology, University of Stellenbosch and NHLS
11h00	Break	
11h15	The role of iron metabolism in health and disease: A clinical overview	Dr Johan van Wyk – Division of Chemical Pathology, University of Stellenbosch and NHLS
11h45	Men of iron, steel magnolias: What you should know about haemochromatosis	Dr Erna Mansvelt – Ampath, N1 City Medical Chambers, N1 City, Goodwood
12h30	The tired patient: Too much or too little iron in the diet?	Dr Dawie van Velden – Dept of Pathology, University of Stellenbosch, Tygerberg
13h00	Lunch break	
13h30	Application of a web-based genetic testing service delivery system: Addressing the iron-heart disease link	Dr Maritha Kotze – Dept of Pathology, University of Stellenbosch, Tygerberg
14h00	The significance of haemochromatosis mutations in patients with non-alcoholic fatty liver (NAFLD) disease	Dr Corne Kruger – Private practicing physician and gastroenterologist, Durbanville Medi-Clinic
14h30	Effects of (iron-containing) Rooibos tea on antioxidant parameters in volunteers at risk of cardiovascular disease	Dr Jeanine Marnewick - Oxidative Stress Research Centre, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology
15h00	Haemochromatosis: Genetic counselling, ethics and insurance	Frieda Louser – Private practicing genetic counsellor, Christiaan Barnard Memorial Hospital and NHLS, University of Cape Town.
15h45	Closure	Kirsten Alberts – Haemochromatosis Society of South Africa

Accredited with 7 CEU's in level 1, including 2 ethics points

All speakers are acknowledged for their contributions to the information provided.

Dear Healthcare Professional

Welcome to the 3rd Applied Genetics Workshop, which forms part of Haemochromatosis Awareness Week.

This GeneTalk event was designed to provide a comprehensive and up-to-date overview of recent advances in the field of iron-related disorders and research. A synopsis of the workshop content is provided in the pages to follow, with particular focus on the preventable genetic disorder hereditary haemochromatosis (HH). For background information on genetics, please download a free copy of the E-book entitled "Genetics in Family Practice" from www.gknowmix.com.

With the completion of the Human Genome Project in 2003, a vast amount of information on genetic variability has become available. Despite the extensive data on the role of genetic variation in human disease, its translation into clinical practice has been slow due to the time required to accumulate population data on mutation frequencies, understand the significance of individual gene variants in disease expression, and develop suitable diagnostic tests.

Despite the above-mentioned limitations, clinicians have very rapidly benefited from molecular genetic research on haemochromatosis, as use of genetic testing in conjunction with assessment of serum iron status has drastically reduced the need for liver biopsy to confirm or exclude disease diagnosis. Early detection of this preventable genetic disorder presents a major healthcare opportunity to reduce the burden of heart disease, cancer, diabetes, arthritis, infertility and many other complications of organ damage in the population.

A web-based genetic testing service delivery system and database tool has been developed for seamless conversion of research and innovation. This process involves interaction with patients (1 and 2), specimen processing, data analysis and reporting back to the consulting healthcare practitioner (3 and 4):

1. Clinicians: Patient consultation, clinical assessment and treatment
2. Genetic counsellors: Genetic risk assessment and family counselling
3. Laboratories: Pathology, molecular genetic testing and evaluation.
4. Medical scientists: Data interpretation and reporting

Your participation is deemed paramount to the success of the Genome Research Innovation initiative launched in collaboration with the Department of Pathology, University of Stellenbosch, in April 2008.

Yours sincerely



Dr Maritha J Kotze
Medical Biological Scientist (Genetics)

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1. INTRODUCTION

The exponential growth in knowledge of human iron metabolism over the last decade resulting mainly from advances in molecular and cell biology has been breath-taking. It has become clear that sequence variation in many different iron-related genes underlying defective proteins or differences in expression levels of alleles at these loci, contributes to individual variability in iron metabolism that, in turn, cause genetic predispositions to numerous diseases involving iron.

The iron status of an individual is determined by a combination of nutritional, environmental and genetic factors. Normal iron status ensures ready availability of metabolic activity for optimal functioning of the immune system, while at the same time impeding uptake by microorganisms and denying growth advantages in tumour cells. The degree of variation on either side of the “normal” situation that can be tolerated without upsetting this fine balance is probably the critical factor in understanding how changes in iron status can predispose to a wide variety of disorders.

Some iron disorders are hereditary in nature and others acquired. Acquired iron overload can be caused by many factors, including repeated transfusions in patients with haematological diseases as represented by chronic anaemias such as thalassaemia major and sickle cell disease.

Prof Akin Abayomi opened the workshop with an introduction to Iron Disorders in Haematology.

2. IRON DEFICIENCY

The most common dietary deficiency worldwide involves iron, which also represents the most abundant metal in the human body. Iron is essential for many metabolic DNA, RNA and protein synthesis, the formation and maintenance of myelin and is a co-factor of many haem and non-haem enzymes.

Iron deficiency may result from inadequate intake, increased iron requirements, increased blood loss or decreased absorption of iron, or a combination of these factors. Clinical consequences of iron deficiency include central nervous system dysfunction, impaired work performance due to chronic fatigue, pregnancy complications, impaired immune response, and gastrointestinal disturbances (e.g. glossitis, stomatitis, gastritis).

To meet their nutritional needs, most cells regulate the expression of ferritin and transferrin, two of the most extensively studied proteins involved in iron metabolism. The iron-regulatory proteins 1 and 2 (IRP1 and IRP2) regulate the expression of multiple iron metabolism genes in order to optimise cellular iron availability. In iron-deficient cells, IRPs bind to iron-responsive elements (IREs) found in the mRNAs of ferritin, the transferrin receptor and other iron metabolism transcripts, thereby enhancing iron uptake and decreasing iron sequestration (Rouault 2006). The physiological role of the IRP-IRE system was illustrated by the following conditions:

- 1) A relatively rare human disorder, hereditary hyperferritinaemia cataract syndrome, in which ferritin L-chain IRE mutations interfere with IRP binding and translational processes.

- 2) A syndrome described in adult mice lacking IRP2, which is characterised by progressive neurodegeneration and anaemia.

Dr Tracey Rouault provided an expert overview of the role of iron regulatory proteins in iron homeostasis, anaemia, and neurodegeneration.

3. IRON OVERLOAD

The predominant feature of iron overload or haemochromatosis is over-absorption of dietary iron. Excess body iron is stored in organs and tissues where it may cause injuries resulting in a variety of disorders, including heart disease, diabetes, arthritis, cancer, cirrhosis, impotence and sterility.

The importance of iron within oxygen-binding and respiratory proteins cannot be overemphasised, but it may also lead to the production of free radicals and consequently tissue damage (Bothwell et al. 1979). Whilst these free radicals may cause DNA strand breakage and disruption of DNA structure (direct mutagenesis), iron can also interfere with immunologic tumour surveillance and macrophage disposal of transformed cells (indirect mutagenesis) (Brock 1994).

Iron accumulation in the brain is believed to contribute to neurodegenerative diseases, including Parkinson and Alzheimer's disease. While an imbalance in brain iron status may cause free radical generation and oxidative damage, the possibility that such iron may be insoluble and unavailable for cellular use must also be considered. In some diseases, the pathology associated with iron accumulation may result from functional iron deficiency (Rouault and Cooperman 2006).

Dr Susan van Rensburg discusses the role of iron and oxidative stress in the context of Alzheimer's disease.

4. WHAT DOCTORS SHOULD KNOW

With patients becoming increasingly aware of available genetic testing options, it is important that the family doctor becomes knowledgeable in identifying and advising patients who may be at increased risk of iron-related disorders.

The role of iron metabolism in health and disease

The importance of iron metabolism now extends beyond the traditional areas of erythropoiesis and nutrition, representing a key factor in pathology, cardiology, oncology, neurological and infectious diseases. Molecular genetic research has revealed that the phenotypic expression of specific mutations in genes involved in iron metabolism may vary significantly. Hereditary iron overload was shown to be of multigenic nature, caused by a genetically determined inability to prevent the excessive influx of iron into the circulatory pool and characterized by progressive parenchymal iron overload with potential for multi-organ damage and disease.

Iron deficiency, iron overload and the anaemia of inflammation are the commonest disorders of iron metabolism:

Nutritional iron deficiency results from a diet that contains insufficient bioavailable iron to meet requirements. In developing countries, traditional foods usually contain large quantities of iron absorption inhibitors, particularly phytates and polyphenols. Conditions that cause blood loss, particularly hookworm infections, have an important contributory role leading to a high prevalence of iron deficiency in many developing countries.

Primary iron overload is far less prevalent than iron deficiency. Primary systemic iron overload (haemochromatosis) is almost always the result of an inherited abnormality of the regulation of iron transport that affects hepcidin or its receptor ferroportin.

The anaemia of inflammation (anaemia of chronic disease) is the result of increased hepcidin expression induced by inflammatory cytokines which is generally considered to be a host response that evolved to make iron less available to pathogens. It is characterized by decreased release from iron stores, low plasma iron and transferrin concentrations, restriction of the available iron supply for red blood cell production and mild or moderate anaemia.

Nutritional anaemia is also characterized by other nutritional deficiencies. Both copper and zinc are essential nutrients and deficiencies of both result in anaemia. Resistance to infections depends on a healthy immune function and copper and zinc are both necessary for normal function of the immune system. It has been found that zinc supplementation may reduce the incidence of malaria. Copper deficit should be included in the differential diagnosis of anaemia unresponsive to iron supplementation.

Although most anaemia in developing countries is due to iron deficiency, a proportion may be due to deficiency of vitamins of B complex, principally folate and vitamin B₁₂. The anaemia is macrocytic but with presence of abnormal red cell precursors in the bone marrow called megaloblasts. Because of the well proven case of increased risk for spina bifida, neural tube defects and other birth defects, folic acid supplementation before, during and after pregnancy is now accepted as being critical regardless of the nutritional status of the woman.

Vitamin B₁₂ enters the human food chain exclusively through animal sources. Its synthesis is completely absent in plants of all kinds, only being present in such foods by way of bacterial contamination or fermentation. For this reason vegetarians and more particularly vegans, are at high risk of insufficient dietary intake.

Dr Johan van Wyk gave a clinical overview of the role of iron metabolism in health and disease.

Hereditary Iron overload: Diagnosis and treatment of haemochromatosis

Clinical characteristics

Haemochromatosis is a common genetic condition but remains largely unrecognised or misdiagnosed. This can be ascribed largely to the wide range of conditions and the non-specific symptoms associated with body iron overload (see below) that complicates a clinical diagnosis. Early features of iron overload such as fatigue, joint pain, abdominal pain and loss of libido are non-specific and are commonly not recognised to be associated with haemochromatosis in primary care settings (Niederer et al. 1996). Many patients with early

iron overload have normal liver function tests, whereas mildly abnormal liver function tests are commonly ascribed to excessive alcohol use.

Symptoms of inherited iron overload include:

- Chronic parenchymal liver disease, cirrhosis, hepatocellular carcinoma
- Cardiomyopathy and arrhythmias
- Diabetes mellitus type I and II
- Infertility, amenorrhoea (no periods), impotence, loss of libido, testicular atrophy
- Anterior pituitary failure
- Arthritis, arthralgia, joint pain
- Porphyria cutanea tarda
- Weakness, chronic fatigue
- Mood swings, depression
- Unexplained abdominal pain, frequent diarrhoea
- Skin pigmentation, bronzing of the skin
- Loss of body hair

Excess iron accumulates in the liver, pancreas, heart and other organs eventually causing organ failure, as the body has no natural way of excreting iron. Symptoms of iron overload could typically appear in middle-age after years of damage, although iron overload may also occur in young persons in their early 20's, as well as children depending on the penetrance of genetic risk factors that may be involved.

The clinical presentation of haemochromatosis has changed over recent years from diagnosing patients with advanced disease (e.g. liver cirrhosis, diabetes) to early detection of patients presenting with abnormal liver function tests, elevated ferritin and/or increased transferrin saturation levels.

Prevalence – who is at risk?

Genetically predisposed individuals occur with an estimated frequency ranging from 1/100 to 1/300 depending on the population studied.

The carrier frequency of the most common mutation underlying hereditary haemochromatosis (HH), C282Y in the HFE gene, is about 1 in 6 in the Caucasian population of South Africa. This means that approximately 1 in 100 (estimated 1/115) individuals of European descent will inherit two copies of the defective gene (de Villiers et al. 1999 a,b). It is uncertain what percentage of these individuals will develop organ damage, as the disease penetrance varies from less than 1% on the one extreme in some populations, to between 40-60% depending on the genetic background and environmental exposures. Gender is also important as females are usually affected about 10 years later than males due to iron loss through menstruation and childbearing.

Many experts do not recommend population screening for detection of abnormal iron-loading genes, because of the low penetrance of the most common late-onset form of haemochromatosis. However, extending genetic investigations to family members of an affected individual may allow accurate genetic diagnosis in the initial asymptomatic phase. Early clinical symptoms include chronic fatigue, joint and muscle pain, etc. An argument for genetic screening of people known to be from high risk populations (e.g. people originating from North Western Europe) has been made and may in some areas be justified. Obviously, individuals with a genetic predisposition for inherited iron overload must have their iron status determined to assess possible gene expression and to monitor response to treatment, if indicated.

Diagnosis of haemochromatosis

Determination of transferrin saturation is recommended as a first line screening method for haemochromatosis and can detect cases of iron overload before organ dysfunction has occurred. However, the use of transferrin saturation requires fasting, is relatively non-specific and will also be elevated in chronic liver diseases due to secondary iron overload. DNA testing, on the other hand, provides a definitive diagnosis in the majority of affected cases with elevated transferrin saturation and ferritin levels, without the need to perform a liver biopsy. The ability to perform rapid mutation analysis on samples that are not C282Y homozygotes is becoming increasingly important also in the South African population (Zaahl et al. 2004), as more novel mutations are found in an increasing number of genes.

The latest classification of hereditary iron overload disorders broadly divides them into HFE-related haemochromatosis, which constitutes about 90% of cases, and non-HFE-related haemochromatosis. Five categories based on different genetic mutations and clinical presentation can be discerned (Brissot et al. 2008):

- Type 1 is classic haemochromatosis, where the affected persons are most often homozygous for the C282Y a mutation in the *HFE* gene causing a substitution of tyrosine for cystine at amino acid 282 in its product.
- Type 2A (with mutations in the *HJV* gene, encoding the hemojuvelin protein) and 2B (mutations in the *HAMP* gene that encodes hepcidin), both presenting at 20-30 years of age
- Type 3 (mutations in the *TFR2* gene, encoding the transferrin receptor 2)
- Type 4 (mutations in the *SLC40A1* gene, encoding ferroportin)
- A(hypo)ceruloplasminemia (a rare autosomal recessive form)

Types 1 to 3 have autosomal recessive inheritance patterns and manifest as parenchymal iron overload with organ failure, targeting mainly the liver, heart and endocrine organs. Type 4 is autosomal dominant and manifests as storage (macrophagal) iron overload.

Hepcidin, the hepatic-derived 25-amino acid peptide has now been recognized to play a key role in the regulation of iron homeostasis. The products of the genes that are associated with the 4 major types of haemochromatosis discussed above, all exert either regulatory effects on the synthesis or affect the function of hepcidin. Most of the clinical, biochemical and pathological features of iron overload disorders can now be explained by hepcidin deficiency or failure.

Inheritance pattern

Except for one rare form of adult-onset haemochromatosis caused by mutations in the ferroportin gene, inherited iron overload follows an autosomal recessive inheritance pattern. This means that patients with haemochromatosis have inherited a defective copy of the gene from both parents. The most common form is caused by mutations in the HFE gene on chromosome 6. Three mutations (HFE C282Y, H63D and S65C) account for the disease in the majority of affected patients.

People with one copy of a defective HFE gene are called heterozygotes or carriers. Mutation carriers do not necessarily develop clinical symptoms and the gene can be passed on in a

family without any one being aware of it. Children of two mutation carriers have a 25% chance of inheriting two copies of the defective gene. Those who inherit a defective copy of the gene from both parents are homozygous and are likely to develop the disease whereas those who inherit from one parent are carriers who are unaffected or may show a lesser increase in iron absorption.

Knowledge about the inherited nature of haemochromatosis and the application of genetic testing is important, because the disease goes undetected in many patients especially in the early preventable phase due to the non-specific symptoms of iron overload such as fatigue, joint aches, abdominal pain, loss of libido and depression.

Genetic testing

Genetic testing is important since it can provide a definitive diagnosis of inherited iron overload without the necessity of an invasive liver biopsy. Several polymerase chain reaction (PCR)-based methods have been developed for detection of mutations underlying haemochromatosis, including a reverse-hybridisation method that allows simultaneous analysis of multiple mutations in a single reaction (Oberkanins et al. 2000; Kotze et al. 2004). Today real-time PCR are mostly used for mutation detection in patients at risk of haemochromatosis. An important consideration in the test design is to be aware of the fact that certain gene regions of relevance to PCR-based tests frequently contain non-functional sequence changes that may interfere with the test procedure and data interpretation (de Villiers and Kotze 1999).

Patients are usually referred for genetic testing to confirm or exclude clinical/biochemical diagnosis, assess carrier status in families or for pre-clinical diagnosis in at-risk family members. To facilitate interpretation of genetic test results, information on clinical symptoms and iron parameters has to be provided when patients are referred for genetic testing.

Treatment

It may be necessary to treat patients with iron overload according to the genetic subtype: venesection is the treatment of choice in patients with haemochromatosis related to hepcidin deficiency, but is poorly tolerated or contraindicated in patients with iron overload due to ferroportin failure.

Standard treatment for HH patients with high transferrin saturation and ferritin levels involve weekly therapeutic phlebotomy of 500 ml whole blood (equivalent to approximately 250 mg iron) (Pietrangelo 2004). Regular venesection should be continued until ferritin levels are <50 ng/ml and transferrin saturation <30%. Although some patients with HH, for reasons that are unclear at this time, do not reaccumulate iron, most patients will require maintenance phlebotomy of 1 unit of blood to be removed every 2-3 months.

Dr Erna Mansvelt gave an comprehensive overview on the following aspects of haemochromatosis: 1) clinical and biochemical features, 2) guidelines how to distinguish hereditary iron overload from other disorders that affect iron metabolism and/or liver function, 3) work-up to the diagnosis and diagnostic tests, and 4) guidelines on disease management and possible complications.

The tired patient: Too much or too little iron in the diet?

Chronic fatigue is one of the most common presenting symptoms in the consulting room of the primary care physician, and the diagnosis could be quite challenging. It is very important

to distinguish between normal physiological causes of fatigue, and pathological causes such as organic (physical) and psychological causes of chronic fatigue. The diagnosis may become quite clear from the anamnesis and though physical examination, but special investigations may be essential to make a definitive diagnosis.

Two clinical entities that have to be considered are iron deficiency anaemia, the most common deficiency disease in the world, and iron overload that may also present with chronic fatigue.

When a patient present with chronic fatigue, serum ferritin is an important investigation to distinguish between the possibility of iron deficiency anaemia and iron overload. Ferritin, a major iron storage protein, is essential to iron homeostasis and is involved in a wide range of physiologic and pathologic processes. Ferritin makes iron available for critical cellular processes while protecting lipids, DNA, and proteins from the potentially toxic effects of iron. Alterations in ferritin are seen commonly in clinical practice, often reflecting perturbations in iron homeostasis or metabolism. In clinical medicine, ferritin is predominantly utilized as a serum marker of total body iron stores. In cases of iron deficiency and iron overload, serum ferritin serves a critical role in both diagnosis and management.

Ferritin is directly implicated in less common but potentially devastating diseases including familial haemochromatosis, sideroblastic anaemias, neurodegenerative diseases, and hemophagocytic syndrome. Elevated serum and tissue ferritin are linked to coronary artery disease, malignancy, inflammatory conditions and poor outcomes following stem cell transplantation. Additionally, recent research describes novel functions of ferritin independent of iron storage.

Iron deficiency, and anaemia resulting from iron deficiency is considered to be one of the top ten contributors to the global burden of disease. Negative effects of iron deficiency include cognitive impairment, decreased physical capacity, reduced immunity and impaired temperature regulation in a cold environment. The global prevalence of anaemia in preschool aged children is approximately 47%, pregnant women is 41%, and non-pregnant women is 30%. Globally, more than 800 million women and children suffer from anaemia, mostly in Africa, Asia and Latin America. Iron deficiency generally occurs in three sequential stages: depleted iron stores, iron deficiency erythropoiesis and iron deficiency anemia. All three stages can be analyzed biochemically with the measurement of haemoglobin (Hb), ferritin and soluble transferrin receptor (sTFR). Although there are some clinical indicators and the evaluation of iron intake might be helpful, the diagnosis relies mainly on these parameters.

The measurement of Hb is essential for the diagnosis of nutritional anaemia and is one of the most common, easiest and least expensive methods. Unfortunately Hb measurement is not very sensitive and specific for iron deficiency (only the third stage affects Hb synthesis). Thus, to determine whether iron deficiency is responsible for anaemia, it is usually necessary to include other indicators.

Ferritin is currently considered the most important indicator of iron status as even in the first stage of iron deficiency, its concentration decreases. Therefore it is the most sensitive indicator of the iron status and the cost of methods for the measurement of ferritin are relatively low. It is important to note that ferritin is increased by many factors, including infection and inflammation, thus a high value does not necessarily indicate a good iron status. It is therefore also valuable in some cases to measure parameters for acute [c-reactive protein (CRP)] and chronic infection [alpha-1-glycoprotein (AGP)].

The recent discovery of hepcidin, a 25 amino acid protein, which is produced in the liver, circulates in the plasma and is excreted in the urine, has revolutionized our understanding of

the regulation of iron absorption and storage. Hepcidin appears to have a primary role in ensuring the maintenance of an optimal iron store, in regulating iron delivery to all body cells in concert with the functional requirements and blocking the absorption of unneeded iron through the intestine. It acts as a negative regulator of release from stores and intestinal absorption. High levels reduce the rate of release from stores and absorption from the intestine by binding to the only known cellular iron exporter, ferroportin, causing it to be degraded. The expression of hepcidin is induced independently by the accumulation of storage iron and by inflammation.

Results from intervention trials seem to indicate that daily iron intake together with multiple micronutrients is most effective in improving anaemia. Iron supplementation administered to deficient individuals was found to increase oxidative stress, but treatment with a combination of iron and vitamins A, C and E proved effective in normalizing oxidative stress.

Recent research on iron supplementation in malaria-endemic areas showed disturbing results of an increased incidence of adverse effects and death. It has been suggested that the adverse effects of iron supplementation on malaria are due to increased peripheral availability of young red blood cells, increased iron reserves for parasite development and the loss of the inhibitory effect of microcytosis on intraerythrocytic parasites. Thus iron supplementation of iron deficient children in malaria endemic areas is not recommended. Mosquito control and avoidance of the insect vector are fundamental.

It is vital to consider the safety of interventions to reduce nutritional anaemias:

1. *Supplementation*

Supplements may contain more than the physiological daily requirements for a nutrient, in particular for iron. It is critical to avoid new imbalances that may arise from iron-induced corrosive effects or oxidative damage as a result of unintended overdosing.

2. *Fortification*

When exogenous nutrients are placed in food, the variation across the population of the consumption of the fortified foods become important. It is therefore important that the fortification level should provide safe exposure for the upper distribution of consumers.

3. *Dietary diversification*

The intervention relating to promoting foods as a source of nutrients are not without potential safety issues and might encourage a dietary pattern that is less than healthy, by increasing the intake of foods promoting chronic disease risk. Distorted intakes of red meat as a natural source of bioavailable iron, for example, could increase the risk of colon cancer and many diseases associated with saturated fat exposure.

Neither supplementation nor fortification can be effective on their own. The promotion of dietary improvement/diversification with a focus on improving the intake of bioavailable iron through greater consumption of products of animal products, fruit and vegetables, especially vitamin C-rich foods, is the preferred intervention as it can lead to sustainable improvements, not only from iron status but also of intakes of other micronutrients. The impact of the consumption of the habitual diet including micro- and macronutrients should be evaluated with priority.

Nutritional anaemias are currently the greatest global nutrition problem. A comprehensive, multiple intervention approach is necessary for sustainable success and must include improved social conditions by poverty alleviation measures, as well as the more direct

measures of fortification, supplementation and improved health care. Recent research points to functional consequences even before the clinical onset of iron deficiency anaemia. Longitudinal studies caution that chronic iron deficiency in infancy permanently retards cognitive, motor, and socio-emotional development.

Worldwide a paradigm shift is happening in healthcare as it is moving from primarily disease management towards a more prominent role for health management. Haemochromatosis is a good example of a preventable genetic disorder caused by an interaction between genetic and environmental factors. We therefore cannot focus only on using genetic testing for diagnosis, but should use it also for risk management.

Dr Dawie van Velden provided a practical approach to the management of patients presenting with chronic fatigue.

5. PATHOLOGY SUPPORTED GENETIC TESTING™

Numerous and major advances characterised the evolution of modern medicine during the past centuries and brought about many changes to the practice of clinical and diagnostic health sciences. The rapidly expanding integration and overlapping of traditionally distinctly separate fields of medicine introduced a new era of interdisciplinary- and team-approaches to clinical practice and patient care. In this regard, clinical human genetics including molecular genetics is indeed no exception (Schneider 2009).

In April 2009 the Department of Pathology at Stellenbosch University launched an initiative to develop pathology supported molecular genetic tests based on an integrated service- and research approach. It is considered important to develop innovative approaches to risk management of complex multi-factorial diseases to be applied in a clinical context, where the genetic test results are fully integrated with relevant clinical information and other diagnostic pathology data. To determine clinical usefulness, careful review of the literature is performed to prevent the use of single nucleotide polymorphisms (SNPs) of uncertain functional significance in genetic tests, following genome wide association studies that may identify risk alleles in the absence of supporting data on relevant metabolic impairments. The aim is to match the disease diagnosis and therapeutic design with the clinical picture, pathology, environmental risk factors and genetic profile of the patient. In order to address the important ethical and scientific issues pertaining to pathology supported gene-based intervention, the information must be captured in a database preferably as part of properly designed and ethical approved research projects that will advance evidence-based medicine.

Haemochromatosis provides an excellent example of a complex disease that is best addressed by a pathology supported genetic testing approach. What this means, is that genetic testing for haemochromatosis is 1) performed within a specific pathology/biochemical and clinical profile that defines the test selection criteria, 2) that both the biochemical and genetic test results are provided in the patient report together with the interpretation for clinical application, and 3) that gene expression and/or response to treatment is monitored through these accompanying genetic test parameters.

Until recently, the HFE gene was considered the only major cause of inherited iron overload. Failure to identify mutations in a relatively large number of patients referred by clinicians for genetic testing contributed to the identification of several other genes involved in iron overload. It also highlighted the importance of a step-wise approach in the diagnosis and treatment of iron overload disorders (Brissot et al. 2008).

Pathology supported genetic testing in patients at risk of iron overload involves the following steps to be discussed at the workshop for future implementation:

1. Consider iron overload based on presenting clinical features and iron status, taking into account the main confounding factors such as alcoholism, inflammatory conditions, acute or chronic hepatitis and polymetabolic syndrome.
2. Evaluate hepatic vs splenic iron load in order to direct the diagnosis to the most likely cause of iron excess.
3. Rule out acquired iron overload due to external factors such as prolonged iron supplementation (e.g. in the setting of competitive sports) or repeated transfusions in patients with haematological diseases, represented by chronic anaemias such as thalassaemia major and sickle cell disease.
4. Identify the genetic origin of iron overload by considering both family and personal information (e.g. plasma ceruloplasmin level when transferrin saturation is normal or low).
5. Treat patients with iron overload according to genetic subtype: venesection is the treatment of choice in patients with haemochromatosis related to hepcidin deficiency, but is poorly tolerated or contraindicated in patients with iron overload due to ferroportin failure.
6. Monitor treatment response by assessment of relevant biochemical parameters and health outcomes.

The ideal test for Haemochromatosis should not only focus on the identification or exclusion of genetic risk factors, but also the documentation of clinical conditions (e.g. non-alcoholic fatty liver disease, NAFLD), abnormal biochemistry and environmental factors that may contribute to or protect against disease development.

Such an approach is considered a logical addition to and expansion of well-established clinical practice to sub-classify complex diseases into treatable entities. A good example is **heart disease**, where correlation between the presence of well-established genetic and lifestyle risk factors and relevant biochemical profiles including an abnormal lipogram, **iron status** and/or folate/homocysteine levels, could be used to determine gene expression and monitor response to the intervention strategy applied.

The iron-heart disease link

The cardiomyopathy of idiopathic or acquired haemochromatosis suggests that the heart may be especially sensitive to toxic effects of excess iron (Sullivan 1990). The features of inherited iron overload include cardiac problems (arrhythmias and heart failure), and/or cirrhosis of the liver, diabetes, arthritis and skin pigmentation. Since haemochromatosis can be easily treated by phlebotomy once diagnosed, this condition is considered a preventable form of heart disease amongst other equally important health risks.

Not only homozygosity for the HFE C282Y mutation, but also the heterozygous state is associated with increased serum iron parameters (Rossi et al. 2001). Individuals with serum transferrin saturation levels above 55% carry an increased all-cause mortality risk especially when combined with high red meat intake (Mainous et al. 2004a,b). Coronary heart disease rates rise sharply from age 20 onwards in males, who begin accumulating excess iron from late adolescence. Healthy women, on the other hand, do not accumulate excess iron in their tissues until after the menopause, when they rapidly develop the same risk of CVD as men of the same age. Detection of an association between heterozygosity for mutation C282Y

and increased risk of acute myocardial infarction in men (Tuomainen et al. 1999), as well as with cardiovascular death in postmenopausal women (Roest et al. 1999), support the iron-heart link. The risk of cardiovascular death in postmenopausal women with at least one copy of the C282Y mutation appeared to be stronger in women who were hypertensive or current smokers, with a nearly 20-fold increased risk when both risk factors were present, compared with nonsmokers, nonhypertensives, and noncarriers. This finding again emphasised the importance of multiple risk factor assessment when low-expression mutations are analysed.

The potential combined effects of elevated body iron stores and hypercholesterolaemia is of particular concern, as two independent studies demonstrated worse clinical outcomes under such circumstances (Salonen et al. 1992; Wells et al. 2004). Persons with both elevated transferrin saturation and elevated LDL showed a significantly greater risk for CVD mortality than persons when both parameters normal or elevated LDL without elevated transferrin saturation.

Recent studies performed in the South African population demonstrated the significance of LDL particle size as a risk factor for CVD in patients with non-alcoholic liver disease (NAFLD), while the presence of HFE mutations may be associated with disease severity in some families (FC Kruger 2008, PhD study). Due to the findings relating to dyslipidaemia, obesity and insulin resistance in the South African study cohort, a diagnosis of NAFLD or metabolic syndrome is regarded an important indication for referral of chronic disease risk management, including the assessment of multiple genetic risk factors for CVD performed in conjunction with a medical and lifestyle/nutrition assessment (Kotze and Badenhorst 2005).

In conclusion, everybody should take notice of the potential danger of inherited iron overload as it starts with the same symptoms as iron deficiency, namely chronic fatigue. When feeling tired, many people assume iron deficiency and take iron supplements. The degree of iron overload, if any, depends on interaction between genetic (low penetrance) and environmental factors. Individuals with a genetic predisposition for haemochromatosis respond differently to iron intake due to gene-diet interaction, a concept termed nutrigenetics.

Dr Maritha Kotze discussed the concept of pathology supported genetic testing in the context of haemochromatosis and heart disease.

Haemochromatosis and non-alcoholic fatty liver (NAFLD) disease

Haemochromatosis can be prevented by regular blood donation or phlebotomy and therefore detection of a genetic predisposition at an early age, before irreversible damage to cardiac, hepatic and endocrine tissue occurs, represents an important clinical goal. HH is characterized by increased iron deposition in different organs of which iron deposition in the liver leads to liver cirrhosis and hepatocellular carcinoma (HHC). Several studies have implicated heterozygous HFE mutations in the exacerbation of other chronic liver diseases to progress to liver cirrhosis. This phenomenon can possibly be explained by the concept that excessive iron accumulation in the liver leads to increased production of reactive oxygen species (ROS). In a liver already compromised by another disease, the increased production of ROS can cause further insult and therefore more severe disease.

The presence and role of HFE mutations in patients with NAFLD and the development of advanced fibrosis remains controversial. The majority of the studies were limited by sample size and referral bias. A recent, large multicentre study from the US and Canada found that the prevalence of HFE mutations was not increased among Caucasians, however the presence of heterozygous C282Y mutation status in Caucasians was associated with

advanced liver fibrosis (Nelson et al. 2007). A Study with Anglo-Celtic subjects from Australia could not confirm these findings, neither could a large study of Caucasians from Italy (Bugianesi et al. 2004). The frequency of the C282Y mutation in the HFE gene was found to be increased in Italian patients from Northern Italy (Valenti et al. 2003). This finding suggests that heterozygosity for C282Y HFE mutation confers susceptibility to NAFLD. The study also concluded that carriers of the C282Y mutation in the HFE gene develop NAFLD in the presence of less severe metabolic abnormalities. In this study they also found that in the presence of increased serum iron the release of insulin was decreased, proposing it as the mechanism involved for the increased susceptibility to NAFLD.

These above-mentioned findings reflect the difficulties in implicating HFE gene mutations in the pathogenesis of advanced fibrosis for patients with NAFLD, especially when the sample size is relatively small. Association does not necessarily equate to causality and is largely dependent on presence or absence of other known risk factors and genetic background of the population.

It seems highly likely that carrier status or inheritance of two copies of a faulty HFE gene could contribute to disease development or severity in South African NAFLD patients, although not in all families. The combined effects of different genetic and environmental risk factors could explain the disease phenotype, but the factors involved could differ between families. In a relatively large South African family with multiple affected cases it seems likely that HFE gene mutations may underlie the familial clustering of NAFLD due to its role in oxidative stress and risk of diabetes, despite normal iron parameters in the majority of cases. Co-existence of NAFLD and HFE mutations was demonstrated in all affected family members subjected to mutation analysis. Mutation-positive family members without NAFLD most likely lack other risk factors required for disease development (including age effects) in the presence of one or more HFE gene mutations. Knowledge of a genetic predisposition for HH in NAFLD patients would nevertheless affect clinical management, as iron levels need to be monitored on a regular basis and phlebotomy treatment implemented to keep levels within the normal range, if necessary.

Patients with fatty liver on ultrasound and/or asymptomatic abnormal liver functions are often referred for specialist investigation. Fatty liver on ultrasound is not only a liver disease, but a marker for the metabolic syndrome. It is therefore important to know when liver biopsies need to be performed on these patients and which patients are at increased risk for CVD and cancer. HH provides a classical example of how advances in molecular technology have led to the replacement of liver biopsy as the diagnostic method of choice for this low-penetrance genetic disease. Identification of the causative HFE gene in 1996 by Feder and co-workers paved the way to universal use of DNA testing in conjunction with serum iron status to make a diagnosis of HH, without the need for an invasive liver biopsy. Today, liver biopsy is only performed in molecularly uncharacterised patients with the HH phenotype.

We know today that patients with NAFLD are not only at risk of developing advanced fibrosis, liver cirrhosis with decompensation, and hepatocellular carcinoma, but they also have a high likelihood of developing CVD and cancers associated with the metabolic syndrome. In a community based study performed in Olmsted County, Minnesota, NAFLD patients followed up for 16 years were found to have liver disease as the third leading cause of death with cancer and CVD in the first and second place (Adams et al. 2005 a,b). NAFLD has been confirmed as an independent risk factor for cardiovascular morbidity. Incorporation of genetic testing in conjunction with assessment of metabolic indicators and lifestyle risk factors may lead to improved patient management in this context.

Dr Corne Kruger discussed the role of HFE gene mutations and disease diagnosis in the context of metabolic syndrome and non-alcoholic liver disease (NAFLD).

Antioxidant effects of Rooibos tea on risk of cardiovascular disease

Rooibos tea contains small amounts of iron in addition to various other nutritional elements. The question was raised whether the tannins in rooibos would have a deleterious effect on iron absorption similar to that of ordinary tea. The tannins in tea bind iron and therefore reduce the absorption of iron. Some teas like black and peppermint tea may inhibit iron as much as 80-90% and may therefore be beneficial in patients with haemochromatosis.

The low tannin content of Rooibos tea is an advantage for people with digestive problems, but the effect on iron absorption in haemochromatosis patients is uncertain. In a study performed by Hesseling et al. already in 1979, it was shown that Rooibos tea does not affect iron absorption significantly.

Exhausted antioxidant defenses in the human body contribute to oxidative stress and when accumulated (as with ageing) may lead to disease development. Oxidative stress is involved in the etiology of various chronic diseases including cardiovascular disease (CVD). Recent studies have demonstrated the cardioprotective effects of Rooibos tea due to its ability to lower plasma cholesterol levels and reduce markers of oxidative stress. These findings have important clinical and nutritional implications.

Dr Jeanine Marnewick provided scientific evidence of the anti-oxidant effects of Rooibos tea in the context of its iron content.

6. GENETIC COUNSELLING

Genetic counselling forms an important aspect of genetic testing, although the level of counselling may differ according to the type of test offered. In patients with haemochromatosis the inheritance pattern of the condition and the risk of close family members to inherit and/or develop the disease is explained. It is made clear that haemochromatosis it is a treatable and even preventable disease if diagnosed at an early stage. It is furthermore explained that medical management such as phlebotomy in those diagnosed with HH or regular blood donation in healthy individuals with a genetic predisposition will reduce future risk of iron overload. Also, mutation carriers with only a single copy of a recessive gene will not develop haemochromatosis, but future generations may be affected. Family members without a HH mutation cannot transfer the determinant gene defect(s) to their children and have a risk similar to that of the general population.

As mutation carriers (in the case of the recessive forms of haemochromatosis) do not necessarily develop clinical symptoms the defective gene can be passed on in a family unnoticed. Offspring of two mutation carriers will have a 25% (1 in 4) chance of inheriting two copies of the defective gene. Since organ damage occur in approximately 40-60% of individuals with a genetic predisposition for haemochromatosis, it is important that testing is offered to all relatives of an HH sufferer (Milani and Kotze 1999). The risk is increased if a family history of arthritis, diabetes, liver disease or heart failure is present.

Registered genetic counsellor Frieda Loubser discussed the importance of genetic counselling in the context of ethical aspects and insurance issues related to haemochromatosis.

Frequently asked questions

1) Why is genetic testing important for diagnosis of haemochromatosis?

Genetic testing is very important to identify haemochromatosis at an early, preventable stage. In patients with serum ferritin levels above 1000 ng/ml the risk of liver cirrhosis is very high.

Increased iron levels occur in approximately 50% of women and 80% of men who with two copies of the most common HFE gene mutation, C282Y. However, in some population clinical expression of this genotype is less than 1% due to gene-gene and gene-environment interaction. Reduced penetrance is caused by interaction with other genes and the environment, which explains why it is possible to prevent this condition. If iron levels builds up to dangerous levels, it poisons the affected person from within.

A simple iron count, including a transferrin saturation (>45%) and serum ferritin test (>300 ng/ml), will show whether a person has too much iron in their blood. A DNA test can detect an increased risk of iron overload even before iron stores increase with age, which provides the opportunity for disease prevention. A positive DNA test in the presence of high iron levels confirms a diagnosis of haemochromatosis without the need to perform a liver biopsy. Determination of serum ferritin and transferrin saturation is recommended as a first line screening method for haemochromatosis in patients with clinical features suggestive of this condition. If haemochromatosis is not diagnosed early, it can lead to organ damage.

2) What effect does the typical South African diet has on disease expression?

A person's diet alone cannot control the iron overload or used for treatment of the condition, but it can help manage the uptake of iron and delay phlebotomy intervals. The use of vitamin C with red meat increases iron absorption and should be avoided in HH sufferers. Restrict (red) meat and alcohol intake, encourage fibres, tannin, anti-oxidants (green tea). Most importantly, haemochromatosis patients should not take iron tablets or other forms of food supplements containing iron.

The low penetrance of clinically manifested haemochromatosis described in some European populations may not apply to African populations that consume lots of red meat, food cooked in iron pots or beer brewed in iron containers in rural areas.

3) Does patients with haemochromatosis have to pay the blood bank to donate blood to help them manage the condition?

Typically, Blood Services in South Africa will accept blood from haemochromatosis patients who are on a maintenance program, which requires them to be bled at intervals of more than 56 days. Patients on maintenance will be accepted in the same way as any other donor. When a person requires to be bled more frequently than the normal interval required by the Blood Services, there are restrictions and there may be a cost for the bleed. It is advisable to confirm the detailed arrangements with the local blood services.

4) What is the best approach to the diagnosis of haemochromatosis in patients from different ethnic groups?

The possibility of haemochromatosis should always be considered when a doctor encounter patients with unexplained mild changes in liver function, abnormal tiredness, right hypochondrial pain, arthritis, diabetes, impotence (particularly if they are young), and unexplained cardiac complaints – particularly if more than one of these are present.

The doctor should be even more wary if the patient can trace his origin back to NW Europe. The screening tests to do first are determination of transferrin saturation and serum ferritin, which can be followed up by genetic testing in cases with high iron stores as appropriate.

5) How is haemochromatosis treated once the diagnosis has been made?

Intervention steps may include venesection, diet modification and chelation. The most common treatment for inherited iron overload is regular therapeutic phlebotomies. A regular, weekly phlebotomy (blood letting) of 500ml blood (which is equivalent to about 250mg of iron) may need to be done until the ferritin levels are less than 50 ng/ml and transferrin saturation <30%. After the levels have stabilised, most patients will require maintenance phlebotomies of one unit of blood every two to three months. Blood letting works because the body uses iron in the production of new blood.

Iron chelation is used mostly to treat patients with secondary haemochromatosis, but occasionally it may be useful in patients with one of the primary hereditary forms of the disorder. In patients with far-advanced haemochromatosis, who may have cirrhosis, hypoalbuminemia and congestive heart failure, the need to remove iron is particularly acute but the patient may not be able to tolerate the removal of a large volume of blood over a short period of time. Chelation therapy may also be preferred in patients in whom venous access is very difficult.

6) Will genetic testing in healthy individuals result in exclusion from life insurance?

There seems to be a misconception that genetic testing in healthy individuals would result in exclusion from life insurance; this does not apply, however, to treatable conditions such as HH identified at an early age if timely preventative measures are implemented. For HH the impact on insurance would depend on the degree of clinical expression at the time of assessment irrespective of a genetic test result. If a family history of HH is present but the applicant has not been tested for this condition, the decision on granting of the policy may be delayed until iron status has been determined, but a DNA test may not be required at this stage. In cases where a genetic predisposition for HH has been identified in the past (due to the presence of two copies of the defective gene) and this finding resulted in precautionary measures being taken to prevent organ damage, the applicant are likely to obtain life insurance at standard rates, provided that no complications occurred over a period of approximately two years prior to submission of the application. In the case of Sanlam and Liberty Life whose medical advisors contributed to an article on genetic testing higher insurance premiums could apply when compliance is not good and where this resulted in development of clinical symptoms related to the genetic defect(s) which have been identified (Kotze et al. 2004).

7) Should people who have been screened for genetic disorders be monitored to avoid stress and anxiety and when is testing most effective?

According to a study by Professor Paul Atkinson of the School of Social Sciences, Cardiff University, published in December 2004 - If someone is told that they are at risk, but have not yet had any symptoms, they are not unduly stressed about their health, provided that they felt they were being looked after. More support for DNA testing without waiting until it is too late to prevent organ damage, comes from a study performed in Australia. Cheek brush samples were taken at the workplace from more than 11000 adults in Australia and 47 people were identified who had two copies of the mutation and about 10% (1 338) who had

one. Self-reported tiredness was higher in C282Y++, 19 (83%) of 23 homozygous men and 11 (48%) of 23 homozygous women had raised fasting transferrin saturation. 46/47 have taken steps to treat or prevent iron accumulation.

Interestingly, almost all the participants were pleased they had been tested and no increase in anxiety was found in the people who had both mutations. This finding is in accordance with a Canadian study published in 2001. In fact, it was shown that anxiety decreased significantly in homozygotes and heterozygotes after genetic testing, and remained constant in C282Y mutation-negative cases. The authors concluded that genetic testing for haemochromatosis is well accepted and should not be discouraged on the basis of potential adverse psychosocial effects.

Population screening can be practicable and acceptable: Delatycki et al. Use of community genetic screening to prevent HFE-associated hereditary haemochromatosis. www.thelancet.com Vol 366 July 23, 2005.]

7. HAEMOCHROMATOSIS SOCIETY

The objectives of the HSSA are to promote awareness of haemochromatosis amongst the public and particularly the medical profession. Through awareness campaigns current information on haemochromatosis is made available and an important aim is to establish support groups for haemochromatosis patients and their families.

If someone has haemochromatosis it is vital that his/her family members are screened for the condition so that treatment can start before serious organ damage occurs. For the same reason, healthcare professionals need to be alerted to the early clinical features of haemochromatosis. People can suffer from the ravages of iron overload yet still be completely unaware of the condition. Haemochromatosis is treatable and the effects are preventable if the correct diagnosis is made before organ damage occurs.

If someone has two or more of the mentioned symptoms or has a relevant family history, refer for testing. The bottom line is that we must have a high index of suspicion in patients presenting with the features known to be associated with haemochromatosis and such patients must have their iron status checked. If indicated, a genetic test must be done to confirm the cause and to screen other at-risk family members.

Public can help by:

- Asking their doctor about iron overload
- Talking to others if have HH
- Joining the Society
- Refer patients for HH (medical professionals)
- Making a Donation to the HSSA

In memory of Paul Bird – by Kirsten Alberts:

My brother died at the age of 24 years, by the time he knew he had haemochromatosis he was dying. The shock for our family was huge and I decided to do all I can to ensure that other families do not have to go through the same trauma. We knew he was ill, but we had no idea that what he had was not only hereditary, but preventable if diagnosed and treated at an early stage.

From my experience in my own family and with HH sufferers through the HSSA, haemochromatosis is frequently diagnosed when it is too late to prevent organ damage because early features of iron overload such as fatigue, joint pain, abdominal pain and loss of libido are non-specific and are commonly not recognised to be associated with HH by primary care physicians. Most cases of early iron overload have normal liver function tests, whereas mildly abnormal liver function tests are commonly ascribed to excessive alcohol use. In the case of my brother he complained of a sensitive stomach and became very aware of what he ate, because he suffered from unexplained diarrhoea. He even thought that he had irritable bowel syndrome or an ulcer and went for a gastroscopy, but it showed nothing. His complexion became darker and he became even thinner. After his death, a friend mentioned that he had had severe joint pains. Twice Paul went to urologists because he experienced impotence problems and twice he was given a clear bill of health. Then, on a Friday afternoon in February 2000, he felt so ill that he could not work. He lay down on the floor in his office, desperately trying to breathe. A doctor referred him for an emergency appendectomy, due to his severe abdominal pains and high blood pressure. In theatre, before the doctors could remove his appendix, they noticed severe organ damage and he was placed in intensive care. His heart and liver were enlarged. There was damage to his pancreas, water in his lungs and most of his glands were swollen. They told us it could be TB, cancer or Aids. Tests were done and on the Monday we heard that it was an iron overload. In the meantime his lungs had collapsed and by the next day Paul was dead.

In my brother's case the initial genetic tests of the HFE gene showed that he had only inherited one gene mutation from one parent, so he should not have developed the disease. But he also had another gene defect which causes juvenile haemochromatosis. That is why he became sick so early in life. All the symptoms were there, but they were not diagnosed – not because the genetic defect was not identified but because his iron status was not checked early on in the course of his illness.

Take-home message

It is now history that the general outcome of haemochromatosis will be gradual iron loading and eventual multi-organ damage, except if the expression of the gene or its effect is altered by interaction with other genetic or environmental factors or is treated by phlebotomy. The pathological changes are fully preventable if the diagnosis is made early and preventative measures introduced timeously. Once HH has been diagnosed in a family using iron studies all close relatives should be screened by DNA testing as part of the iron studies done to identify those with a genetic predisposition before iron loading occurs so they can stay healthy by donating blood on a regular basis.

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