Preventing organ damage by genetic testing for hereditary haemochromatosis

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Abstract

The rapid discovery of several iron-related genes in the last 10 years has led to the development of cost-effective genetic assays for early diagnosis of hereditary haemochromatosis (HH). A genetic predisposition for this relatively common autosomal recessive disease has been identified in approximately 1 in 100 South Africans of European descent. If left untreated, this condition may lead to organ damage presenting as cirrhosis, liver cancer, diabetes, arthritis, impotence, sterility and/or cardiac disease. Due to the fact that serum iron parameters are frequently affected by factors such as liver disease and inflammation, direct mutation detection has become the method of choice for accurate diagnosis of inherited iron overload in patients with elevated iron stores. 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.

Introduction

Hereditary haemochromatosis (HH) 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 HH complicating early clinical diagnosis. Early features of iron overload such as fatigue, joint pain, abdominal pain and loss of libido are non-specific and not commonly recognised to be associated with HH by primary care physicians (Niederau et al. 1996). Most cases of early iron overload have normal liver function tests, whereas mildly abnormal liver function tests are commonly ascribed to excessive alcohol use. Conditions and symptoms associated with iron overload include:

- Chronic parenchymal liver disease, cirrhosis, hepato cellular carcinoma
- Cardiomyopathy and arrhythmias
- Diabetes mellitus type I and II
- Infertility, amenorrhoea, 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

How does iron accumulation lead to disease?

Iron is absorbed from the gut enterocyte, and transported bound to the carrier protein transferrin to most organs of the body. Because of iron toxicity, stored iron is mainly compartmentalised as ferritin in the bone marrow where it is available for haem synthesis.

If iron absorption is dysregulated iron will accumulate. Iron deposited in organs will lead to organ dysfunction. In haemochromatosis organs most commonly affected include the liver, skin, pancreas, joints, heart and pituitary gland.

Who is at risk?

Genetic studies performed in South Africa by de Villiers et al (1999a,b) have identified the genetic defects in more than 80% of HH patients and have shown that 1 out of 6 Caucasians are carriers of a common iron-related mutation (C282Y) in the HFE gene (Feder et al. 1996). This means that an estimated 1/115 Caucasian individuals in South Africa are homozygous for the C282Y mutation. As mutation carriers do not necessarily develop clinical symptoms the defective gene can be passed on in a family unnoticed. HH is the most common autosomal recessive disorder that affects humans. This means that both genes must be inherited (one from each parent) to develop clinical disease. 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.

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 in the South African population (Zaahl et al. 2004) as more novel mutations are found in an increasing number of genes (Table I).
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). The haemochromatosis strip-assay currently includes 17 mutations in three genes and validation of this assay in the South African population (Kotze et al. 2004) accurately produced the correct genotype. This is an important consideration, because the gene regions of relevance to PCR-based tests for HH frequently contain sequence changes that may interfere with the test procedure and data interpretation (de Villiers and Kotze 1999). Guidelines for genetic testing and diagnosis of haemochromatosis are provided in the flow diagram in Figure 1.

Table I: Different genes underlying iron overload disease subtypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Subtype</th>
<th>Main Organs affected</th>
<th>Organ damage</th>
<th>Symptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human iron (HFE)</td>
<td>Type I, AR</td>
<td>Liver, endocrine glands, heart</td>
<td>Variable</td>
<td>4-5th decade</td>
</tr>
<tr>
<td>Hemojuvelin (HJV)</td>
<td>Type II, AR</td>
<td>Liver, endocrine glands, heart</td>
<td>High</td>
<td>2-3rd decade</td>
</tr>
<tr>
<td>Hepcidin (HAMP)</td>
<td>Type IB, AR</td>
<td>Liver, endocrine glands, heart</td>
<td>High</td>
<td>2-3rd decade</td>
</tr>
<tr>
<td>TF receptor (TFR-2)</td>
<td>Type III, AR</td>
<td>Liver, endocrine glands, heart</td>
<td>Variable</td>
<td>4-5th decade</td>
</tr>
<tr>
<td>Ferroportin (SLC40A1)</td>
<td>Type IV, AD</td>
<td>Liver spleen</td>
<td>Low</td>
<td>4-5th decade</td>
</tr>
</tbody>
</table>

AR, autosomal recessive inheritance; AD, autosomal dominant inheritance

Figure 1: New guidelines for genetic testing and diagnosis of Haemochromatosis

TARGET HIGH-RISK POPULATIONS FOR TESTING
Diabetes, cardiac disease, hepatomegaly, male sexual dysfunction, hyperammonotransferasemia, liver disease with chronic hepatitis C and nonalcoholic steatohepatitis (NASH), early onset alipycal arthropathy, unexplained fatigue

Determine serum transferrin saturation and ferritin levels

PRE-TEST ASSESSMENT
Fasting transferrin saturation >45% and haematologic disorders or liver disease excluded

Strip-assay analysis of multiple iron-related mutations in a single reaction

Mutation-positive

Mutations-positive or carrier

Mutation-negative

Interpretation of test result:
• Evaluate functional effect(s) of mutation(s) identified and possible gene-gene interaction within the context of clinical and biochemical information provided at referral
• Iron overload genotype confirmed for HFE, TFR-2, SLC40A1 genes

Interpretation of test result:
• Re-evaluate clinical features, document presence of conditions suggestive of iron overload in close family members, repeat testing of serum iron parameters
• Consider liver biopsy for diagnosis when serum ferritin and transferrin saturation remains persistently elevated
• If hepatic iron index and distribution indicative of HH

Determine serum transferrin saturation and ferritin levels

DIAGNOSIS
Adult-onset haemochromatosis

Mutation-positive

Mutation-negative or carrier

Determine serum transferrin saturation and ferritin levels

DIAGNOSIS
Probable haemochromatosis

Family screening
• Screen DNA samples of close family members for haemochromatosis gene mutations

Long-term research project
Screen DNA sample of index patient for undefined mutations in all 5 genes using direct sequencing
• Sequence HJV and HAMP genes in patients with early-onset iron overload
• Consider more extensive analysis of the HFE, TFR-2 and SLC40A1 genes in mutation-negative patients

Genetic counselling
Explain that:
• Mutation carriers with only a single copy of a recessive gene will not develop haemochromatosis, but future generations may be affected
• Mutation-negative family members cannot transfer the determinant gene(s) to their children and have a risk similar to that of the general population
• Pre-clinical diagnosis of a genetic predisposition for haemochromatosis will not have an impact on insurance, provided that iron levels are kept within the normal range
• Medical management (e.g. phlebotomy or regular blood donation in healthy individuals) will reduce future risk or iron overload in relatives with the haemochromatosis genotype

References