

Leading article

Population screening for haemochromatosis

Introduction

Results of many studies from around the world have revealed that haemochromatosis is the most prevalent genetic disease in people of European descent. However, it continues to be regarded as an uncommon disease by many physicians and is not listed as a common cause of death. The gene for haemochromatosis was discovered in 1996 and most typical patients are homozygous for the C282Y mutation of the HFE gene.¹ Studies using genetic testing have estimated the prevalence to be in the 1:188 to 1:327 range (table 1).^{2–5} The two most likely explanations for the apparent discrepancy between prevalence studies of haemochromatosis and the clinical impression are: (a) the failure to consider the diagnosis and to order the appropriate diagnostic tests (underdiagnosis) and (b) absence of iron overload and clinical disease in patients homozygous for the haemochromatosis gene (incomplete penetrance).

The percentage of C282Y homozygotes with iron overload as defined by an increased serum ferritin in population screening studies has ranged from 19% (blood donors) to 75% in the general population (table 1). However, an elevated serum ferritin is not synonymous with the inevitable progression of the disease to cirrhosis, diabetes, heart disease, and other haemochromatosis related morbidity. In a study of patients with haemochromatosis from our hospital, treated at the time of diagnosis and followed over a 30 year period, 43% of men and 28% of women developed life threatening complications.⁶ This is likely to be an overestimate of the morbidity of haemochromatosis because of a referral bias. A major impediment in the implementation of population screening for haemochromatosis has been this uncertainty about the burden of disease and the natural history of disease in an asymptomatic C282Y homozygote detected by screening. The unique design of the screening study by Olynyk *et al* in Busselton, Australia, allowed for serial testing of serum ferritin in untreated C282Y homozygotes. This study showed that in 60% of homozygotes serum ferritin increased over the four year observation period, but four cases had normal ferritin concentrations.³ As randomisation to a no treatment arm is considered unethical, this observation in untreated C282Y homozygotes may form the basis of future predictions and cost models on screening for haemochromatosis.

Case definition

A fundamental controversy in the area of screening for haemochromatosis is whether the disease should be defined on the basis of genetic testing independent of iron status or should be defined on the basis of the degree of iron overload in the absence of other known risk factors. A

position on this issue must be taken in any screening study to avoid circular reasoning.

PHENOTYPIC DEFINITION

The diagnosis of haemochromatosis was previously based on a combined clinical and laboratory assessment that included history and physical examination, elevated transferrin saturation (TS; thresholds have ranged from 45% to 62%), serum ferritin, parenchymal iron overload on liver biopsy, elevated hepatic iron concentration, quantity of iron mobilised by venesection therapy, and pedigree studies identifying other family members with iron overload. The pedigree studies have been shown to have the best correlation of close to 100% with genetic testing⁷ but are the most difficult to perform in many countries. Iron overload within a single pedigree in the absence of the typical C282Y mutation of the HFE gene has been reported in Italy but remains an uncommon observation.⁸ As the cornerstone of the *phenotypic* diagnosis of haemochromatosis was iron overload, it was self-fulfilling that biochemical markers of iron overload (transferrin saturation, ferritin) would be elevated in these cases. As physicians became aware of the diversity of liver conditions that could be associated with secondary iron overload (alcoholic liver disease, chronic viral hepatitis, cirrhosis of many aetiologies),⁹ the phenotypic diagnosis based on iron overload in an isolated patient became problematic with many false positives or overdiagnosis of haemochromatosis.

GENOTYPIC DEFINITION

It has become apparent that >90% of typical patients with haemochromatosis have the C282Y mutation of the HFE gene.^{10 11} The centres that are the most enthusiastic about the C282Y genetic test as a diagnostic test for haemochromatosis are those centres where pedigree studies have been an integral component of the diagnostic evaluation (London, Canada, Brisbane, Australia, Rennes, France, Salt Lake City, Utah). At our own centre we have found that 96% of our patients that were classified as homozygotes before the discovery of the gene are C282Y homozygotes. The centres that are the least enthusiastic about the C282Y test are those that have a much lower prevalence of the gene (Italy) and centres where the phenotypic case definition was based on iron overload without pedigree studies. It is this diversity of phenotypic assignment that has led to the uncertainty in the medical community about the C282Y test as a diagnostic test for haemochromatosis.

Abbreviations used in this article: TS, transferrin saturation; UIBC, unbound iron binding capacity.

Table 1 Prevalence of haemochromatosis in screening studies using genotyping and phenotyping

Year	Population sample	Number screened	Location	Initial test	Liver biopsy	Prevalence of C282Y homozygotes	Iron Overload*	Reference
1998	Electoral roll	1064	Christchurch, New Zealand	TS%, C282Y	Yes	1 in 213	60%	Burt and colleagues ²
1999	Primary care (HMO)	1653	Missouri, USA	TS%, C282Y	Yes	1 in 276	50%	McDonnell and colleagues ⁵
1999	Blood donors	5211	London, Canada	UIBC, C282Y	Yes	1 in 327	19%	Adams and colleagues ⁴
1999	Epidemiology survey	3011	Busselton, Australia	TS%, C282Y	Yes	1 in 188	75%	Olynyk and colleagues ³

*Iron overload is defined as the percentage of C282Y homozygotes with an elevated serum ferritin. TS, transferrin saturation; UIBC, unbound iron binding capacity.

At the same time, geneticists marvel at the observation that a single genetic mutation explains most of the cases of haemochromatosis in the world. A second mutation, H63D, has been more difficult to implicate in the pathogenesis of haemochromatosis. Compound heterozygotes (C282Y/H63D) have mild to moderate iron overload, often in association with another risk factor. It has been estimated that only 1.5% of compound heterozygotes will develop significant iron overload.¹¹ H63D homozygotes are common in the general population and rarely have iron overload.¹²

Screening methods

The ideal population for screening is young adults (<40 years) of European descent. Selective screening in patients with symptoms of haemochromatosis has not been an optimal strategy because organ dysfunction is often already established. Screening strategies include phenotypic screening, phenotypic/genotypic screening and genotypic/phenotypic screening (table 2). The most established screening test for haemochromatosis is transferrin saturation. This can be determined using (1) the serum iron/total iron binding capacity, (2) serum iron/serum transferrin, or (3) serum iron/serum iron plus unbound iron binding capacity (UIBC). The transferrin saturation is raised in most but not all haemochromatosis homozygotes. The sensitivity of transferrin saturation for the diagnosis of haemochromatosis has been reported to be 94% in the recent population screening study from Busselton, Australia.³ The UIBC is a one step automated biochemical test that can be done at a substantially lower cost than measuring the transferrin saturation. A population screening study in blood donors using the UIBC to detect C282Y homozygotes found that the UIBC outperformed the transferrin saturation test with a higher sensitivity and fewer false positives.⁴ More studies are required in the general population to confirm these observations. Previous screening studies prior to genetic testing advocated the recall of patients to have a fasting transferrin saturation test.^{13 14} The objective was to reduce the number of false

positives that would proceed to liver biopsy. However 20% of participants will not return for testing which leads to a large number of missed homozygotes.¹⁵ The number of C282Y homozygotes missed below the transferrin saturation threshold cannot be calculated in a study unless genetic testing is done on all participants. Although the goal of most screening studies is to detect homozygotes requiring venesection treatment, in our pedigree studies in 299 families we have found that 3% of C282Y homozygotes had a TS% <45% despite a ferritin >300 µg/l. Our current direction in screening methods is towards sequential testing on the original blood sample rather than sequential visits with blood sampling to minimise the missed homozygotes. Our preferred strategy is initial screening with the low cost UIBC, ferritin on cases with a UIBC <28 µmol/l and then genetic testing on those cases with both a low UIBC and a raised ferritin (phenotypic/genotypic—one step; table 1).

An alternative to phenotypic screening is initial testing with genotyping for the C282Y mutation in adults. This detects both iron loaded and non-expressing homozygotes. Genetic testing could technically be done in neonates but this raises complex ethical issues and long term follow up arrangements. Management decisions in adults can be based on a serum ferritin measurement in C282Y homozygotes. An estimate of the number of cases that have the gene without iron overload is essential to study the cost implications of this strategy. The cost-effectiveness profile for screening for haemochromatosis is within acceptable guidelines even if only 20% of patients will develop life threatening symptoms.¹⁶ Siblings of non-expressing homozygotes have been found to have iron overload.^{17 18} There is a concern that non-expressing homozygotes detected through genotyping may be at risk of losing health insurance, life insurance, employment, or suffer from the stigma of being labelled with a disease. Further studies are required to assess the psychosocial impact of genetic testing in asymptomatic patients. In most cases the benefits to the patient and their family outweigh these potential risks. Legislation to prevent genetic discrimination is forthcoming.

Table 2 Comparison of population screening strategies for haemochromatosis

	Phenotypic (sequential visits)	Phenotypic/genotypic (sequential visits)	Phenotypic/genotypic (one step)	Genotypic/phenotypic (one step)
Initial test	TS or UIBC	TS or UIBC	TS or UIBC	C282Y
Secondary tests	Returns for fasting TS then ferritin	Returns for fasting TS then ferritin followed by C282Y	Ferritin on original sample followed by C282Y	Ferritin on original sample
Liver biopsy	All	Selected cases (liver dysfunction, atypical genetic test)	Selected cases (liver dysfunction, atypical genetic test)	None
Overdiagnosis	Alcoholic liver disease, chronic viral hepatitis	No	No	Detects non expressing homozygotes
Underdiagnosis	Will exclude homozygotes with TS <45% and those that do not return for fasting TS	Will exclude homozygotes with TS <45% and those that do not return for fasting TS	Will exclude homozygotes with TS <45 % or UIBC >28 µmol/l	Will exclude non-C282Y haemochromatosis
Age dependent	Yes	Yes	Yes	No
Detects iron deficiency	Yes	Yes	Yes	No
Cost considerations	Cost of liver biopsy	Cost of lost homozygotes that do not return for fasting TS	More C282Y and ferritin tests but fewer homozygotes missed	Cost of initial genetic test

TS, transferrin saturation; UIBC, unbound iron binding capacity.

ing and should help alleviate the anxiety related to this problem. Education of the insurance industry on the excellent prognosis in the absence of organ dysfunction is essential.

An impediment to the implementation of population screening has previously been the need for liver biopsy, particularly in those that do not have haemochromatosis. Genetic testing has replaced the need for liver biopsy in many cases, particularly in young adults without liver dysfunction. A C282Y homozygote with a normal aspartate aminotransferase, without hepatomegaly and a serum ferritin <1000 µg/l has a very low probability of having liver fibrosis.¹⁹ There will still be a role for liver biopsy to assess the severity of liver disease in patients with liver dysfunction and as a diagnostic method in the iron loaded patient without the typical genetic profile. As the number of liver biopsies decreases, gastroenterologists will become less involved in the diagnosis and management of haemochromatosis.

A central national screening programme seems to be an unattainable goal in most countries. A more feasible one is to increase both the public's and doctors' awareness of the magnitude of the problem so that primary care physicians at the initial contact point with a healthy adult patient at risk could screen for haemochromatosis. Screening for hypercholesterolaemia or breast cancer has already reached this level of awareness. Haemochromatosis is a model disease for prevention, and early screening will prevent the fatal complications of this disease. The uncertainty about the clinical expression of disease is likely to be resolved by the population screening studies that are already in progress in over 15 countries.

To move forward with population screening for haemochromatosis, the gap between public health experts who do not support universal screening^{20 21} and haemochromatosis physicians must narrow. Haemochromatosis physicians can benefit from the expertise used in other public health screening initiatives that have emphasised attributable risk and the need for cross-sectional studies with control groups. At the same time, public health officials should become more directly involved with patients with haemochromatosis to understand the challenges of phenotypic assignment and the morbidity of the disease. Since the discovery of the gene and the subsequent rapid growth of publicity on haemochromatosis, we have already begun to see an increase in early referrals for this condition. The combination of increased awareness amongst physicians and patients may ultimately lead to the same goal as population screening, namely the early diagnosis and treatment of disease.

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