

REVIEW

Is genetic screening for hemochromatosis worthwhile?

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Abstract. Hereditary hemochromatosis is an iron overload disorder and is the most common recessive disease in Caucasians. About 80% of hemochromatosis patients are homozygous for the C282Y mutation in the *HFE* gene. Since iron accumulation can be prevented by phlebotomy, there is increasing interest in screening populations for hemochromatosis. Hemochromatosis is a disease that meets all the criteria for screening as set by the World Health Organization (WHO) or the US preventive services task force criteria for a screening program. However, there is no consensus on the value of a screening program for hemochromatosis. Moreover, there is no agreement on whether this screening should be based on the

phenotype i.e. biochemical levels of serum iron parameters or on the genotype i.e. based on the presence of mutations in the *HFE* gene. Other important concerns are the lack of important data in evaluating screening as well as the psychosocial impact of a screening program. The present review analyses the current situation from a genetic-epidemiological perspective. We conclude that general population screening may be helpful to identify high-risk groups or individuals in the early stage of the disease so that treatment can be started. We suggest a two-phase screening program based on the first instance on serum iron levels and then a genetic test to only those with elevated serum iron parameters.

Key words: Genetic epidemiology, Hemochromatosis, HFE, Penetrance, Screening

Introduction

Hemochromatosis includes several disorders of iron metabolism characterised by pathological accumulation of iron in tissues [1]. Although there is no consensus on the definition of hemochromatosis, the disease is usually categorised into primary and secondary forms [2]. Primary hemochromatosis is referred to as hereditary hemochromatosis. It is an inherited disorder resulting from an inborn error of iron metabolism that leads to progressive iron loading of the parenchymal cells in the liver, pancreas and heart [1]. Secondary hemochromatosis referred to as acquired hemochromatosis is an iron overload disorder that occurs as a result of chronic disorders of erythropoiesis such as thalassemia or sideroblastic anemia [2]. Hereditary hemochromatosis is one of the most common genetic diseases in populations of northern European descent with a prevalence of 0.2–0.5% [2–6]. Hemochromatosis can lead to multiple diseases like cirrhosis, hepatocellular carcinoma, diabetes mellitus, amenorrhoea, impotence, cardiomyopathies, arthritis, pituitary hypogonadism and skin hyper pigmentation [3, 7, 8]. Early symptoms and complaints include joint pain, abdominal pain, weakness and fatigue [8]. Expression of the disease is modified by several factors, in particular dietary iron intake, blood loss associated with menstruation and pregnancy, and blood donation. The disease is 5–10 times more frequent in men than women and the age of onset is delayed in

women [9]. Hemochromatosis does not usually express before 20 years of age, although with genetic screening and periodic health examinations, asymptomatic subjects with iron overload can be identified in adulthood.

For long, the diagnosis of hemochromatosis was based on the presence of excess iron in a liver biopsy in combination with serum iron, serum transferrin, and total iron binding capacity (TIBC) [10, 11]. In 1996, Feder et al. [12] reported that two mutations in the *HFE* gene, the C282Y and the H63D are associated with hereditary hemochromatosis [12]. The C282Y mutation is found in about 85% of patients with hereditary hemochromatosis. Since the discovery of these mutations, diagnostic procedures have shifted to biochemical and genetic tests. Biochemical tests including serum iron, ferritin and transferrin saturation level are now widely used in combination with genetic tests [13]. Hemochromatosis has been regarded as a model disease for large-scale genetic screening. The aim of this paper is to critically review the potential of genetic testing in hemochromatosis. Before we turn to preventive screening we will start with a brief review of the genetic epidemiology of hemochromatosis.

Genetic epidemiology of hemochromatosis

In 1935, Sheldon [14] suggested that hemochromatosis is an inborn error of iron metabolism. Studies of

familial aggregation have extended from the 1970s up to the 1990s. Hemochromatosis is indeed found more commonly in relatives of patients [15–22]. Studies of the transmission of the disease in families suggest that hemochromatosis segregates usually as an autosomal recessive trait [15, 18, 23]. Genetic and phenotypic heterogeneity are well-recognised features in hemochromatosis and it is becoming more and more evident that several genes or environmental factors may lead to the disease. Depending on the localisation of the genetic defect and the clinical phenotype, several types of hemochromatosis are distinguished.

Type 1 hemochromatosis

Type 1 hereditary hemochromatosis (*HFE1* or simply *HFE*) is by far the most common form of hemochromatosis [12, 24–26]. The culprit gene, termed *HFE*, is located on human chromosome 6p21.3 and has two major mutations, c.845G → A (C282Y) and c.187C → G (H63D) [12]. Since its identification, over 37 other allelic variants of the *HFE* gene have been described [27]. The localisation of the *HFE* protein in the crypt cells of the duodenum, the site of dietary iron absorption and its association with the transferrin receptor in those cells are consistent with a role in regulating iron absorption [28, 29]. In *HFE* associated forms of hemochromatosis, the progression of iron overload is usually slow and affected individuals do not often present with clinical signs or symptoms until the fifth or sixth decade of life [30]. Type 1 hemochromatosis explains for a large part the prevalence of hemochromatosis (0.2–0.5%) found in northern Europeans [3, 5, 31]. *HFE* segregates in families as an autosomal recessive trait [16, 18] and about 80% of clinically diagnosed probands of hemochromatosis patients are homozygous for the C282Y mutation in the *HFE* gene [1, 12, 32].

Type 2 hemochromatosis

Type 2 hemochromatosis (*HFE2*), also called juvenile hemochromatosis, differs distinctly from type 1 hemochromatosis [33, 34]. This is a rare recessive form with a more severe disease phenotype that affects both sexes equally in the second decade of life. There is rapid iron loading and early onset of cardiac symptoms, endocrine dysfunction (hypogonadotropic hypogonadism) and premature death [33, 35, 36]. Kelly et al. [37] reported a mean onset of 22 years in patients from three pedigrees. The gene responsible has not yet been cloned but several genomic screens suggest it is located on human chromosome 1q in the region of D1S498 and D1S2344 [38]. By homozygosity mapping the critical candidate region was defined in an interval of 4 cM between markers D1S442 and D1S2347. It has been recently suggested that more than one gene may underlie the phenotype of juvenile hemochromatosis. This genetic

type of hemochromatosis is referred to as chromosome 1q unlinked juvenile hemochromatosis [39].

Type 3 hemochromatosis

Type 3 hemochromatosis (*HFE3*) is phenotypically similar to *HFE1*. The disease has been associated to the transferrin receptor 2 (*TFR2*) gene on human chromosome 7q22 [40]. Five *TFR2* homozygous mutations have been documented in *HFE3* patients, a nonsense mutation (Y250X), a missense mutation (M172K), a C insertion that causes a frameshift and a premature stop codon (E60X), a missense mutation in exon 17 (Q690P) and a 12 bp deletion in exon 16 that causes four amino acid loss (AVAQ 594-597del) in the extra cellular domain of *TFR2* [41]. The *TFR2* gene is homologous to transferrin receptor (*TFRC*) gene and is able to bind transferrin with lower affinity than *TFRC*. The *TFR2* function is still unclear. *TFR2* is spliced in two alternative forms, Alfa and Beta. The Alfa form is strongly expressed in the liver. The Beta form, coded from a start site in exon 4 of the Alpha has a low and ubiquitous expression [40, 41]. *TFR2* mutations are very rare mutations.

Type 4 hemochromatosis

Contrary to the previously described forms of hemochromatosis, type 4 hemochromatosis or *HFE4* segregates as an autosomal dominant trait [42–44]. The clinical phenotype of patients in this case is quite similar to that of patients with *HFE1* hemochromatosis but differs in that the disease is less severe and the pattern of iron loading is distinct [42–44]. Iron accumulates predominantly in Kupffer cells and other macrophages. Type 4 hemochromatosis (*HFE4*) is associated with various mutations (N144H, A77D, V162 del) in the *SLC11A3* gene encoding the metal transporter called ferroportin (*FPN1*) alias, iron regulated transporter (*IREG1*) or metal transporter protein (*MTP1*) on human chromosome 2q [43–47]. The exact mechanism by which mutations in the *SLC11A3* gene causes autosomal dominant iron overload is still not known. Gain of function and loss of function of the protein have both been suggested [43, 44], but it is becoming more apparent that interactions between *SLC11A3* protein and other proteins involved in iron metabolism occur and can lead to iron accumulation [48].

A form of autosomal dominant iron overload clinically distinct from type 4 hemochromatosis and which is due to a single point mutation (A49U) in the iron responsive element of the H ferritin mRNA has been reported in a single Japanese family [49].

Other types of hemochromatosis

Other forms of hereditary iron overload include neonatal hemochromatosis, hypoferritinemia cata-

ract syndrome, aceruloplasminemia, congenital atransferrinemia and African iron overload. African iron overload is common in sub-Saharan Africa and is a distinct type of iron storage disorder [50, 51]. It is believed to result from increased dietary iron derived from traditional home-brewed beer. The etiology of neonatal hemochromatosis and hereditary hyperferritinemia cataract syndrome is not well understood. Aceruloplasminemia, and congenital atransferrinemia are due to the absence of ceruloplasmin and transferrin respectively and are secondary forms of iron overload. The pattern of iron deposition in patients suffering from these diseases is clearly different from that of classical hemochromatosis. Each of these disorders is rare.

Occurrence of mutations involved in hemochromatosis

HFE is the most widely studied gene that is involved in hemochromatosis. In the general Caucasian population, the carrier frequency of the C282Y mutation is estimated to be 10%, and for the H63D mutation, 22% [3, 5, 12, 24, 25, 30, 31, 52, 53]. In Caucasians, the most common form of hemochromatosis is due to homozygosity for the C282Y mutation or compound heterozygosity for the C282Y and H63D mutations in the *HFE* gene [20, 34, 36, 52]. The proportion of hemochromatosis due to *HFE* mutations varies in different parts of the world. Figure 1 summarises the published frequencies of carriers of *HFE* mutations in different populations (adapted from Hanson et al. 2001) [31]. Most C282Y and H63D carriers are found in the United States of America and Europe. About 65% of the population of these two continents are homozygous for the wild type or normal allele com-

pared to 85% in India, and about 95% in Africa, Middle East, and Asia.

Up until now all other mutations involved in hemochromatosis are found to be rare, the contribution of *HFE2* gene to the occurrence of hemochromatosis is thought to be limited to a few families [37, 38]. TFR2 mutations are rare but may occur frequently in the Italian population [40]. Although several mutations have been reported for the SCL11A3 gene, these mutations are thought to be rare in the general population.

Is genetic testing worthwhile?

In recent years there has been increasing interest in screening populations for hemochromatosis [5, 54–56]. Hemochromatosis is an excellent example of a disease that meets the World Health Organisation recommendations and the US preventive services task force criteria for a screening program [57, 58]. The disorder is common, it has a prolonged asymptomatic and early symptomatic phase, and if untreated can result in serious morbidity and premature death. Simple and effective screening tests for iron overload are available and there is a reliable confirmatory test. The treatment is safe and acceptable and in some countries the blood collected from venesection treatment is utilised by the blood transfusion services. It is still a matter of debate whether we should screen for hemochromatosis and if yes whether the test should be based on biochemical levels of serum iron parameters or based on the presence of common mutations in the *HFE* gene [54, 59–61]. On the one hand, screening using DNA analysis is simple to carry out and has the additional advantage of

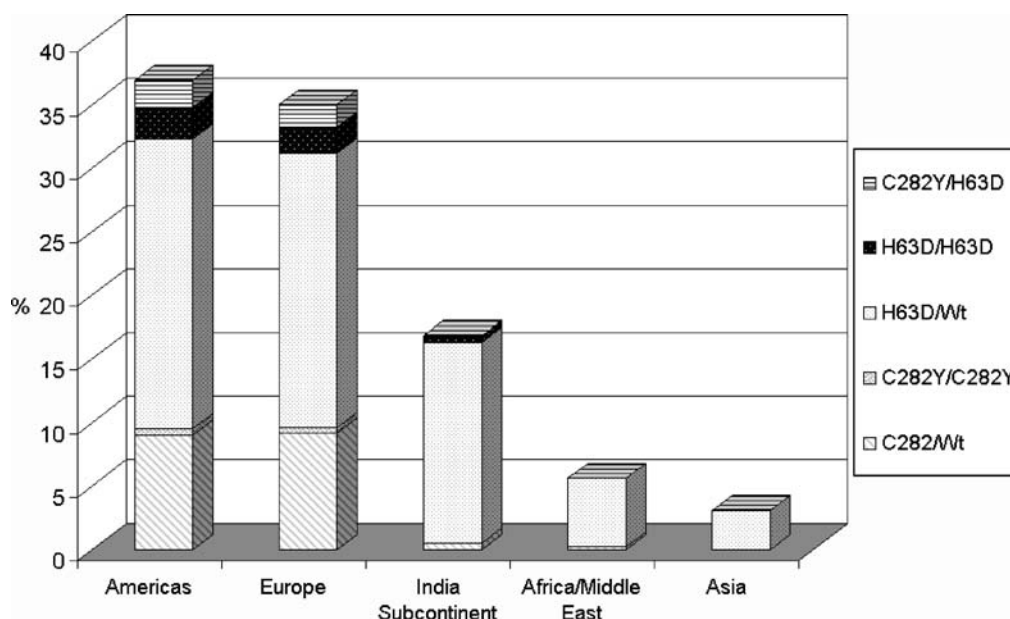


Figure 1. Frequencies of *HFE* C282Y and H63D mutations in different populations.

detecting subjects with delayed or incomplete penetrance, allowing diagnosis at an early age and treatment to prevent clinically significant iron overload [62]. However, not all subjects with iron overload carry the C282Y mutation. This mutation is mainly found in Caucasians. This limits the application of this screening test to other ethnic groups. On the other hand, phenotypic measures such as biochemical iron levels are early indicators of disease but they have a low specificity and are less valuable for a screening strategy. Phenotypic expression of hemochromatosis is very much influenced by age, diet, blood loss and menstruation, pregnancy and gene-gene interactions.

An important factor to be considered in screening is the cost of the test used. Screening for hemochromatosis based on the genotype are more expensive compared to phenotypic screening. This is important whether the cost of screening is paid by the beneficiary or by a third party.

Another important parameter in evaluating a screening program is the cost-effectiveness of the latter. This is assessed by comparing the total diagnostic costs to the extra costs arising from managing the disease. Studies have shown that population screening for hemochromatosis is cost effective [63, 64]. However, screening for hemochromatosis like many other diseases has several disadvantages other among ethical concerns, psychological troubles, over-medicalisation, and if screening is based on the genotype, many subjects with iron overload due to other reasons will be missed. Little is known of the psychosocial impact and ethical implications of a screening program for hemochromatosis. There is still lack of information on the natural history of the disease and the age-related penetrance of the disease is still unknown.

In deciding whether or not to screen, important quantitative parameters that should help in the decision are the positive predictive value (PPV), the sensitivity and the specificity of the test used. The PPV, the probability that a person with a positive test result will develop the disease is approximately equal to the penetrance of the disease and is a function of the frequency of the susceptibility-conferring genotype, the relative risk of the disease and the risk of disease in a given population [65]. It can be calculated as follows: $PPV = [R(D) \times 100] / [G(R - 1) + 1]$, where R is the relative risk, D the incidence of the disease and G the frequency of the susceptibility conferring genotype [66].

Our study in the elderly population has shown that for all HFE mutations, the PPV was 10% in men and 5% in women. The sensitivity (the probability that the test correctly classifies people with preclinical disease as positive) was 70% for men and 52% for women and the specificity (the probability that the test classifies as negative those who will not have the disease) was 62% for men and 64% for women [67].

A more or less important quantitative parameter is the population attributable risk (PAR). This is the proportion of cases of a disease that can be attributed to the susceptibility-conferring genotype. It can be calculated as follows: $PAR = [G(R - 1) \times 100] / (G(R - 1) + 1)$, where G is the frequency of a susceptibility conferring genotype and R is the relative risk [66]. Only in the case of polymorphisms that have frequencies in the range of 10–30% and that increase susceptibility to disease will the PAR be appreciable. Single, highly penetrant gene mutations cause only a small proportion of the disease [68]. Our results in a population-based setting suggest that many sub-clinical cases of hemochromatosis will be missed when screening based on the HFE genotypes. These findings in the general elderly population suggest that the value of screening for high iron based on HFE genotypes is limited in that only a small percentage of subjects with elevated levels of iron will be detected. However, the aim of a population-based screening is to identify at an early stage individuals at risk of developing serious iron overload, to prevent organ damage. Although not all patients may be found, the implications for those who are found are high despite the controversy of the role of HFE in disease.

One reason why genetic screening for complex diseases is not advocated is that the risk for disease does not only depend on the gene but also on other factors like the environment, nutrition and genetic modifiers. Penetrance depends on at least six factors: (1) The importance of the function of the protein encoded by the gene, (2) the functional importance of the mutation, (3) the interaction with the environment, (4) onset of somatic mutations, (5) interaction with other genes and (6) existence of alternative pathways that can substitute for the loss of function.

Another point of concern is the definition of the phenotype. There is no consensus on the definition of hemochromatosis and also no agreement on the clinical features of the disease among clinicians and experts. This situation has led to several approaches in estimating the penetrance of HFE mutations and thus controversial results. While some authors consider clinical hemochromatosis as the end point, others have used combinations of signs and symptoms of hemochromatosis as endpoint to estimate the penetrance while other investigators have used phenotypic measures such as serum iron indices. These diverging outcomes have obviously lead to diversity in quantification and estimates of penetrance. Four stages of the disorder are generally recognised; the genetic predisposition but without any abnormality, iron overload without any symptom, iron overload with early symptoms (lethargy, arthralgia), and iron overload with organ damage (cirrhosis especially) [1]. Some authors [69–71] have argued that the excess of iron may not

translate to associated diseases of hereditary hemochromatosis such as diabetes, but other disorders such as atherosclerosis, cancer and joint pathologies. This hypothesis is supported by our own data suggesting that HFE is involved in atherosclerosis, particularly in smokers [72]. We have studied the association between HFE gene mutations, carotid atherosclerosis, and stroke. We observed that in the presence of additional risk factors (smoking and hypertension), there is increased risk of carotid atherosclerosis and stroke in carriers of HFE mutations [72]. HFE mutations only showed an overall weak association with stroke (odds ratio (OR) 1.3; 95% confidence interval (CI) 0.8–2.2). But patients with hypertension who were also carriers of the HFE mutations showed a significantly increased increased risk of stroke (OR: 3.0; 95% CI: 1.9–4.6). Also HFE carriers who were also smokers had an risk of stroke (OR: 2.6; 95% CI: 1.4–5.0) [72]. We concluded that HFE mutations modify the risk of stroke in subjects who already carry traditional risk factors. We also found in our population-based study that subjects C282Y homozygous have a two–three-fold increased risk of myocardial infraction. Concerning the relationship with diabetes, we have conducted a meta-analysis of the association between HFE mutations and diabetes and did not find any indication of an increased risk of diabetes in carriers of the HFE mutations (Figures 2 and 3). Also in a population-based sample of elderly, we observed that 11% of patients with type 2 diabetes and 10.6% of controls were carriers of the C282Y mutation (OR: 1.0; 95% CI: 0.6–1.7). For the H63D mutation, 25.7% of type 2 diabetes patients and 28.5% of control subjects were carriers (OR: 0.8; 95% CI: 0.6–1.1) [73].

Conclusion

Hemochromatosis is a common genetic disease in Western populations. The potential for genetic screening for mutation carriers is *a priori* high. Although findings have been negative in the largest study to date, the possible biases in this study leave the question to be answered whether screening for the HFE mutations is worthwhile. Our findings in the general elderly population suggest that the value for screening for high iron based on HFE genotypes is limited in that only a small percentage of subjects with elevated levels of iron will be detected. However, the aim of a population-based screening is to identify at an early stage individuals at risk of developing serious iron overload is that treatment can be started to prevent organ damage. Although not all patients may be found, the implications for those who are found are high. Thus, screening for C282Y homozygosity is helpful to identify high-risk groups.

In assessing the feasibility of screening for hemochromatosis, attention should not be directed only to the disease genotype or phenotype but also to the human being as end beneficiary. The translation of genetic and epidemiological advances in the field of hemochromatosis calls for studies on the cost-benefit and cost-utility of screening for hemochromatosis to be carried out. From this point of view, all information critical for the assessment and implementation of population screening for hemochromatosis are still lacking and need the input and cooperation of scientists in several fields of research. Although many consider hemochromatosis as a good example of a disease that meet the criteria for genetic screening, some key information are still necessary before genetic screening can be assessed.

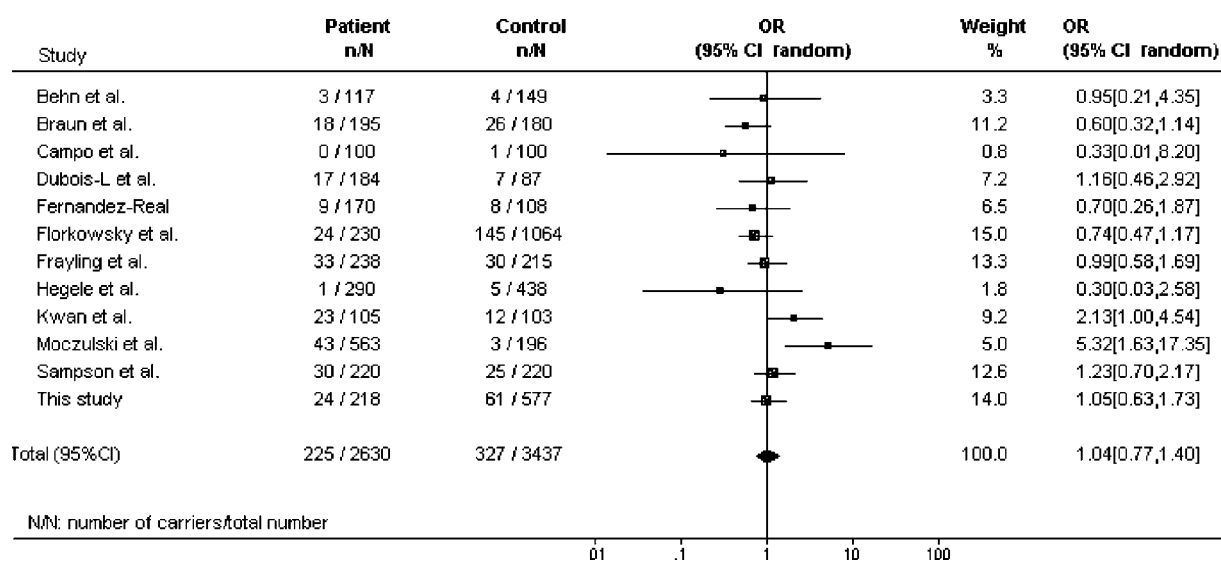


Figure 2. Meta-analysis of the frequency of the C282Y mutation in patients with type 2 diabetes.

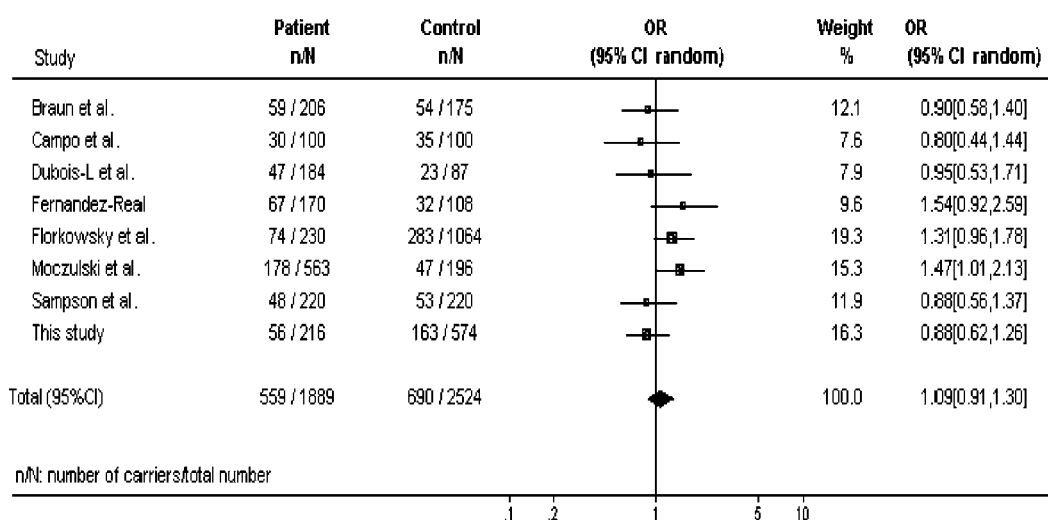


Figure 3. Meta-analysis of the frequency of the H63D mutation in patients with type 2 diabetes.

What is clear is that there will be a long road ahead translating findings of new genes into preventive medicine. The differential risk of disease seen with different genotypes and the evidence of incomplete penetrance for the genotype conferring the highest risk make genetic screening for hemochromatosis less worthy. A screening program in first instance based on phenotypic iron measures and a genotypic test only for those with elevated serum iron measurements appears to be the best alternative for the time being.

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