Penetrance in hereditary hemochromatosis

The HFE Cys282Tyr mutation as a necessary but not sufficient cause of clinical hereditary hemochromatosis

Ernest Beutler

It is not unusual for a disease to be considered rare when first described and to prove to be much more prevalent as the medical profession becomes aware of its existence. This seemed to be the case with hereditary hemochromatosis. But now the perception that hemochromatosis is a common disease has undergone a sudden reversal. Although everyone does not yet agree, the data that have accumulated in the past year provide compelling evidence that the actual disease, as contrasted with the genotype or biochemical phenotype, is quite rare after all. In this perspective I will review how an exaggerated impression of the clinical penetrance of the Cys282Tyr mutation of HFE developed, consider some of the factors that may be important in determining whether a person with HFE mutations develops the clinical stigmata of hemochromatosis, and suggest what further studies are needed to better understand the low penetrance of the HFE mutations.

Hemochromatosis as a rare disease

In a classical 1935 monograph that raised the profession's awareness of the disease Sheldon1 wrote, “It may be accepted that haemochromatosis is a rare disease.” Twenty years later, a classical review by Finch and Finch2 concluded, “In general hospitals idiopathic hemochromatosis is recognized only in about 20000 and once in about 7000 hospital deaths. These figures suggest that in the United States there are about 20000 people with idiopathic hemochromatosis of which only a small fraction are in the symptomatic stage.” These reviews did much to raise our awareness of this interesting disorder, but reflected the general belief that hemochromatosis was a very uncommon disease.

Hemochromatosis as a common disease

By the early 1980s hemochromatosis had come to be regarded as quite a common disorder in northern European populations.3 I believe that it is no accident that this new perception coincided with establishment of linkage with human leukocyte antigen A (HLA-A) and HLA-B on chromosome 6 and with the development of facile methods for the measurement of serum iron, transferrin saturation, and ferritin levels. An authoritative review4 written by the Practice Guideline Development Task Force of the College of American Pathologists proposed that “Diagnosis of HH (hereditary hemochromatosis) requires observation of elevated TS [transferrin saturation] 60% on at least 2 occasions in the absence of other known causes of elevated TS.” This report, written shortly before the HFE gene was cloned, cited 3 large studies in which hemochromatosis was detected by screening for transferrin saturations. The prevalence was 2.4, 5.6, and 8.3 per 1000 in these surveys. The cloning of HFE gene removed all doubt regarding the gene frequency; the estimate of 5 homozygotes per 1000 proved to be an accurate one.

Gradually and subtly the definition of hemochromatosis had shifted from a clinical disorder to a diagnosis based on the saturation of plasma transferrin with iron. The distinction between the biochemical changes that are associated with the hemochromatosis genotype, on the one hand, and the disease that causes grave illness and shortens life span, on the other, had become blurred. Reflecting this trend, Felitti and I wrote in 1999, “Hereditary hemochromatosis is the most common hereditary disease of Northern Europeans with a prevalence of approximately 5 per 1000.”5 The literature is now replete with exhortations such as, “Both phenotypic and genetic screening are highly cost effective for detection of iron loaded individuals in the general population”6 and “. . .to benefit all those at risk there is an ethical imperative to implement screening for the major mutation causing hemochromatosis now rather than wait years for confirmation of what is currently known—that at least half of those with predisposing genotype will develop some form of the disease.”7

Because the disease is readily treated by phlebotomy, population screening would, indeed, be an imperative if most persons who are homozygous for the Cys282Tyr mutation became ill from it. And it has been written that they do. For example it was concluded from a meta-analysis of data from 7 studies that clinical manifestations were present in 50% of male and 44% of female homozygotes.8 Edwards et al9 wrote, “Although the time required to become iron loaded is variable, it is clear that most homozygotes will eventually become symptomatic.” More recently, Olynk et al10 reported that “8 of the 16 homozygous subjects had clinical findings that were consistent with the presence of hereditary hemochromatosis, such as hepatomegaly, skin pigmentation, and arthritis.” Bulaj et al11 reported that 52% of males older than 52 years and 16% of females older than 50 years with the homozygous genotype had at least one “disease-related condition.” An expert panel12 held that 95% of patients older than 45 years and homozygous for the Cys282Tyr mutation have significant morbidity from hemochromatosis. Earlier this year Niederau and Strohmeyer6 wrote, “Genetic hemochromatosis is one of the most frequent inborn errors of metabolism. Up to one half of these patients will already have an irreversible complication such as liver cirrhosis, diabetes mellitus or cardiomyopathy.” Those without cirrhosis were characterized as “precirrhotic,” with the clear implication that cirrhosis was sure to follow.

All of these investigations of the penetrance of hemochromatosis had one flaw in common; the prevalence of the
findings attributed to hemochromatosis was not reported in matched controls.

**Hemochromatosis as a rare disease**

Although the idea that the homozygous state for hemochromatosis was clinically highly penetrant had become firmly entrenched, there were reasons to doubt such a premise. It had been found that the prevalence of homozygotes for the Cys282Tyr HFE mutation seemed to be no lower in the aged than in the young, a finding that seemed difficult to reconcile with the concept of a disease that killed frequently.

Although the studies that concluded that the penetrance of hereditary hemochromatosis was high did not compare clinical manifestations of homozygotes with matched controls, there was a single investigation in which an attempt had been made to compare patients with hemochromatosis with a sample from the general population with respect to arthritis, fatigue, liver disease, or diabetes. No difference could be found except possibly for an earlier age of onset. This result was especially surprising since the patient group consisted entirely of persons who had been diagnosed with hemochromatosis, a particularly biased sample. What was needed was a population-based screening program in which the health status of homozygotes could be documented prior to their diagnosis. We have now had the opportunity to conduct such an investigation. In a study performed on 41,238 subjects attending a health appraisal clinic at Kaiser-Permanente in southern California all were genotyped and comparison made between 152 Cys282Tyr homozygotes and 22,347 ethnically matched subjects with the wild-type HFE.

Many of the subjects were retirees, and the average age was about 58 years. Each had a relatively comprehensive laboratory evaluation, and extensively validated questionnaires were completed by all subjects before a genetic diagnosis had been established. This removed the bias that would be introduced once the patients had been informed that they were homozygous for the hemochromatosis mutation. A possible confounding variable was created by the fact that phenotypic screening for hemochromatosis had been carried out previously at the same clinical facility; 45 of the homozygotes had previously been diagnosed, not because of apparent disease, but because an elevated transferrin saturation had been detected earlier. However, the results were the same whether these patients were included or excluded. The only difference detected between homozygotes and controls was a significantly higher prevalence of abnormal liver-function tests, aminotransferase activities, and serum collagen IV levels. Only 1 of the 152 subjects had multiple stigmata of classical clinical hemochromatosis, including diabetes, cardiomyopathy, and cirrhosis. Having been convinced, or as it now seems, misled, into believing that hemochromatosis was a common scourge, we tried to find extenuating reasons for the lack of patients with severe hemochromatosis in our cohort. Had they all died, or were they hospitalized on a liver unit or in a diabetic ward? Apparently not. The number of homozygotes was actually slightly lower than predicted by the Hardy-Weinberg equilibrium (Figure 1), and the age distribution of our patients was the same as that of controls. Not only had we not lost patients to nonattendance or death, but also the effect of homozygosity for the Cys282Tyr mutation on life span was sufficiently small that it could not be detected in a cohort of 152 patients. Thus, it seemed unlikely that the aberration of liver function that we had detected in 10% to 20% of the patients had a significant effect on life span and presumably on health.

Other uncontrolled studies reported within the past year support the conclusions from the Kaiser study, providing confidence that the results do not represent a conclusion based on a geographic anomaly or a methodologic flaw. In the largest of the recent studies, Asberg et al examined 65,238 Norwegians. Given the reported gene frequency of 0.078 among Norwegians, one would expect to find 400 homozygotes among these subjects. In fact 1698 persons with high transferrin saturation on first measurement were found. After genotyping and repeating transferrin saturation measurements and performing serum ferritin determinations, 179 homozygotes for the Cys282Tyr mutation were selected for biopsy because of high serum ferritin levels. Severe organ damage was found in only 4 of the homozygotes for the Cys282Tyr mutation (1.0% of all of the putative homozygotes; 8.8% of those actually biopsied) and “fibrosis at least moderate” in an additional 12. Thus, the numbers from the San Diego study are very similar to those emerging from Norway. In England, too, penetrance is very low. Here 63 homozygotes were found among 10,556 blood donors. All were interviewed and none showed classical signs of iron overload. In the northeastern United States 4865 patients attending HMOs or general practice were genotyped; 12 homozygotes were found, and none had cardiac dysfunction or cirrhosis.

**Future challenges**

It is true, of course, that the exact clinical penetrance of the hemochromatosis mutations remains unknown, but arguing over whether it is 1% or 4% of homozygotes will avail us little. It is better for us to accept the fact that we have been mistaken concerning the high penetrance of this disorder. The hemochromatosis mutation is common; the hemochromatosis disease is, as Finch and Sheldon originally thought, rare. The challenge now is to understand why a few homozygotes for the Cys282Tyr mutation develop devastating iron-storage disease, while the majority go through life unscathed by this genotype.

Understanding the reason for the different expression of the disease in different people is an example of the challenge that we...
face in human genetics. As it has become easy to genotype patients with common single-gene disease we have become increasingly aware of the fact that in many disorders, not just hemochromatosis, but Gaucher disease, cystic fibrosis, hemophilia, pyruvate kinase deficiency, and virtually every other disorder, patients with the same genotype have different clinical phenotypes even though their mutant genotype is identical.22 There are 3 potential explanations for this variable penetrance—epigenetic, environmental, and genetic.

Epigenetic mechanisms

An epigenetic mechanism that could account for differences in phenotypes in genotypically identical individuals has recently been proposed. There are many retrotransposons in the mammalian genome, and some of these may influence the expression of nearby genes. Whitelaw and Martin23 recently suggested that silencing of retrotransposons occurs during early embryogenesis, but that this process is incomplete and random, producing a mosaic pattern of retrotransposon expression in somatic cells. This is an elegant concept that requires further evaluation, but one that we cannot yet approach in human disease.

Environmental factors

There are surely environmental variables that affect the expression of hemochromatosis. One might expect that the level or type of iron intake might have an influence, but normal diet provides a rather narrow range of iron and most patients with hemochromatosis have not taken iron medication. It therefore seems doubtful that the role of diet is an important one.

The effect of alcoholism has always been recognized. Sheldon1 found that about one fifth of all patients and one third of females had a history of alcoholism. In fact, some earlier investigators considered alcohol to be the cause of hemochromatosis, and later its role was emphasized by MacDonald24 who believed that hemochromatosis had no genetic basis at all.

The data from our study of the Kaiser-Permanente population show an effect of alcohol intake. Table 1 shows the effect of the frequency of alcohol intake on serum collagen IV levels, a measure of hepatic fibrosis. There is a statistically highly significant effect (P < .001), but the data also make clear that alcohol intake cannot be the most important or the only variable. Of the homozygotes with elevated collagen IV levels, 8 never drank and 7 stated that they hardly ever used alcohol. We have much additional information regarding the relationship of alcohol intake to manifestations of hemochromatosis in the Kaiser population, including the number of drinks taken and the frequency of very heavy drinking, and this is under analysis. There seems, for example, to be no relationship at all between serum ferritin levels of the homozygotes in our study and frequency of drinking. Clearly there must be other risk factors that are of major importance. Indeed, other hepatotoxic influences may be important. Hepatitis C may well worsen the disease,25 but most patients with clinical hemochromatosis do not have hepatitis C.

Genetic modifiers

The possibility that mutations or polymorphisms in other genes are involved in determining a severe disease phenotype is an attractive one. Examples of mutations that produce a severe genotype only when another mutation is also present exist but are not commonly appreciated. In the case of thrombophilia, the combination of 2 defects, each of which alone have a modest thrombophilic effect, gives rise to more severe disease.26 For example, patients who inherit both factor V Leiden and protein C deficiency are much more likely to develop thrombotic disease than are those with only one or the other mutation.27 Alone, neither glucose-6-phosphate dehydrogenase (G6PD) deficiency nor the uridine diphosphate (UDP) glucuronosyl transferase mutation that gives rise to Gilbert syndrome produce severe jaundice, but it is the combination that produces potentially deadly hyperbilirubinemia in the newborn.28

Defects in the HFE gene are by no means the only cause of iron-storage disease. It seems likely that mutations of other genes involved in iron homeostasis occurring in homozygotes for the Cys282Tyr HFE mutation may be the ones who develop severe iron-storage disease. In humans, mutations of transferrin,29 transferrin receptor-2,30,31 ferroportin,32 ceruloplasmin,33,34 and hepcidin35 have all been associated with iron-storage disease. In mice, knock-outs of β2-microglobulin37,38 and of Usp2 causing hepcidin deficiency result in excess iron storage. All of these candidates have already been sequenced in sufficient numbers of patients with hemochromatosis to rule out an important role in determining the phenotype in patients; others need to be investigated. It has been suggested that the tumor necrosis factor promoter polymorphism might be a factor affecting severity of liver damage in hemochromatosis,40 but we have not seen a significant effect in our own larger series.41 What, then, are some of the other mutations that need to be investigated? The common duplication polymorphism of haptoglobin has been associated with increased ferritin levels but not with increased hepatic fibrosis in homozygotes for the Cys282Tyr HFE mutation.42 The studies reported so far are small, and the patients with hemochromatosis selected for study were heavily biased toward those with clinical manifestations. A particularly attractive gene to attempt to identify is the one that causes juvenile hemochromatosis. This gene was mapped to chromosome 1q several years ago43 in several Italian families, and the critical interval seems to have been narrowed in more recent studies.44,45 It is probably only 1 to 2 megabases in size, but unfortunately it is not accurately represented by the human genome project in GenBank. There seem to be major duplications in this region, and the published assembly, although continually undergoing revision, is not correct. Finding the gene that causes juvenile hemochromatosis would certainly shed some new light on the regulation of iron metabolism, since none of the genes that have been found so far to produce dysregulation of iron homeostasis are in this interval. It seems entirely possible that mutations of the same gene that causes juvenile hemochromatosis may help to explain why only few patients homozygous for the Cys282Tyr mutation develop severe iron-storage disease. Perhaps heterozygotes for the same mutation that causes juvenile hemochromatosis in the homozygous state develop severe hereditary hemochromatosis when they are homozygous for the Cys282Tyr mutation, or homozygotes or compound heterozygotes for other mutations of the same gene may be the ones who are at risk. If this proved to be the case, then one

Table 1. The distribution of homozygotes for the Cys282Tyr mutation with respect to elevations of serum collagen level to greater than 187 ng/mL.

<table>
<thead>
<tr>
<th>Frequency of alcohol intake</th>
<th>Serum collagen IV level normal (%)</th>
<th>Serum collagen IV level elevated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never/hardly ever</td>
<td>50 (56)</td>
<td>15 (41)</td>
</tr>
<tr>
<td>Less than 3 times/week</td>
<td>26 (29)</td>
<td>6 (16)</td>
</tr>
<tr>
<td>More than 3 times/week</td>
<td>4 (4)</td>
<td>10 (28)</td>
</tr>
<tr>
<td>Daily</td>
<td>9 (10)</td>
<td>5 (14)</td>
</tr>
</tbody>
</table>
might even aver that HFE is not the real hereditary hemochromato-
sis gene but merely a risk factor.

Certainly much needs to be done. The HFE mutation seems to be a necessary but not sufficient condition for the development of clinical disease. Finding the factor(s) that are required in addition to the HFE mutation in producing clinically significant iron-storage disease will be an important step forward. It would provide us with further insights into the regulation of iron homeostasis, and enable us to more precisely distinguish those few homozygotes for HFE mutations who need treatment from the large number who do not.

Acknowledgment

This is manuscript number 15074-MEM from the Scripps Research Institute, La Jolla, CA.

From the Scripps Research Institute, La Jolla, CA.

Submitted July 11, 2002; accepted January 8, 2003.

Supported by National Institutes of Health grants DK53505-04 and RR00833 and the Stein Endowment Fund.

Reprints: Ernest Beutler, The Scripps Research Institute, Department of Molecular and Experimental Medicine, 10550 N Torrey Pines Rd, La Jolla, CA 92037; e-mail: beutler@scripps.edu.

References


chro-matosis type 3 due to a novel mutation in transferrin receptor 2 gene. Gas-


19. Fargion S, Valentini L, Dongiovanni P, et al. Tumor necrosis factor alpha pro-
moter polymorphisms influence the phenotypic expression of hereditary hemo-


25. Waelen J, Feiltiti V, Gelbart T, Ho NJ, Beutler E. Penetration of hemochromato-
ant antimicrobial peptide hepcidin is associated with severe juvenile hemo-