Clinical consequences of iron overload in hemochromatosis homozygotes

Richard S. Ajioka and James P. Kushner

Hereditary hemochromatosis is an autosomal recessive disorder characterized by hyperabsorption of dietary iron and accelerated recycling of iron by macrophages. Homozygosity for the disease trait occurs in approximately 5 per 1000 whites of northern European descent. The causative gene, designated \( HFE \), was identified in 1996. Most individuals with hemochromatosis are homozygous for a guanine to adenine transition at nucleotide 845 in the coding sequence of \( HFE \) resulting in a cysteine to tyrosine conversion at amino acid residue 282 of the HFE protein (Cys282Tyr). Wild-type HFE protein interacts with the transferrin receptor, but the mechanism by which the Cys282Tyr mutation leads to increased iron absorption and recycling remains unclear.

Most hemochromatosis homozygotes display a common laboratory phenotype, namely an elevated transferrin saturation. Iron stores in excess of normal eventually occur in most men and some women but the prevalence of organ damage due to iron overload remains a controversial issue. The basis of the controversy, in our view, is that ascertainment bias has affected the findings in most of the published studies. Retrospective studies have been biased in favor of individuals with morbid complications, whereas screening studies in groups such as blood donors have favored the inclusion of healthy subjects.

The issue of ascertainment bias

Hemochromatosis was first recognized as a clinical entity by Trousseau in 1865, but the first comprehensive description of the disorder was offered by Sheldon in 1935. Autopsy studies in Europe and the United States between 1921 and 1985 reported that hemochromatosis occurred with a frequency of 1 to 2 per 1000 but, by the nature of these studies, death from hemochromatosis was the ascertainment criterion. In contrast, in a review published in 1955, Finch and Finch estimated that hemochromatosis was responsible for only 1 per 7000 hospital deaths and 1 per 20,000 hospitalizations. More recently, an analysis of data from the National Hospital Discharge Survey for the United States indicated that between 1993 and 1997, the hemochromatosis-associated hospitalization rate for men older than 60 years was 11.3 per 100,000, but even this figure is far lower than the 5 per 1000 incidence of the homozygous Cys282Tyr hemochromatosis genotype. In these retrospective studies, full penetrance of the clinical phenotype was required for ascertainment. Numerous reports have described the frequency of symptoms, physical findings, and laboratory abnormalities in patients with a clinical diagnosis of hemochromatosis. Estimates of the frequency of cirrhosis have ranged from 30% to 94%; of arthropathy, from 44% to 68%; of hypogonadism, from 16% to 40%; and of diabetes, from 12% to 82%. The ascertainment bias in these studies, due to the requirement of a clinical diagnosis of hemochromatosis, is clear.

Recognition of the utility of an elevated transferrin saturation as a phenotypic marker for the hemochromatosis homozygous genotype made it feasible to perform prospective screening studies designed to determine the frequency of the homozygous genotype and the incidence of disease-related morbidity associated with homozygosity. A transferrin value of 62% or greater was used as a screening probe in a study of more than 11,000 predominantly white blood donors by Edwards et al. who estimated the frequency of homozygosity at 8 per 1000 men and 3 per 1000 women. It was later recognized that the threshold value used for the transferrin saturation was too high in women and that a value of 50% would have been more appropriate. The highest ferritin value detected in this study was 549 \( \mu \)g/L, but many of the homozygotes detected were young (mean age of 37.5 years for men and 34.7 years for women) and had donated blood multiple times. Nearly all the homozygotes detected underwent liver biopsy. The finding of increased liver iron stores was not rare, but no individual had evidence of liver damage, a finding clearly the result of ascertainment bias as blood donors represent a population preselected to be healthy and young. The homozygotes detected in this study later underwent \( HFE \) genotyping, and more than 90% proved to be homozygous for the Cys282Tyr mutation.

Another large screening study in 10,500 blood donors was reported from Wales, United Kingdom. Donors were screened by \( HFE \) genotyping and the incidence of homozygosity for the Cys282Tyr mutation was 6.8 per 1000. The age of the donors was similar to that reported by Edwards et al., and multiple donations were common. The highest ferritin concentration observed was 410 \( \mu \)g/L. Liver biopsies were not done in this study, but none of the 63 homozygotes evaluated had physical manifestations of iron overload. The ascertainment bias inherent in studying blood donors is again apparent.

Avoiding ascertainment bias

Ascertainment bias can be avoided by studying all members of a population, regardless of their health status. In one of the largest population-based screening projects reported, over 65,000 individuals older than 20 years in a Norwegian county underwent determination of the transferrin saturation. Individuals with a confirmed transferrin saturation above a threshold value of 55% were invited to undergo genotyping at the \( HFE \) locus. Already known to have hemochromatosis were 3 women and 6 men with transferrin saturations above the threshold value. The incidence of the homozygous Cys282Tyr genotype was estimated at 4.1 per 1000 women and 6.8 per 1000 men. There were 322 women and 300 men who had confirmed transferrin saturations above the threshold level, and, of these, 137 women and 205 men had an elevated serum ferritin concentration. Liver biopsies were planned on all those with elevated serum ferritin concentrations but were actually performed on only 50 women and 129 men. Increased liver iron stores were found in 97% of the liver biopsy specimens but at least moderate fibrosis and/or cirrhosis was found in only 9.8% (11 of 129) of men and 2.4% (1 of 50) of women. Serum ferritin values in these 12 newly discovered homozygotes ranged from 311 to 3511 \( \mu \)g/L with 8 values more than 1000 \( \mu \)g/L. The incidence of histologically documented, iron-induced liver damage of approximately 10% in male homozygotes represents a minimal estimate as liver biopsy findings in the 6 homozygous men with previously diagnosed hemochromatosis were not reported.

In a smaller population-based study free of ascertainment bias, a much higher incidence of hemochromatosis-associated morbidity was found. Olynyk et al studied 3011 unrelated individuals older than 20 years living in Busselton, Australia, a town with a population of 10,888 residents of predominantly Anglo-Celtic
Of the 3011 subjects screened, 16 (5 per 1000) were homozygous for the Cys282Tyr mutation. Of the 16 Cys282Tyr homozygotes, 8 had clinical signs or symptoms associated with hemochromatosis, and 11 underwent liver biopsies (7 men and 4 women). The 4 biopsies, all from men, revealed fibrosis or cirrhosis. There were 2 Cys282Tyr homozygotes with elevated ferritin values (805 and 1200 μg/L) who declined liver biopsy. Only if the 4 homozygotes with hepatic fibrosis or cirrhosis were considered to have morbidity complications of the homozygous genotype, this study would indicate that 25% of homozygotes are likely to become ill. The explanation for the higher frequency of hemochromatosis-associated morbidity in this Australian study compared with the larger Norwegian study described above is not clear.

An alternate approach to avoid ascertainment bias was taken by our group at the University of Utah and reported by Bulaj et al. We studied family members (mainly siblings) with human leukocyte antigen (HLA) identity to a family proband with established diagnosis of hemochromatosis. The sole ascertainment criterion used related to the HLA haplotype. Health status at ascertainment was not a consideration. Assignment of the hemochromatosis homozygous genotype was confirmed later by demonstration of homozygosity for the Cys282Tyr mutation of HFE. Nearly all newly identified homozygotes underwent liver biopsy. Criteria for disease-related morbidity were restricted to 4 objective findings: cirrhosis, hepatic fibrosis, elevated aminotransferase values with no apparent cause other than iron overload, and, finally, radiographically proven hemochromatotic arthropathy of the metacarpal-phalangeal joints. The presence or absence of subjective symptoms such as fatigue, impotence, joint pains, and palpitations were not considered nor were findings that might be due to factors other than iron overload (eg, heart failure, diabetes, abdominal pain). Identified were 214 clinically unselected homozygotes (113 men and 101 women). In men older than 40 years, 52% (27 of 52) had at least one criterion for disease-related morbidity, as did 16% (7 of 43) of postmenopausal women (all older than 50 years). If criteria for morbidity were restricted to biopsy-proven hepatic fibrosis or cirrhosis, the incidence was 24% for men and 6% for women.

Bulaj et al extended the morbidity analysis to address the possibility that ascertainment bias had been introduced by examining the relatives of probands. This possibility was considered because of the suggestion that modifier genes influence penetrance of the hemochromatosis phenotype. The presence of modifier genes was supported by a logistic-regression analysis that indicated that siblings of probands with liver biopsy abnormalities were approximately 3 times more likely to have liver biopsy abnormalities than siblings of probands with normal biopsy results. To minimize effects of putative modifier genes favoring penetration, an additional analysis was restricted to homozygous relatives of healthy probands (probands with no histologic evidence of iron-induced hepatic injury or arthropathy). These probands were detected through screening programs involving blood donors or through annual health assessment visits. In these homozygous relatives, 29% of men older than 40 years and 11% of women older than 50 years met our criteria for disease-related morbidity.

The pedigree studies by Bulaj et al presented the opportunity to study a large control population, namely 1058 heterozygous relatives of the pedigree probands. In addition to a complete clinical assessment, percutaneous liver biopsies were performed on a subset of 39 of these heterozygotes. Evidence of hepatic fibrosis was found in 2 men with porphyria cutanea tarda (both of whom drank heavily) and in 2 men who were alcoholics. None of the heterozygotes studied had radiographic evidence of hemochromatotic arthropathy.

Powell et al prospectively studied heterozygous relatives of hemochromatosis probands for prolonged periods (up to 24 years). The heterozygous genotype was assigned to pedigree members sharing one HLA haplotype with the pedigree proband. Identified were 98 heterozygotes (49 men and 49 women), and liver biopsies were performed on a subset of 13 heterozygotes with modestly elevated serum ferritin concentration. No evidence of iron-induced liver damage was found. None of the 98 heterozygotes identified had clinical evidence of iron overload.

A third approach aimed at avoiding ascertainment bias was undertaken by Beutler et al. In this study, 41,038 individuals attending a health appraisal clinic operated by the Kaiser-Permanente San Diego (KPSD) health plan underwent genotyping at the HFE locus. Identified were 152 individuals homozygous for the Cys282Tyr mutation (73 men and 79 women), an incidence of 3.7 per 1000 in this racially mixed population. Assessment of Cys282Tyr homozygotes was accomplished with a questionnaire, a panel of laboratory tests and an interview, and physical examination by a physician or a physician’s assistant. No liver biopsies were performed and there was no radiographic assessment of the joints. The authors concluded that subjective symptoms that might be due to hemochromatosis occurred no more frequently in Cys282Tyr homozygotes than in age- and sex-matched controls homozygous for wild-type HFE alleles. The symptoms included fatigue, joint pain or stiffness, darkening of the skin, abdominal pain, impotence, weight loss, loss of body hair, and palpitations. Of note is that 41.4% of controls felt their general health was limited to some degree (as did 41.9% of Cys282Tyr homozygotes).

There were 2 objective laboratory measures compared between Cys282Tyr homozygotes and controls, namely the aspartate aminotransferase value and plasma collagen IV concentrations (a surrogate “marker” for hepatic fibrosis). Elevated aspartate aminotransferase values were found twice as often in Cys282Tyr homozygotes and over 3 times as often in Cys282Tyr homozygotes older than 55 years. The median plasma collagen IV concentration was also significantly higher in Cys282Tyr homozygotes than in controls. In spite of the differences in objective measurements, only 1 of 152 Cys282Tyr homozygotes was considered to manifest clinical penetrance of hemochromatosis, a value lower than any previously reported study without a presumed ascertainment bias. A summary of the findings in biased and unbiased studies is shown in Table 1.

The controversy related to disease penetrance

The nearly total lack of penetrance reported by Beutler et al has engendered considerable controversy. A series of letters to the editors of The Lancet commented upon the lack of liver biopsy data, varied definitions of penetrance, high frequency of symptoms in the control population, failure to consider previous blood donations, and exclusion of Cys282Tyr homozygotes identified before the questionnaire was developed. More importantly, the population studied by Beutler et al was enrolled in the KPSD health plan when a hemochromatosis screening program was established in 1997. Felliti reported in 1999 that more than 200 patients with hemochromatosis identified through the KPSD screening program were already enrolled in a therapeutic phlebotomy program. Felliti reported that one third of these Cys282Tyr homozygotes were older than 65 years and that 90% of these older patients had signs or symptoms attributable to iron overload. Felliti concluded...
that most KPSD program members with hemochromatosis would become symptomatic over time. The widely discrepant conclusions reported by Felliti and by Beutler et al, using data collected from the same patient population, is difficult to reconcile. It seems likely that the differences reflect variations in the criteria used to establish the presence of disease-related morbidity and the implication that if a sign or symptom is present with equal frequency in a control group, the abnormality noted in Cys282Tyr homozygotes is not due to iron overload.

A study by Willis et al has been cited in support of the concept that penetrance of the hemochromatosis phenotype might be low. The frequency of Cys282Tyr homozygotes in elderly men proved to be no different than the frequency of this genotype in younger men.27 Willis et al concluded that death due to hemochromatosis had not depleted the aged population of Cys282Tyr homozygotes and that “life-threatening” complications associated with the homozygous genotype must be rare. The criterion for hemochromatosis-associated morbidity in this study was death, and other disease-related manifestations were not considered. In contrast, in a study done in Denmark, Bathum et al found only half the expected number of elderly Cys282Tyr homozygotes and even noted an age-related reduction in the frequency of Cys282Tyr heterozygotes, suggesting that both homozygotes and heterozygotes have a shorter than normal life expectancy.28

Dr Beutler has proposed that all of the studies reporting high penetrance of hemochromatosis have a common flaw, namely the lack of matched controls without hemochromatosis.29 We propose that appropriate controls, Cys282Tyr heterozygous relatives of homozygous probands, were evaluated in the large studies from Utah and Australia.20,21 In our view the widely different estimates of penetrance are due mainly to ascertainment bias. The report by Bulaj et al16 made every effort to eliminate ascertainment bias, even restricting the disease-related morbidity analysis to homozygous relatives of healthy homozygous probands. This restricted analysis yielded a minimal estimate of disease-related morbidity to 29% in homozygous men older than 40 years and 11% of women older than 50.

**Conclusions**

What conclusions can be drawn from the data reviewed here? Is the penetrance of hemochromatosis less than 1%, is it 25%, or is it higher? If penetrance is low, are large-scale screening programs justified? These are difficult questions, and this communication and Dr Beutler’s accompanying paper make it clear that there is considerable disagreement about the correct answers. One approach to a resolution would be to identify a cohort of Cys282Tyr homozygotes, perhaps 20-year-old men, and follow them for 30 to 40 years without phlebotomy therapy. This approach, however, is both impractical and unethical. Once cirrhosis due to hemochromatosis is established, life expectancy is shortened even if phlebotomy therapy is initiated.30

The hemochromatosis research clinic at the General Clinical Research Center (GCRC) at the University of Utah was established more than 25 years ago when the population in Utah was approximately 1.25 million.31 The population of Utah is now approximately 2.2 million, but the average population over the past 25 years was approximately 1.7 million. Approximately 90% of the Utah population is of northern European descent.32 We estimate that the number of white men in Utah over the past 25 years averaged 765,000 and this should include 3825 Cys282Tyr homozygotes. The report by Bulaj et al described 179 homozygous men with disease-related morbidity (136 clinically affected probands and 43 clinically unselected homozygous relatives) identified between 1975 and 1998.16 Over the past 4 years an additional 16 clinically affected homozygous men have been identified (data not shown). The 195 men with disease-related morbidity due to hemochromatosis represent 5.1% of the assumed population of 3825 homozygous men. This figure clearly represents an underestimate of disease penetrance for 2 reasons. First, no correction has been made for the age distribution in Utah males. Currently 66% of males in Utah are younger than 18 years.32 Second, only 350 male homozygotes have been evaluated at the GCRC. Many homozygotes have been identified in practices not affiliated with the university and these homozygotes have not been evaluated by our group.

Resolution of the controversies concerning the clinical and pathologic sequelae of hemochromatosis may be achieved by a multicenter, population-based study undertaken by the National Heart, Lung, and Blood Institute of the National Institutes of Health. In the study, 100,000 individuals older than 25 years will be screened for hemochromatosis using an elevated transferrin saturation as the screening probe, followed by HFE genotyping and a full clinical assessment. This study will be free of ascertainment bias and will include epidemiologic and family studies designed to define factors affecting disease penetrance. Until results are available, the pragmatic approach is to continue to screen for hemochromatosis in the primary care setting and to maintain serum ferritin values at approximately 100 μg/L or lower with phlebotomy therapy.
References

8. Lindmark B, Eriksson S. Regional differences in the idiopathic hemochromato-
17. Jackson HA, Carter K, Darce C, et al. HFE mutations, iron deficiency and over-
18. Asberg A, Hveem K, Thorstensen K, et al. Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,236 per-
24. Cox T, Rochette J, Camaschella C, Walker A, Robson K. Clinical haemochro-

Rebuttal to Ajikoa and Kushner

Ernest Beutler

Drs Richard S. Ajikoa and James P. Kushner provide an able review of the studies that have led them to conclude that the penetrance of hereditary haemochromatosis is much higher than our study of Kaiser-Permanente patients has shown it to be. They suggest that the cause of the discrepancy is ascertainment bias affecting the population that we investigated. I believe that there is strong evidence against ascertainment bias in our population and that the differences that have been reported in various populations are due, in part, to observer bias. But the differences in findings are much smaller than might appear. It is the difference in defining “penetrance” that is responsible in large measure for the disparate results that have been reported.

Distribution of genotypes and analysis of age distribution demonstrates that the Kaiser population shows no ascertainment bias

It is, indeed, important to ensure that the results are not due to such bias, and this is a consideration that we addressed carefully and from several points of view in our studies. Nonetheless, it appears to be an aspect of our study frequently singled out by those who found it difficult to reconcile our results with their own notions of the penetrance. The authors of one of the letters cited by Drs Ajikoa and Kushner averred that our patients were “sickly”;1 another letter2 suggested that the subject population was too healthy. Both criticisms cannot be correct. But selection biases can occur, and we have examined the data using 2 independent methods to determine whether such a bias existed.

The distribution of genotypes fits the Hardy-Weinberg equilibrium

As pointed out in “The Cys282Tyr mutation as a necessary but not sufficient cause of clinical hereditary hemochromatosis,” the first of these methods examines the distribution of genotypes among white subjects according to the Hardy-Weinberg equilibrium. If there were a hereditary disease in which half the homozygotes were healthy and the other half died, a population such as the one we studied could lead to the erroneous conclusion that the disease had no phenotype, since those who had perished would not attend a health appraisal clinic. Conversely, if we were studying a disease such as hereditary hemochromatosis from the vantage point of a liver clinic, as was done, for example, by Niederer and Stroh-
meyer,1 we might accumulate an excess of homozygous patients.