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Commentary

Targeted disruption of the *HFE* gene

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Iron is not a trace element. The 2 liters of erythrocytes that circulate through a 70-kg man contain 2 gm of iron, and another gram of reserve iron is stored in the liver and in macrophages throughout the body. All living organisms require iron. The same unique chemical properties that make iron metabolically useful in facilitating electron transfer make an excess of iron hazardous to health by generating activated oxygen species. Although living organisms are forced to extract iron efficiently from their environment they must also protect themselves against accumulating too much. Mammals do not excrete iron. Instead, iron is lost from the body in an essentially unregulated fashion through bleeding, pregnancy, and cell desquamation. Control of body iron content is achieved through adjustment of the amount of iron absorbed from the gastrointestinal tract. In humans either a deficiency or an excess of iron produces disease. Iron deficiency is a debilitating disorder that is particularly common in women. It is characterized by anemia and fatigue. Hemochromatosis, on the other hand, is the result of iron excess. It is common among those of Northern European ancestry, particularly men, and is characterized by cirrhosis of the liver, diabetes mellitus, skin pigmentation, cardiac arythmias and failure, and arthropathy.

The genetic nature of primary hemochromatosis had been proposed more than 60 years ago by Sheldon (1), but it was not until Simon *et al.* (2) demonstrated unequivocally the HLA linkage of this disorder that its genetic basis was clear. The gene involved proved to be particularly elusive. Several groups of scientists invested enormous effort in attempting to identify it, but because the genomic region in which it is located seems particularly resistant to crossing over, it was only in 1996 that a large group of investigators at Mercator Genetics succeeded by positional cloning in identifying the gene and the mutations that were responsible for most cases of hemochromatosis (3). Although the possibility that an HLA-like gene might be involved had been proposed (4) largely because of the iron storage disease that had been found to occur in mice with targeted disruption of the β-2-microglobulin gene (5), most investigators were astonished to find that the culpable mutation was in a gene closely resembling major histocompatibility complex (MHC) class I genes. The gene was designated *HLA-H* but, in deference to the WHO Nomenclature Committee for Factors of the HLA System (6), is now known as *HFE*. Initially skepticism was expressed whether this "candidate gene" was the correct one, and there were calls for direct proof that this gene was involved in iron metabolism (7). However, the almost immediate confirmation (8–11) of the extraordinarily high percentage of patients with hereditary hemochromatosis who are homozygous for a single mutation, 845A(C282Y), or compound heterozygotes for 187G(H63D) and 845A made it very clear that the gene identified by Feder *et al.* (3) was the one that had been sought.

In the current issue of the *Proceedings* Zhou *et al.* (12) report the production of a mouse in which the *HFE* gene has been disrupted. The "knockout" mouse showed an increased serum transferrin saturation and increased hepatocellular iron deposition, the stigmata that are characteristic of human hemochromatosis. The major significance of the production of this elegant model is not merely the formal proof that *HFE* is the major gene that causes hereditary hemochromatosis; there was no longer any doubt of that. Instead, the most immediate importance of this model is that it clearly indicates that the *HFE* mutations that produce hemochromatosis in humans do so by causing a deficiency of the *HFE* protein, not by changing its characteristics or location in the cell. Until the production of the knockout mouse by Zhou *et al.*, this conclusion was far from obvious. The spectrum of mutations in patients with hemochromatosis appears to be more narrow than that found for other autosomal recessive diseases. In balanced polymorphisms such as Tay–Sachs disease, Gaucher disease, cystic fibrosis, and galactosemia, among many others, one or two major mutations predominate but large numbers of other less common mutations exist in the population. These mutations usually come to light when one of them is inherited in the compound heterozygous state with a common mutation. The situation initially has seemed to differ in hemochromatosis. Although the coding region of at least 24 *HFE* genes of patients with hemochromatosis who do not have two copies of the known *HFE* mutations have been sequenced (13, 14), no mutations beyond 845A and 187G have been detected. Such a limited repertoire of mutations suggested the possibility that these mutations caused hemochromatosis through a gain-of-function (15), analogous perhaps to the situation in sickle cell anemia where only a valine substitution for Glu-6 in the β-globin chain can produce the sickling phenotype. Although one expects gain-of-function mutations to be dominant rather than recessive, such a mutation might have been one explanation for the singular lack of other mutations in HFE. Now, it appears that other mutations involving *HFE* will surely be found and these will play a role in causing hemochromatosis either in the homozygous state or as compound heterozygotes with one of the other two known mutations.

Another potentially valuable contribution that the knockout mouse may make to our understanding of hemochromatosis is in investigation of pathogenesis. How can a class I MHC molecule regulate iron absorption? Does its cleft bind a peptide? Heme? Some other compound? Or nothing at all? Does HFE act as a transport molecule or does it signal like the Fc receptor family to which it is related? A faulty signal to the gastrointestinal tract from a peripherally located iron detector in a cell such as a macrophage might result in an inappropriate increase in iron absorption. Does HFE affect the function of the transferrin receptor, to which it has recently been reported to bind (16)? What is the role of calreticulin (mobilferrin) (17), which has been shown to play a role in iron absorption and which also binds class I MHC proteins (18)? And where does Nramp2 (19, 20), a newly described iron transport protein, fit in? A further question is raised by the finding that in the small intestine, the primary site of iron absorption, the distribution of HFE seems to be mainly intracellular and perinuclear and limited to cells in deep crypts (21). In other parts of the gastrointestinal tract (21) and in tissue culture cells (22) it is exposed on the cell membrane. Does this imply that HFE may have different functions in different tissues, perhaps prevent-

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ing absorption at the gastrointestinal tract level and enhancing transport elsewhere? It is notable, although Northern blot studies indicated that *HFE* mRNA is broadly expressed in many tissues (3), there is so little of the protein in most cell lines that virtually all of the studies on which our current understanding is based have been performed in cells transfected with *HFE* or a combination of *HFE* and β-2 microglobulin genes. Conclusions drawn from investigation of cells with such unphysiologic overexpression of the protein may prove to be misleading, and the simulation of the deficient state in cells derived from the knockout mouse may provide more robust data bearing on many of the questions that need to be answered.

The mouse model may also aid in understanding more clearly the pathogenesis of the diverse clinical findings in patients with hemochromatosis. The massive accumulation of iron and the fact that phlebotomy stops or even reverses most of the manifestations of hemochromatosis has focused attention on iron as the main culprit in pathogenesis. It is often not appreciated, however, that the absorption of iron and some other metals may be controlled coordinately and may even traverse a common pathway. When iron absorption is increased in iron deficiency, that of other metals such as cobalt and copper seems to be enhanced (23), and the newly described iron transporter, Nramp 2 (20), manifests activity with many different metals. In hemochromatosis the tissue levels of lead and copper are increased and the levels of aluminum are very low (24). Copper was at one time believed to play an important role in causing the cirrhosis that occurs in hemochromatosis (25). But do other metals actually play a role in the pathogenesis of some or all of the manifestations of the disease? It is notable in this regard that the arthropathy of hemochromatosis does not respond to phlebotomy. This is a question almost impossible to answer in humans, but the importance of metal other than iron might well be approached in an animal model now developed by Zhou *et al.* (12).

The study of genetic disorders has often led the way in understanding normal physiology. We do not understand what regulates iron absorption. The study of hemochromatosis, the most common disorder of iron absorption, will very likely make major contributions to the unraveling of this complex system. Important new discoveries often raise more questions than they answer, and this has certainly been the case with the discovery of mutations of *HFE* as a cause of hemochromatosis. The production of an *HFE* knockout may well be an important tool in helping to delineate that pathway of iron absorption and its regulation.

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