GENETICS OF IRON STORAGE AND HEMOCHROMATOSIS

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ABSTRACT:
The regulation of total body iron is important to all organisms. In mammals, the iron content of the body is controlled almost entirely through regulation of absorption. The precise mechanism by which iron is absorbed and the manner in which the absorption is regulated is unknown, but a number of different proteins that are involved either in the transport process itself or its regulation have been identified. These include HFE, a class 1 HLA molecule involved in hereditary hemochromatosis, the divalent metal transporter (DMT-1), hephaestin, the transferrin receptor, and mobilferrin. Iron overload occurs in a number of hereditary disorders including transferrinemia, aceruloplasminemia, X-linked hereditary sideroblastic anemia, thalassemia major, congenital dyserythropoietic anemia, and various red cell enzyme deficiencies. In Europeans, most cases of hereditary hemochromatosis are due to mutations of the HFE gene. There are two major mutations of this gene c.845G→A (C282Y) and c.187C→G (H63D). These mutations have extraordinarily high prevalence in northern Europe and approximately five in a thousand Europeans are homozygous for the 845A mutation. The penetration of even the homozygous state for the 845A mutation is very low and that for the compound heterozygote 845A/187G, which is also associated with hemochromatosis, is even lower. The reason for the markedly variable penetrance that exists in this disorder remains unknown.

Evolutionary Forces and the Regulation of Iron Absorption

Iron, the second most common mineral on Earth, is essential to all forms of life, and even primitive forms of life have evolved mechanisms for obtaining the supply they need for growth and metabolism (Fiss et al., 1994). There is a constant struggle, however, to maintain the optimal level of iron. A deficiency of iron results not only in anemia, but also in the depletion of many iron enzymes (Fairbanks et al., 1971; Beutler and Fairbanks, 1980). Most common in children and in women in the childbearing age group, there is little doubt that it can have a major effect on fitness and probably on reproduction. Although it has sometimes been proposed, although hardly established, that iron deficiency may in some circumstances be advantageous (Weinberg, 1984), evolutionary pressure surely favors iron replete individuals. At the same time, excess iron accumulation has an adverse effect, as exemplified by patients with hereditary hemochromatosis, some of whom die at an early age from cirrhosis of the liver, diabetes, and cardiac failure. Evolutionary forces act here on a population of individuals whose members are diverse in their iron requirements. The iron requirements of childbearing and menstruating women and of growing children are much greater than those of men. In the case of iron, it may truly be said that “One man’s meat is another’s poison”.

The Regulation of Iron Absorption

Our understanding of the hereditary disorders that lead to accumulation of excess iron has been greatly hampered by the fact that neither the method by which iron passes across the intestinal cells into the blood nor how this absorption is regulated is known. The matter is complicated by the fact that the absorption of heme iron appears to follow a pathway that is different, at least in its initial portion, than that followed by inorganic iron.

Iron is a highly reactive metal that is very unlikely to pass through the intestine in an unbound form. Conrad and colleagues (Umbreit et al., 1998) have proposed that inorganic iron is bound by intestinal mucin and then absorbed into the intestinal cell. Purification of the microvillus membrane-associated proteins indicated that iron is associated with a dimer of polypeptides, one approximately 90 kDa and the other 150 kDa. These reacted with monoclonal antibodies to known integrins. The iron-binding protein mobilferrin binds to the cytosolic carboxyl-terminal end of the α chain of an integrin; pulse-chase experiments suggest that a complex consisting of iron, mobilferrin, integrin(s), a flavin-monoxygenase, and β2-microglobulin, which has been designated paraferritin (based on similarity in chromatographic migration to ferritin) is formed. It is not clear how the iron in the paraferritin complex reaches the transferrin at the abluminal membrane. A major problem with respect to details of this pathway is that it does not take into account recently described proteins that clearly play a role based on genetic data. These proteins are HFE, deficient in hereditary hemochromatosis (Feder et al., 1996), the divalent metal transporter (DMT-1), deficient in the mk mouse (Fleming et al., 1997) and the Belgrade rat (Fleming et al., 1998), and hephaestin (Vulpe et al., 1999), deficient in the sla mouse. HFE is known to bind to the transferrin receptor (Feder et al., 1998; Lebron and Bjorkman, 1999; Roy et al., 1999), but the proposals of how this affects iron trafficking in cells do not fully explain why increased amounts of iron are absorbed in hemochromatosis (Roy et al., 1999). DMT-1, formerly known as Nramp2, seems to be involved in intracellular iron transport, and hephaestin is a ceruloplasmin-like protein that presumably serves to oxidize iron at the luminal side of the membrane so that it may be bound by plasma transferrin. However,
TABLE 1

Forms of primary hemochromatosis

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cause of Iron Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-linked hereditary hemochromatosis</td>
<td>Defect or deficiency in HFE</td>
</tr>
<tr>
<td>Non-HLA-linked hereditary hemochromatosis</td>
<td>Unknown</td>
</tr>
<tr>
<td>Juvenile hemochromatosis</td>
<td>Unknown</td>
</tr>
<tr>
<td>African hemochromatosis</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

The precise role of these proteins and their possible role in iron storage disease remain unknown.

Hereditary Human Disorders in Which There Are Abnormalities of Iron Homeostasis

Tables 1 and 2 summarize some of the disorders of man in which there is an increased iron burden. Table 1 lists those disorders that may be regarded as “primary” hemochromatosis, disorders in which iron loading occurs without any underlying disease. Secondary hemochromatosis, on the other hand, is the consequence of increased iron absorption because of a stimulus to absorption by a disease process, often compounded by increased body iron due to transfusions.

It is apparent that ineffective erythropoiesis is a common factor in many of the anemias that cause iron overload. Transfusion also may play an important role in loading the body with iron. In the other cases, the cause has not been found; in a number of cases, sequencing of candidate genes such as DMT-1 (Lee et al., 1998), calreticulin (Beutler et al., 1997), β2-microglobulin (Beutler et al., 1997), or the transferrin receptor (Tsuchihashi et al., 1998) have been disappointing.

Only the gene that causes HLA-linked hemochromatosis, viz. HFE, has been identified. Therefore, this discussion of the genetics of hemochromatosis will, of necessity, deal only with the HLA-linked type of hemochromatosis.

HFE Mutations

Tight linkage of hereditary hemochromatosis with the HLA complex has been recognized for over 20 years (Simon et al., 1975). However, the HFE gene was cloned positionally only in 1996 by studying a large number of markers in the HLA region and sequencing the area with the greatest linkage disequilibrium (Feder et al., 1996). Two mutations, c.845G→A (C282Y) and c.187C→G(H63D) were found in hemochromatosis patients and, of course, in some normal individuals. Subsequently a few other mutations affecting the HFE gene have been documented. The most prevalent of these is 193A→T (S65C) (Mura et al., 1999), and a few private mutations have been encountered, as well (Barton et al., 1999; Wallace et al., 1999).

Distribution of Mutations from the Perspective of the Hemochromatosis Patient. Genotyping patients of European ancestry with proven hemochromatosis has shown an extraordinarily high prevalence of HFE mutations. Table 3 summarizes the results of some of the larger studies that have been conducted. It is clear that the great majority of patients with the established diagnosis of hereditary hemochromatosis are homozygous for the 845A mutation.

Although the role of the 187G mutation was recognized by Feder et al. (1996) in their original study of the HFE gene, and immediately confirmed (Beutler et al., 1996), the role was misinterpreted in two subsequent studies (Jazwinska et al., 1996; Jouanolle et al., 1996) and deemed to be absent or uncertain. This erroneous conclusion was based upon the fact that the frequency of this mutation was no higher in the hemochromatosis population than in the normal population. What had not been taken into account is that the 845A and 187G mutations are in complete linkage disequilibrium, and that therefore the only chromosomes “at risk” for bearing the 187G mutation are those that do not have the 845A mutation. When the calculation is made in this way it becomes obvious that the frequency of the 187G mutation is greatly increased in patients with hemochromatosis (Beutler, 1997a,b). The importance of the 187G mutation is also obvious from Table 3, which shows a great excess of patients who have the 845A/187G genotype. In all but one of the studies, the number of patients with the 845A/187G was greater than the number of 845A/wild-type genotype. Yet, the frequency of the wild-type genotype is six times higher than that of the 187G genotype. Based upon the relative prevalence of the 845A/845A and the 845A/187G genotypes

three patients who had previously been diagnosed and phlebotomized have been excluded. The open symbols represent homozygotes that gave a history of having donated at least 20 units of blood during their lifetime. The horizontal lines indicate the upper limit of normal given for the transferrin saturation and for the ferritin level.

![Figure 1](transferrin_saturation_and_ferritin_levels_in_all_845A/845A (C282Y/C282Y) homozygotes_dected_in_a_DNA-based_screening_program_for_hereditary_hemochromatosis.jpg)

FIG. 1. Transferrin saturations and ferritin levels in all 845A/845A (C282Y/C282Y) homozygotes detected in a DNA-based screening program for hereditary hemochromatosis.

Three patients who had previously been diagnosed and phlebotomized have been excluded. The open symbols represent homozygotes that gave a history of having donated at least 20 units of blood during their lifetime. The horizontal lines indicate the upper limit of normal given for the transferrin saturation and for the ferritin level.

TABLE 2

Genetic disorders that are associated with increased iron storage (secondary hemochromatosis)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cause of Iron Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atransferrinemia</td>
<td>Mutations in transferrin</td>
</tr>
<tr>
<td>Aceruloplasminemia</td>
<td>Mutations in ceruloplasmin</td>
</tr>
<tr>
<td>X-Linked hereditary sideroblastic anemia</td>
<td>Mutations in ALA52/ ineffective erythropoiesis</td>
</tr>
<tr>
<td>Thalassemia major</td>
<td>Ineffective erythropoiesis</td>
</tr>
<tr>
<td>Congenital dyserythropoietic anemia</td>
<td>Ineffective erythropoiesis</td>
</tr>
<tr>
<td>Pyruvate kinase deficiency and other red cell enzyme deficiencies</td>
<td>Transfusions and ineffective erythropoiesis</td>
</tr>
</tbody>
</table>

* 5-aminolevulinate synthase.
in the general population and the population of hemochromatosis patients, we have inferred that the relative penetrance of the compound heterozygous genotype is only about 1.5% of that of the homozygous 845A/845A genotype (Beutler et al., 1996). Indeed, studies of patients with hemochromatosis have tended to show that the amount of iron accumulation was less in the compound heterozygote than in the 845A/845A homozygote (Ryan et al., 1998).

**Distribution of Mutations from the Perspective of the General Population.** Surveys of several populations show that the gene frequencies of the common HFE mutations are highest in the British Isles and northern France, consistent with a putative Celtic origin, at least of the 845A mutation. Some of the previously published gene frequency data are shown in Table 4.

We have recently carried out large scale genotyping on patients attending a Health Appraisal Clinic. The frequency of the 845A, 187G, and 193T mutations have been determined on 7823 DNA samples, and the serum transferrin saturation and ferritin level was determined. Because several of these subjects either did not disclose their ethnic origin or were of mixed race, the gene frequency analysis has been limited to 6473 subjects. The results are given in Table 5.
Phenotypic Effects

845A/845A Homozygotes. Figure 1 shows the distribution of transferrin saturation values and ferritin levels for homozygotes for the 845A mutation. It is clear that even in patients over 50 years of age, approximately half of the homozygotes have normal transferrin saturations and ferritin levels. Thus, many of the homozygotes detected in a screening program do not have the standard biochemical stigmata of hemochromatosis. Nor did any of the subjects have clinical stigmata of the disease.

Other Genotypes. Because of the differences in the normal hematologic values of persons of different ethnic groups, the present analysis has been limited to those patients who identified themselves as “white”. The average hemoglobin values in each group and the mean corpuscular volume are presented in Table 6. The differences between groups is due to a shift in the entire distribution, not just a tail of patients with microcytosis, as might be expected if there were an excess of patients with frank iron deficiency or of thalassemia in one of the groups.

Discussion

The existence of a broad range of phenotypes is characteristic of many or most genetic diseases. Thus, patients with the same Gaucher disease (Beutler, 1992), cystic fibrosis (National Institutes of Health Consensus Development Conference Statement, 1999), or pyruvate kinase deficiency (Lenzner et al., 1997) genotypes may vary widely in their clinical manifestations. In these diseases, no clues exist to guide us in understanding this variability. In the case of hereditary hemochromatosis, the most common form of iron storage disease, marked variability is the rule. Although it has been suggested that the penetrance of HLA-linked homozygous hemochromatosis is high, exceeding 50% in males (Edwards et al., 1994), the contrary seems to be the case, based on the data that we now present. If the ferritin level actually accurately predicts the total body iron burden, then it would seem that the majority of patients with this genotype do not have excess iron stores, even when they are over 50 years of age.

In the case of most genetic disorders, little is known of genetic factors that determine the degree of expression of the disease phenotype. A recent exception with respect to genetic interactions is the interaction glucose-6-phosphate dehydrogenase deficiency and uridine-5′-diphosphoglucuronic acid glucuronyltransferase-1 to cause neonatal icterus (Kaplan et al., 1997) and similar interaction of this gene, which causes Gilbert disease, with other disorders (Galanello et al., 1997; Sampietro et al., 1997; Iolascon et al., 1998, 1999), but in the case of other genetic diseases, it is difficult to know where to begin to seek modifying factors. Environmental factors have been easier to identify in a number of disorders, such as glucose-6-phosphate dehydrogenase deficiency, acute intermittent porphyria, and cholelithiasis anaplasa. A considerable number of such interactions have been explored in this symposium. In the case of hemochromatosis, a number of candidate environmental conditions seem logical and bear examination. These include dietary or medicinal iron intake, bleeding, hepatitis, and a family history of diabetes. The database that we are accumulating will ultimately make such comparisons possible.

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