Hemochromatosis Caused by Mutations in the Iron-Regulatory Proteins Ferroportin and H Ferritin

Although hemochromatosis in northern European white populations is almost always (>90%) the result of common mutations in the transferrin-binding protein HFE, non-northern Europeans with inherited iron overload syndromes often fail to have detectable HFE alterations. Three recent reports detailed the genetic basis of autosomal dominant iron overload in Dutch, Italian, and Japanese families. Two independent groups of investigators each recently identified mutations in the same gene (SLC11A3) encoding the iron-transport protein ferroportin as the pathogenic alteration causing iron overload in a Dutch and an Italian family [1,2]. Ferroportin is a basolateral integral membrane iron-transport protein known to be a critical regulator of iron release, both into plasma from the basolateral surface of the intestinal enterocyte and from body stores within reticuloendothelial cells. Although both the Dutch and Italian family members with hemochromatosis had nonconservative missense mutations in ferroportin, the mutations were in different areas of the protein (N144H and A77D, respectively). Whether these iron-loading ferroportin mutations result in a gain or loss of iron transport function (and in which compartment) remains to be determined.

In a third recent report, a Japanese family with autosomal dominant iron overload was shown to carry a missense mutation (A49U) in the iron-response element within the 5′ untranslated region of the H ferritin gene [3]. The H and L subunits assemble to form the functional iron-storage protein ferritin, the synthesis of which is tightly regulated by binding of the iron-regulatory protein (IRP) to the 5′ untranslated region iron-response element of ferritin messenger RNA. The A49U H ferritin mutation has been shown to have greater binding affinity for IRP (than wild-type H ferritin) and, when over-expressed, causes reduced uptake of iron into the ferritin complex, with the excess unbound iron found in the cytosol. The A49U H ferritin mutation may abolish the ferroxidase activity of H ferritin that is necessary for the incorporation of iron into the ferritin shell. The massive iron overload of H ferritin knockout mice further attests to the iron-loading nature of a loss of H ferritin function.

The discovery of these two new genes that, when mutated, cause phenotypic iron overload brings the number of confirmed genes causing hemochromatosis to four: HFE, TFR2, and now ferroportin and H ferritin. Whether the three non-HFE genes should be examined in cases of non-HFE hemochromatosis awaits further important details on the frequency of these alterations in normal and iron-overloaded cohorts, their ethnic and geographic distributions, and their biochemical mechanisms of controlling iron homeostasis.

Crohn's Disease Caused by Mutations in the Bacterial Response Protein NOD2

Three teams of researchers each recently showed a strong association between mutations in the bacterial response and apoptosis regulatory protein NOD2 and development of a common inflammatory bowel disease, Crohn’s disease [4–6]. Crohn’s disease is a complex multigene disorder with a high population prevalence of approximately 0.1% that affects primarily young adults. Although predisposition to this disease is known to be familial (at least in part), environmental factors (such as smoking and bacterial infections) are believed to significantly modify preexisting genetic risk. The strongest genetic susceptibility locus for Crohn’s disease on chromosome 16 now has been shown, in a classic positional cloning approach, to be the location of the NOD2 gene [4]. Three rather common NOD2 mutations, two missense mutations (R702W and G908R) and a frameshift truncation mutation (3020insC), each with population allele frequencies
from 1% to 4%, were found at excess frequency in patients with Crohn’s disease, confirming a relative risk of approximately 3 for heterozygous carriers of any of these mutations and a relative risk of approximately 40 for homozygotes or compound heterozygotes. NOD2 (expression restricted to monocytes) is known to be part of the innate immune response and functions as a cytosolic receptor for pathogenic bacterial components, in part, by upregulating the nuclear factor-κB (NF-κB) signaling pathway. Crohn’s disease–associated mutations in NOD2 are in a region of the protein that, when deleted, causes enhanced NF-κB signaling in response to bacterial lipopolysaccharide. The pathogenic role of NF-κB signaling in Crohn’s disease (but not ulcerative colitis) has been documented by the therapeutic efficacy of pharmacological agents targeting this pathway, including corticosteroids and sulfasalazine. Although investigators estimate that approximately 20% of cases of Crohn’s disease may be directly attributable to NOD2 mutations, most cases more likely will turn out to be the result of environmental risks combined with a preexisting genetic predisposition. Crohn’s disease may then be the first example of the use of positional cloning using dense postgenomic markers for the discovery of a single gene defect that has a major contributory role in a common complex genetic disease.

**New Food and Drug Administration–based Oversight Over Genetic Testing**

Despite the near-unanimous objections of the diagnostic laboratory community, the US government is moving forward with plans to create additional, potentially burdensome, regulatory oversight over genetic testing. A particularly onerous proposed new regulation, and one specifically contested by the pathology community, was described in a January 19, 2001, directive by the outgoing Clinton administration to the Secretary’s Advisory Committee on Genetic Testing (http://www4.od.nih.gov/oba/sacgt/gt-documents.html) and would categorize laboratories developing in–house genetic tests as medical device “manufacturers.” These laboratories would then be subject to direct Food and Drug Administration (FDA) review, to register their establishments with the FDA, and list their tests in accordance with section 501 of the Federal Food, Drug, and Cosmetic Act. In a May 2001 letter cosigned by various pathology professional organizations (American Association of Clinical Chemists, College of American Pathologists, American Society of Clinical Pathologists, Association for Molecular Pathology, American Clinical Laboratory Association, and American Society for Investigative Pathology), the pathology community argued that additional FDA regulation of genetic testing is not warranted because the proposed new FDA regulations “overlap with existing regulations and may stifle the development and introduction of new genetic tests.” Furthermore, the letter contended that “these tests are already subject to the most stringent standards under CLIA’88. The existing regulatory framework, which we support, currently provides effective federal oversight without hindering patient access to care or unnecessarily increasing laboratory costs.” Furthermore, “some of the recommendations, such as registering all genetic testing laboratories, are redundant since HCFA already certifies all laboratories performing patient testing, while others, such as developing and implementing a genetic testing categorization process may become very burdensome and costly and force many quality laboratories to stop performing genetic testing.” Despite these objections (and the potentially new attitudes of the Bush administration), an August 2001 response to the pathology community by the Department of Health and Human Services (DHHS) continues to assert that “CLIA does not, and was not designed to, address the premarket review of the analytical and clinical validity of clinical laboratory tests. These matters fall within the regulatory purview of the FDA.” The DHHS will therefore continue to pursue additional FDA-based regulatory mechanisms over genetic testing, but “will be judicious in developing any new oversight mechanisms for genetics tests and such proposals will be subject to appropriate public notice and comment.”

**References**