The C282Y Mutation Causing Hereditary Hemochromatosis Does Not Produce a Null Allele

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**Results and Discussion**

**Materials and Methods**

**Commentary**

**Hereditary Hemochromatosis** is a common, autosomal recessive disorder affecting approximately one million people in the United States. The gene responsible for most cases was identified by positional cloning in 1996. Termed HFE, it encodes an atypical member of the class I major histocompatibility protein family that heterodimerizes with β2-microglobulin. Most affected patients are homozygous for a unique missense mutation that results in a tyrosine for cysteine substitution at amino acid 282 (C282Y). It is not clear whether heterozygosity for the C282Y mutation, by itself, can predispose to iron overload. The C282Y mutation disrupts an intramolecular disulfide bond, and may interfere with β2-microglobulin binding. Mice deficient in β2-microglobulin and mice deficient in Hfe both develop systemic iron overload. The function of HFE and its role in iron metabolism remain unknown. We created two mutant mouse strains to investigate whether the C282Y mutation results in total loss of protein function, and to develop a model system to study the pathogenesis of hemochromatosis.

**targeted mutagenesis** was used to produce two mutations in the murine hemochromatosis gene (Hfe) locus. The first mutation deletes a large portion of the coding sequence, generating a null allele. The second mutation introduces a missense mutation (C282Y) into the Hfe locus, but otherwise leaves the gene intact. This mutation is identical to the disease-causing mutation in patients with hereditary hemochromatosis. Mice carrying each of the two mutations were bred and analyzed. Homozygosity for either mutation results in postnatal iron loading. The effects of the null mutation are more severe than the effects of the C282Y mutation. Mice heterozygous for either mutation accumulate more iron than normal controls. Interestingly, although liver iron stores are greatly increased, splenic iron is decreased. We conclude that the C282Y mutation does not result in a null allele.

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Fig 1. Targeting constructs used to create mutant Hfe alleles. The structures of the two targeting constructs are shown, with reference to the murine Hfe locus. In each case, the intron/exon structure of the genomic clone is shown on the top line, the structure of the targeting construct is shown on the second line, and the structure of the correctly targeted mutant locus is shown on the third line. Black boxes are Hfe exons. Translational start (ATG) and stop (STOP) sites are indicated. 5' homology (5' hom) and 3' homology (3' hom) regions are indicated for each targeting vector. The locations of the neomycin resistance (NEO) and cytosine deaminase (CD) cassettes are shown. Hatched boxes represent loxP sites. The asterisk (*) shows the site of codon 282. (a) Summarizes the strategy used to make the null allele; (b) summarizes the strategy used to introduce the C282Y missense mutation. The final line in (b) shows the structure of the C282Y allele after vector sequences have been removed by Cre-mediated recombination between loxP sites.

Fig 2. Iron metabolism in Hfe mutant mice. (a) Liver iron content was determined for 4-week-old 129/SvEvTac mice as previously described10 and expressed as micrograms per gram wet weight ± standard error. Genotypes are abbreviated as follows: wild-type (+/+), Hfe C282Y homozygous (Y/Y), and Hfe null homozygous (−/−). All differences between genotypes were statistically significant when P values were determined by Welch correction of the unpaired t-test. (b) Hepatic iron was visualized by Prussian blue staining of tissue sections from wild-type and mutant F2 mice with a C57BL/6j × 129/SvEvTac background. Nonheme iron deposits appear blue. (c) Spleen iron was determined for 4-week-old 129/SvEvTac mice using the same method as was used for liver iron, and expressed as microgram per gram wet weight ± standard error. Differences between wild-type mice and each mutant strain were statistically significant according to P values determined by Welch correction of the unpaired t-test. The mutant strains were not significantly different from each other.
cardiac puncture; it remains possible that portal transferrin saturations are higher.

Mice homozygous for each Hfe mutation had less splenic nonheme iron than wild-type mice, despite systemic iron overload (Fig 2c). This may be because mouse spleen is an active site of erythropoiesis, and most splenic storage iron is found in macrophages. This may be analogous to human hemochromatosis, where patients have been shown to have decreased iron in bone marrow macrophages.9

In conclusion, the C282Y mutation clearly predisposes to iron loading, but is not as severe as a null allele. Heterozygosity for either mutant Hfe allele results in increased iron stores, consistent with the notion that the prevalence of the human mutation may result from heterozygote advantage. Analogous to human patients, mice carrying Hfe mutations also have depleted splenic iron stores, further confirming that these mouse mutants offer a valid system for studying the human disease. Finally, liver iron loading begins before circulating transferrin is fully saturated, suggesting that it may occur in the absence of significant levels of nontransferrin-bound iron. This has implications for the mechanism of hepatic iron loading, and suggests that it may begin earlier than generally appreciated in hemochromatosis patients.

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