CORRESPONDENCE

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Genes for Gestational Iron Loading?

Dear Sir:

In a recent report,[1] Bensaid et al present data from a genetic mapping study on the polygenic inheritance of hepatic iron levels in HFE-deficient mice. The study design consisted of breeding mice homozygous for a targeted disruption (knockout or −/−) of the HFE gene that had been extensively back-crossed on to the C57BL/6 (B) and the DBA/2 (D) backgrounds. Normal HFE replete B and D mice are known to differ markedly in hepatic iron levels,[2] a difference that Bensaid et al show is maintained in HFE nullizygotes. A backcross strategy was used to breed the disrupted HFE allele on to the B and D genetic backgrounds. The resulting B and D HFE −/− mice were then bred to produce BXD F1 and F2 HFE −/− homozygotes. Based upon the data presented in Figure 1, the F1 mice were severely iron overloaded as expected. However, this result has potentially significant implications for the interpretation of the iron overload phenotype in the F2 progeny. With iron overload and presumed elevated transferrin saturation in the blood, the potential for substantial gestational transfer of iron from F1 mother to F2 fetus could occur. This would result in F2 mice that were born with increased iron stores.

In human hereditary hemochromatosis, most HFE mutant homozygotes are born to mothers who are heterozygous and not iron overloaded. The chance of a homozygous HFE C282Y woman carrying a homozygous fetus is less than 1/20 (1/10 incidence of heterozygous father x 1/2 chance of inheritance of mutant allele). In addition, women of reproductive age who are homozygous may not yet be significantly iron overloaded, may be subfertile,[3] or may already be treated. One report measured the iron status of an infant born to a treated hemochromatotic mother.[4] The cord blood samples of the infant had elevated transferrin saturation (88%) and a raised ferritin concentration (250.2 μg/L). The mother had elevated transferrin saturation (66%) but normal ferritin level (91.6 μg/L). In another study,[5] the amount of isotopic iron found in the circulation of human neonates was shown to be significantly related to maternal iron absorption following maternal oral dosing. These results imply that elevated maternal blood iron levels during pregnancy may result in increased iron transfer to the fetus.

Maternal age may have also affected iron levels. The ages of the BXD F1 HFE −/− homozygotes used for breeding the F2 mice may have varied, resulting in F1 female mice with potentially widely different degrees of iron overload due to age effects. Thus, each F2 litter may have developed in widely disparate maternal iron environments.

A related point is the age at which hepatic iron levels were determined in the F2 mice. Since the mice were only 7 weeks old at the time of sacrifice, the amount of iron acquired during the approximately 3 weeks spent in utero could represent a substantial portion of the total iron stored. The subsequent 3 weeks in which the pups presumably fed on breast milk until weaning may also have had an impact on iron levels. Iron intake or supplementation does not seem to have a major impact on the iron levels in breast milk of normal individuals,[6,7] but little data is available on the iron content of breast milk in patients with hemochromatosis. A potential evolutionary explanation for the continued high prevalence of HFE mutations rests upon increased iron transfer during both gestation and lactation.[8] The variables impacting hepatic iron levels in the phenotypic extremes of the large F2 population selected for genotyping may therefore be very different than those affecting adult patients with hereditary hemochromatosis or mice derived from HFE heterozygotes. We argue that the modifier loci identified in their study may need to be considered in a different physiological light, which, nonetheless, may provide insight into the contribution of genetic variation on iron metabolism during and after gestation.

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1. Bensaid M, Fruchon S, Mazeres C, Bahram S, Roth MP, Coppin H. Multigenic control of hepatic iron loading in a murine model of hemochromatosis. Gastroenterology 2004;126:1400–1408.
2. Gerhard GS, Kaufmann EJ, Wang X, Erikson KM, Abraham J, Grundy M, Beard JL, Chorney MJ. Genetic differences in hepatic lipid peroxidation potential and iron levels in mice. Mech Ageing Dev 2002;123:167–176.
3. Bradley RJ, Rosen MP. Subfertility and gastrointestinal disease: 'unexplained' is often undiagnosed. Obstet Gynecol Surv 2004; 59:108–117.
4. Baynes RD, Meyer TE, Bothwell TH, Lamparelli RD. Maternal and fetal iron measurements in a hemochromatotic pregnancy. Am J Hematol 1991;36:48–49.
5. O'Brien KO, Zavaleta N, Abrams SA, Caulfield LE. Maternal iron status influences iron transfer to the fetus during the third trimester of pregnancy. Am J Clin Nutr 2003;77:924–930.
6. Vuori E, Makinen SM, Kara R, Kuitunen P. The effects of the dietary intakes of copper, iron, manganese, and zinc on the trace element content of human milk. Am J Clin Nutr 1980;33:227–231.
7. Arnaud J, Prual A, Preziosi P, Cherouvrier F, Favier A, Galan P, Hercberg S. Effect of iron supplementation during pregnancy on trace element (Cu, Se, Zn) concentrations in serum and breast milk from Nigerian women. Ann Nutr Metab 1993;37:262–271.
8. Jankovic GM, Petrovic MD, Colovic MD, Milosevic RA, Janosevic S, Trpinac DP. Increased transplacental and breast milk iron delivery in hereditary hemochromatosis: an evolutionary enigma resolved? Am J Hematol 1991;38:153–155.

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Reply. In their response to our article, "Multigenic control of hepatic iron loading in a murine model of hemochromatosis," Gerhard and Chorney raise an interesting issue for discussion. They suggest that the increased iron stores found in the *Hfe*$^{-/-}$ F2 progeny could result from gestational transfer of iron from iron-overloaded *Hfe*$^{-/-}$F1 mothers. The genetic loci we mapped would then control susceptibility to gestational iron loading rather than modulate hepatic iron loading in response to *Hfe* disruption.

We have, however, unpublished results that do not corroborate this possibility. First, we compared hepatic iron levels of *Hfe*$^{-/-}$ mice born

from either heterozygous $Hfe^{+/-}$ or homozygous $Hfe^{-/-}$ mothers of similar ages. $Hfe^{-/-}$ mice of DBA/2 background had 2442 ± 341 μg iron/g dry weight (n = 3) when their mother was $Hfe^{+/-}$ and 2461 ± 311 (n = 3) when their mother was $Hfe^{-/-}$. These figures were 643 ± 44 (n = 3) and 620 ± 137 (n = 3), respectively, in $Hfe^{-/-}$ mice of C57BL/6 background. No influence of the mother's *Hfe* genotype on the degree of iron loading of the $Hfe^{-/-}$ progeny could thus be detected, whatever the genetic background.

Furthermore, a C57BL/6 $Hfe^{-/-}$ mother crossed with a $Hfe^{+/-}$ heterozygous male produced $Hfe^{+/-}$ mice that were not significantly iron loaded (250 ± 39 μg iron/g dry weight; n = 3) compared to their $Hfe^{-/-}$ siblings (550 ± 60; n = 2). Therefore, the eventual influence of iron transfer from the mother to the fetus on the progeny's hepatic iron levels would only be marginal compared to that of the progeny's *Hfe* genotype.

Finally, Gerhard and Chorney suggest that the maternal iron environment of each litter, which might influence iron loading in the progeny, is dependent on the age of the F1 $Hfe^{-/-}$ mice used for breeding the F2. We examined 4 successive litters obtained from a F1 $Hfe^{-/-}$ mother aged 9, 13, 16, and 19 weeks when she gave birth. Hepatic iron levels in the F2 mice of the different litters, measured at 7 weeks of age, were 1582 ± 667 μg iron/g dry weight (n = 8), 1289 ± 510 (n = 10), 1561 ± 646 (n = 8), and 1452 ± 500 (n = 6), respectively. In each litter, mice were found, who contributed to the 2 phenotypic extremes of the whole F2 population. No influence of the mother's age on the hepatic iron loading of the $Hfe^{-/-}$ progeny could thus be observed.

Although the issue raised by Gerhard and Chorney deserved consideration, our data unequivocally prove that the genetic loci we mapped in this study modulate the severity of iron accumulation in response to *Hfe* disruption and are not merely genes for gestational iron loading.

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1. Bensaid M, Fruchon S, Mazeres C, Bahram S, Roth MP, Coppin H. Multigenic control of hepatic iron loading in a murine model of hemochromatosis. Gastroenterology 2004;126:1400–1408.

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Chronic Diarrhea

Dear Sir:

I have read with interest the comprehensive review of Dr. Schiller on chronic diarrhea published in July 2004 issue of GASTROENTEROLOGY.[1] The author directs us systematically to a correct diagnosis and management of this complex entity, and provides the reader with efficient tools to solve difficult cases.

My only criticism for this excellent paper is about the absence of M2A endoscopic capsule (Given Imaging, Israel) in the list of investigative armament. The capsule endoscopy is the most sensitive method for diagnosis of Crohn's disease and tumors of the small bowel, such as carcinoid,[2,3] for exclusion of pathology in suspected irritable bowel syndrome,[4] and may contribute to accurate transit time measurement. For any pathology mentioned this procedure is more sensitive than small bowel barium studies, CT, MRI, or enteroscopy.[5]

I believe that no paper dealing with chronic diarrhea can ignore this new investigative modality.

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1. Schiller LR. Chronic diarrhea. Gastroenterology 2004;127:287–293.
2. Eliakim R, Adler SN. Capsule video endoscopy in Crohn's disease – the European experience. Gastrointest Endosc Clin North Am 2004; 14:129–138.
3. de Franchis R, Rondonotti E, Abbiati C, Becari G, Signorelli C. Small bowel malignancy. Gastrointest Endosc Clin North Am 2004; 14:139–148.
4. Hagenmuller F, Keuchel M. Video capsule endoscopy in the work-up of abdominal pain. Gastrointest Endosc Clin North Am 2004;14:195–206.
5. Friedman S. Comparison of capsule endoscopy to other modalities in small bowel. Gastrointest Endosc Clin North Am 2004;14: 51–60.

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Reply. I share Dr. Niv's enthusiasm about capsule enteroscopy, but its place in the evaluation of patients with chronic diarrhea is uncertain at present.

While capsule enteroscopy allows visualization of the entire length of the small bowel, it does not permit biopsy of the mucosa or collection of an aspirate for quantitative culture, 2 procedures that can be of great help in the diagnosis of more common causes of chronic diarrhea. Moreover, many of the conditions causing chronic diarrhea produce pathological findings within reach of an upper endoscope or colonoscope, making visualization of the midgut of much less urgency.

For now it makes sense to proceed with colonoscopy with ileoscopy and upper gastrointestinal endoscopy or push enteroscopy with appropriate biopsies relatively early in the evaluation of patients with chronic diarrhea, reserving capsule enteroscopy for cases that remain undiagnosed after initial testing. This approach would permit diagnosis in most patients with chronic diarrhea who have structural lesions in the gut.

This recommendation may change as formal studies of the utility of capsule enteroscopy in patients with chronic diarrhea are published. A few small studies suggest that capsule enteroscopy picks up more lesions than traditional tests, but the clinical impact of discovering these lesions is less well described.[1–3] What is needed is a large head-to-head trial in this patient population with clinically meaningful endpoints.

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1. Eliakim R, Fischer D, Suissa A, Yassin K, Katz D, Guttman N, Migdal M. Wireless capsule video endoscopy is a superior diagnostic tool in comparison to barium follow-through and computerized tomography in patients with suspected Crohn's disease. Eur J Gastroenterol Hepatol 2003;15:363–367.