Available online at www.sciencedirect.com
SCIENCE DIRECT®
Blood Cells, Molecules, and Diseases 32 (2004) 283–289
BLOOD CELLS, MOLECULES, & DISEASES
www.elsevier.com/locate/ybcmd

Strain and gender modulate hepatic hepcidin 1 and 2 mRNA expression in mice

Brice Courselaud,[a] Marie-Bérengère Troadec,[a] Séverine Fruchon,[b] Gennady Ilyin,[a] Nicolas Borot,[b] Patricia Leroyer,[a] Hélène Coppin,[b] Pierre Brissot,[a,c] Marie-Paule Roth,[b] and Olivier Loréal[a,*]

[a] *Unité INSERM U522, CHRU Pontchaillou, 35033 Rennes Cedex, France*
[b] *INSERM U563, CHU Purpan, Toulouse, France*
[c] *Service des Maladies du foie CHRU Pontchaillou, 35033 Rennes Cedex, France*

Submitted 5 November 2003

(Communicated by E. Beutler, M.D., 20 November 2003)

Abstract

Hepcidin (HEPC) plays a key role in iron homeostasis and an abnormally low level of hepcidin mRNA has been reported in HFE-1 genetic hemochromatosis. Considering the well-known phenotypic variability of this disease, especially between men and women, it is important to define factors susceptible to modulate hepatic hepcidin expression and, consequently, to influence the development of iron overload in HFE-1 hemochromatosis. Therefore, our aim was to analyze the effects of strain and gender on hepatic hepcidin expression in the mouse. C57BL/6 and DBA/2 wild-type mice were included in this study. Liver and splenic iron contents were measured. Specific hepatic *Hepc1* and *Hepc2* mRNA levels were quantified using real-time reverse transcription polymerase chain reaction (RT-PCR). C57BL/6 mice expressed predominantly *Hepc1* mRNA, whereas *Hepc2* mRNA was the main form in DBA/2 mice. In both strains, females had higher levels of iron stores and Hepc mRNAs compared to males. Our results demonstrate that the expression of both hepcidin mRNAs varies according to strain and gender. They suggest that sex and genetic background, which are regulators of hepcidin expression, could play a role in the phenotypic expression of genetic hemochromatosis.

Keywords: Liver; Hepcidin; mRNA expression; Mice

Introduction

Hepcidin (HEPC, HAMP; OMIM 606464), also named LEAP-1 [1], originally described as having an antimicrobial activity, is mainly produced by the liver, and its mature form (20–25 amino acids) is found in the plasma ultrafiltrate [1] and urine [2]. Humans and rats have a single identified hepcidin gene [3], whereas two functional genes, *Hepc1* (hepcidin 1) and *Hepc2* (hepcidin 2), which have only 68% identity at the protein mature form level, are present in the mouse genome [3–5].

A link between hepcidin and iron metabolism in the mouse was first highlighted by Pigeon et al. [3], who pointed out a relationship between iron stores and hepatic hepcidin mRNA expression in induced or β2-microglobulin knockout ($\beta 2m^{-/-}$) iron overload models. Later on, Nicolas et al. [4] found that $Usf2^{-/-}$ mice developed an iron overload phenotype similar to that observed in $Hfe^{-/-}$ mice, and that the hepcidin 1 and 2 genes, which are located close to *Usf2* genes, were also totally silent in these animals. They hypothesized from these observations that hepcidin could be an iron metabolism regulating hormone acting on enterocytes and macrophages [4,6]. This key role of hepcidin in iron metabolism has been further supported by the iron deficiency phenotype observed in transgenic mice hyperexpressing *Hepc1* [7] and by the association of severe juvenile hemochromatosis with a mutation or a deletion in the hepcidin gene [8]. From the two hepcidin genes identified in the mouse, only hepcidin 1 has been clearly implicated in iron metabolism. The role of *Hepc2* remains to be characterized.

* Corresponding author. Fax: +33-2-99-54-01-37.
E-mail address: olivier.loreal@rennes.inserm.fr (O. Loréal).

1079-9796/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcmd.2003.11.003

HFE-1 genetic hemochromatosis (HGH) is a frequent inherited disease mainly related to the C282Y mutation of the HFE gene in populations of northern European origin [9]. Patients who are homozygous for the mutation classically develop iron overload, which is at least partly related to intestinal iron hyperabsorption. In the liver, early stages of iron overload involve parenchymal cells, whereas Kupffer cells (i.e., resident macrophages) are belatedly overloaded during the disease, conversely to secondary iron overload [10]. Therefore, both enterocytes and macrophages have an altered iron metabolism leading to the specific phenotype of HGH. Classically, HGH leads to visceral complications involving predominantly the liver, pancreas, and heart, but also joints, skin, and endocrine system [11,12]. However, the spectrum of the disease is variable and the penetrance incomplete [13–15], as recently strengthened by large epidemiological studies [16,17]. Such a variability in the phenotypic expression of homozygotes for the HFE C282Y mutation might be at least partly related to genetic factors. The role of these factors is indirectly supported by studies performed in wild type [18,19] and in genetically modified iron overloaded mice, including β2-microglobulin deficient mice and *Hfe*$^{-/-}$ mice [20–23]. Thus, multiple genes could play a role in the modulation of iron overload phenotype related to *Hfe* mutation.

The key role of hepcidin in the development of iron overload during HGH and its murine models has recently been emphasized. Indeed, the hepatic *Hepc* mRNA level was found to be abnormally low in young *Hfe*$^{-/-}$ mice [24]. In humans, Bridle et al. [25] reported a decrease of the hepatic hepcidin mRNA level in C282Y homozygous patients with clinical manifestations of the disease and Gehrke et al. [26] found an absence of liver hepcidin mRNA increase in HFE-1 genetic hemochromatotic patients, despite their marked iron overload. All these data suggest that an inadequate regulation of hepcidin mRNA expression may contribute to the development of iron overload in HGH patients. This hypothesis was reinforced by the correction of the iron overload phenotype of *Hfe*$^{-/-}$ animals by a constitutive expression of *Hepc1* mRNA [27].

Taken together, these observations suggest that the level of hepcidin expression could be one of the critical factors accounting for the phenotypic expression of iron overload during HGH. Therefore, our aim was to evaluate the influence of the genetic background, including strain and gender, on the expression of the two hepcidin genes in the mouse liver.

Material and methods

Animals

Eleven week-old C57BL/6 and DBA/2 mice were obtained from CERJ (France) and housed in the IFR30 animal facility in Toulouse. Twenty animals (five per group) were included in this study. All animals were maintained in accordance to French law and regulations in a temperature- and light-controlled environment. They were given free access to tap water and food. Mice liver and spleen samples were frozen in liquid nitrogen and kept at −80 °C until further processing.

Iron load measures

Liver (LIC) and splenic (SIC) iron concentrations were evaluated biochemically [28] on frozen specimens.

Total RNA isolation and reverse transcription (RT)

Total RNA was extracted from livers of C57BL/6 and DBA/2 mice using the SV total RNA isolation system (Promega, Charbonnières, France). cDNA first-strand synthesis was performed with the Advantage RT-for-PCR kit (Clontech, Palo Alto, CA), using 1 μg of total RNA and oligo dT primers. Reverse transcription products were diluted fivefold in pure sterile water before use for real-time PCR (polymerase chain reaction) assays.

cDNA probes

Mouse hepcidin cDNA probe was obtained by cloning in pCRII-TOPO vector (TOPO™ TA Cloning® kit, Invitrogen Life Technologies, Cergy Pontoise, France), the iron-induced hepcidin cDNA isolated by suppressive subtractive hybridization as described previously [3]. A 232-bp hepcidin cDNA fragment was then released by *Bam*H1 and *Xho*I digestion and used as probe for Northern blot analysis.

Northern blot analysis

For all templates, 10 μg of total RNA were loaded on a denaturing 1.2% agarose gel and transferred onto Hybond N+ filters (Amersham, Buckinghamshire, England). Filters were then hybridized with the mouse hepcidin cDNA probe labeled with [α^{32}P] dCTP (3000Ci/mmol) (Amersham and Random Primers DNA Labelling System™, Invitrogen Life Technologies). Equal mRNA loading was checked by methylene blue staining (not shown).

To assess hepcidin mRNA expression level, we exposed Northern blot membranes to overnight autoradiography and then performed densitometry measures with the assistance of Densylab v2.0.5 software (Microvision Instruments, Evry, France).

Real-time RT-PCR analysis

Primers validated in previous studies [4,23] have been used to amplify *Hepc1* (5′-CCTATCTCCATCAACAGATG-3′ and 5′-AACAGATACCACACTGGGAA-3′), *Hepc2* (5′-CCTATCTCCAGCAACAGATG-3′ and 5′-AACAGATACCACAGGAGGGT-3′), and β-actin (5′-GACGGCCA-

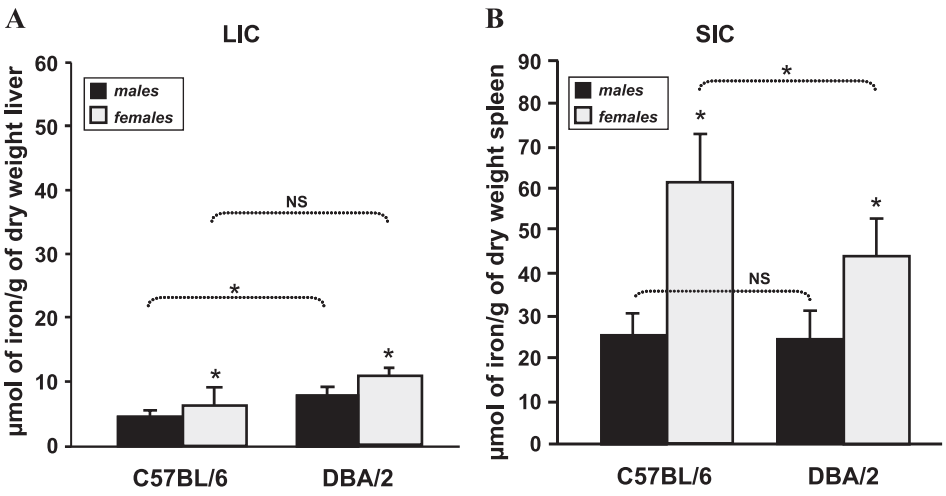

Fig. 1. Influence of strain and gender on iron store parameters. Liver iron concentration (LIC, panel A) and spleen iron concentration (SIC, panel B) were evaluated biochemically in C57BL/6 and DBA/2 mice. Iron concentrations are expressed as µmoles of iron/g of dry weight tissue. Statistical significance (*$P < 0.03$) of the comparison between male (black bars) and female (grey bars) mice is shown above the boxes.

AGTCATCACTATTG-3′ and 5′-CCACAGGATTCCATAC-CCAAGA-3′). These three primer pairs lead to 171-, 171-, and 88-bp amplification products, respectively. To avoid coamplification of genomic DNA, the forward *Hepc1* and *Hepc2* primers have been designed to overlap exons I and II and the reverse primer was positioned on exon III.

TaqMan® probes for real-time PCR analysis were designed with the Primer Express Software (PE Biosystems) and purchased from Eurogentec (Seraing, Belgium). Probe 5′-FAM-CCCTGCTTTCTTCCCCGTGCAAAG-TAMRA-3′ was used to detect *Hepc1* and *Hepc2* and probe 5′-FAM-TCCGATGCCCTGAGGCTCTTTTCC-TAMRA-3′ to detect β-actin [5].

All quantitative real-time PCR assays were performed in triplicate in 96-well microplates using the qPCR™ Core reagent kit (Eurogentec) and an ABI PRISM 7700 Sequence Detection System (PE Biosystems). Each microplate included the target cDNAs, a negative no-template control, and two standard curves prepared from normal adult mouse liver cDNA. For each cDNA sample, the difference between the threshold cycle for Hepc1 or for Hepc2 amplification and the threshold cycle for β-actin has been calculated. This allows normalization of the amount of target to the endogenous reference, β-actin. To compare the expression of the two hepcidin messengers according to strain or gender, we expressed the amount of targets relative to a calibrator Hepc1 mRNA level, which is specified on each figure.

Statistical analysis

Results were expressed as mean ± SD. Nonparametric Mann–Whitney tests were performed to test that the distributions of Hepc1 or Hepc2 mRNA levels were the same in the two strains, or the two genders, or that the distributions of Hepc1 and Hepc2 were the same within each strain/gender group. A significance probability less than 0.05 was considered as an indication of a significant mRNA expression difference between the compared groups.

Results

Strain and gender modulate hepatic and splenic iron concentrations in mice

Hepatic and splenic iron loads were evaluated biochemically and analyzed according to the mouse strain, that is,

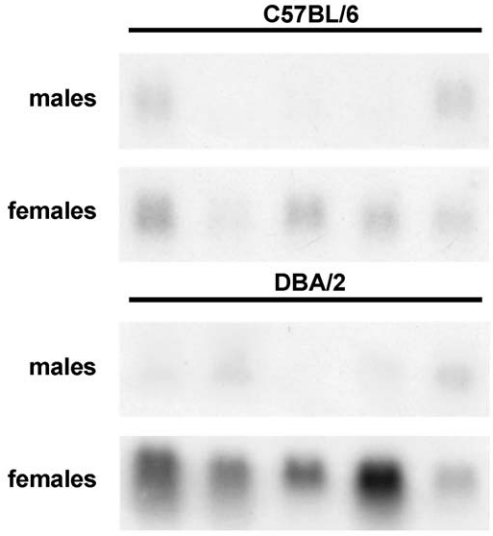

Fig. 2. Hepcidin mRNA expression is related to strain and gender in mice. For the two strains, C57BL/6 (upper panel) and DBA/2 (lower panel), total RNA was extracted from the livers of males and females, and subjected to northern blot analysis using mouse hepcidin cDNA probe. Equivalence of RNA loading was assessed by methyl blue staining (not shown). Autoradiography was exposed overnight.

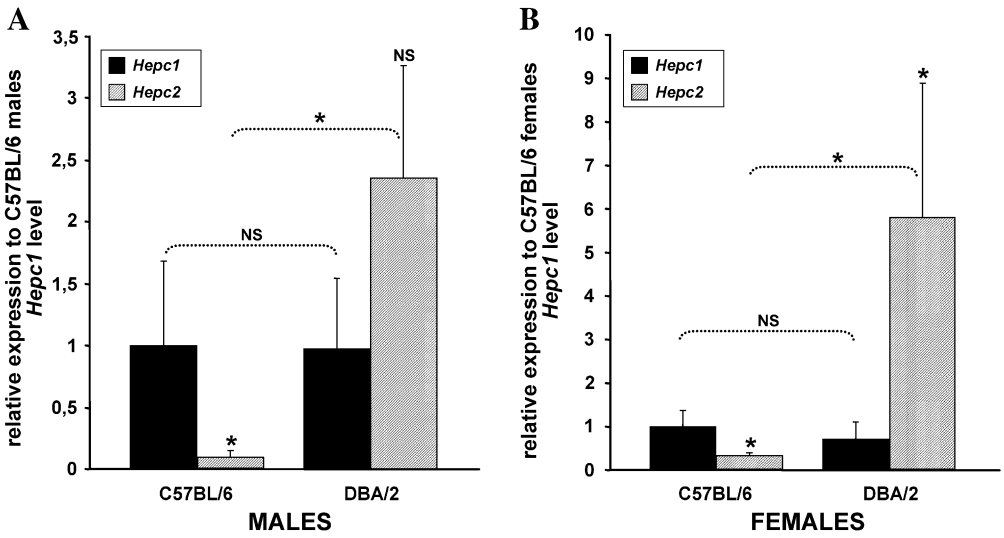

Fig. 3. Differential expression of hepcidin 1 and 2 mRNAs according to strain. The relative amount of specific Hepc1 (black bar) and Hepc2 (hatched bar) transcripts was determined by real-time RT-PCR analysis in males (A) and females (B) of the C57BL/6 and DBA/2 strains. For each strain/gender group, the histograms illustrate the expression of Hepc1 and Hepc2 relatively to the mean Hepc1 mRNA expression in males C57BL/6 (A) or females C57BL/6 (B). Statistical significance (*$P < 0.03$) of the comparison between Hepc1 and Hepc2 mRNA expression within the same strain and gender, or the comparison of the expression of a single messenger between C57BL/6 and DBA/2 mice of same sex, is shown above the boxes. NS means no significant difference. Note that due to the technology used, which required a separate PCR plate per couple of studied parameters (strain or gender), graphs cannot be cross-compared and must therefore be read independently.

C57BL/6 or DBA/2, and gender. As can be seen in Fig. 1A, females of both strains had significantly higher LIC than the corresponding males. In addition, LIC of DBA/2 males were significantly higher than those found in C57BL/6 males.

Similarly, as can be seen in Fig. 1B, females of either the C57BL/6 or the DBA/2 strain exhibited higher SIC than the corresponding males. However, SIC were significantly lower in DBA/2 females than in C57BL/6 females.

Total hepatic hepcidin mRNA level is modulated by gender and strain

Northern blot analysis (Fig. 2) indicated that females of both strains expressed higher *Hepc* mRNA levels than males. In addition, DBA/2 females expressed higher levels of hepcidin mRNA than the corresponding C57BL/6 group, and clearly were the mice with the highest hepcidin mRNA

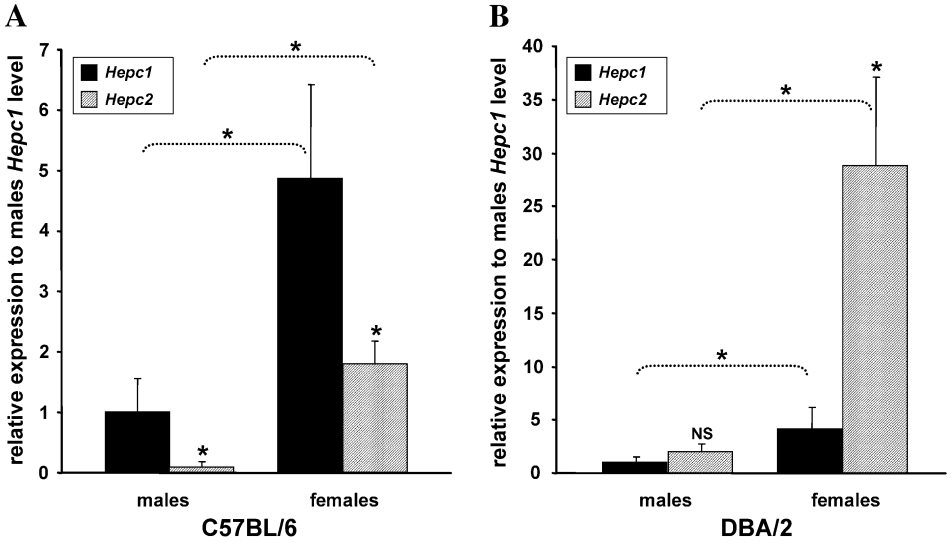

Fig. 4. Differential expression of hepcidin 1 and 2 mRNAs according to gender. The relative amount of specific Hepc1 (black bar) and Hepc2 (hatched bar) transcripts was determined by real-time RT-PCR analysis in wild-type C57BL/6 (A) and DBA/2 (B) mice of both genders. For each gender and strain group, the histograms illustrate the expression of Hepc1 and Hepc2 relatively to the mean Hepc1 mRNA expression in males C57BL/6 (A) or males DBA/2 (B). Statistical significance (*$P < 0.03$) of the comparison between Hepc1 and Hepc2 mRNAs within the same gender and strain, or the comparison of the expression of a single messenger between males and females of the same strain, is shown above the boxes. NS means no significant difference. Note that due to the technology used, which required a separate PCR plate per couple of studied parameters (strain or gender), graphs cannot be cross-compared and must therefore be read independently.

Table 1
Schematic representation of *Hepc1* and *Hepc2* mRNA expression in C57BL/6 and DBA/2 mice

	C57BL/6		DBA/2	
	Males	Females	Males	Females
Hepc1 mRNA	++	+++	++	+++
Hepc2 mRNA	+	++	+++	+++++

Expression levels of Hepc1 and Hepc2 according to each strain and gender are symbolized by crosses. A growing number of crosses indicates an increase in the mRNA expression level.

expression among the different strain/gender combinations studied.

Since Northern blot analysis reflects global expression of both hepcidin 1 and hepcidin 2 mRNA, we investigated the expression level of each mRNA separately, using real-time RT-PCR with probes specific of each mRNA to discriminate the two messengers.

Hepatic hepcidin 1 mRNA expression is modulated by gender

Males of the two C57BL/6 and DBA/2 strains expressed *Hepc1* mRNA at similar levels. The same held true for females of the two strains (Fig. 3). However, in both strains, *Hepc1* mRNA levels were significantly higher in females (Fig. 4).

Hepatic hepcidin 1 and hepcidin 2 mRNAs are differentially expressed

Whatever the gender, the predominant hepatic hepcidin mRNA was *Hepc1* in C57BL/6 and *Hepc2* in DBA/2 mice. Moreover, we found that DBA/2 males and females expressed *Hepc2* mRNA at very high levels compared to C57BL/6 males and females, respectively (Fig. 3). For both strains, *Hepc2* mRNA levels were significantly higher in females than in males (Fig. 4). Modulations of *Hepc1* and *Hepc2* mRNA expression by gender and strain are schematically summarized in Table 1.

Discussion

The role of hepcidin in iron metabolism has been demonstrated in mice [4,7] and in humans [8]. Therefore, it is suggested that elements regulating its expression could strongly modulate iron absorption and recycling [4]. In mice, two closely related hepcidin genes have been identified. Whereas the role of hepcidin 1 gene product on iron metabolism is established [4,7], biological functions of hepcidin 2 remain to be fully characterized. Our goal was to evaluate the effects of genetic factors, including strain and gender, on the expression of the two hepcidin genes in the mouse.

We found that C57BL/6 and DBA/2 strains differentially expressed hepcidin 1 and 2 mRNAs. Indeed, although both strains expressed *Hepc1* mRNA at similar levels, we found that *Hepc1* mRNA was the predominant messenger in C57BL/6 strain, whereas *Hepc2* mRNA was the main species in DBA/2 strain. These data demonstrate that despite strong homologies in the 5′ flanking region of the two hepcidin genes [5,29], there are important regulatory differences between the two genes, likely related to the genetic background. Such a differential regulation of *Hepc1* and *Hepc2* mRNAs has also been recently reported in mice between the liver and the pancreas [5]. It must be pointed out that, to date, there is no data depicting that the two polypeptides, which exhibit the eight cysteine motif but have only 68% homology, exert the same biological activities. Therefore, the impact of this differential expression between hepcidin genes on iron metabolism remains to be evaluated.

Females of both strains expressed both hepatic hepcidin mRNAs at higher levels than males, suggesting that there are gender-linked factors that are involved in the hepcidin 1 and 2 mRNA expression control. This increase of both mRNAs in females did not change the predominance of *Hepc1* and *Hepc2* mRNAs in the C57BL/6 and DBA/2 strains, respectively.

The high levels of global hepcidin mRNA in females were associated with higher LIC and SIC values compared to males, suggesting that a relationship between iron stores and hepcidin expression does exist. Such a link was previously reported [3,27,30]. However, in some cases, including anemic atransferrinemic mice, whereas livers exhibited significant iron overload, hepatic hepcidin mRNA expression was paradoxically decreased [30], suggesting a role of other factors, independent of iron stores, on hepcidin mRNA expression regulation. Accordingly, the impact of lipopolysaccharides [3], bacterial challenge [31], inflammatory cytokines [32], and hepatocyte phenotype [29] on hepcidin mRNA expression has been described, as well as the decreasing effect of hypoxia on its expression, likely partly mediated via erythropoietin pathway [33,34]. Whether the high level of hepcidin mRNA in females is a consequence of higher iron stores remains to be established.

In mice, the role of genetic background, including strain and gender, in the control of iron metabolism is supported by studies performed in wild type [18] as well as in β2-microglobulin deficient mice and $Hfe^{-/-}$ mice. In $\beta 2m^{-/-}$ mice, in which absence of Hfe expression at the cell surface leads to an iron overload similar to that observed in $Hfe^{-/-}$ mice [20], it has indeed been suggested that strain and gender may influence the iron overload phenotype [22]. Moreover, Dupic et al. [23] pointed out that strain may have a significant impact on the development of iron overload in $Hfe^{-/-}$ mice, through an effect on the expression of duodenal genes involved in iron absorption. Overall, our results, together with the finding of abnormally low levels of hepcidin mRNA in HFE-1 genetic hemochromatosis, suggest that variations in the basal hepcidin levels of hepcidin could modulate the impact of the HFE C282Y mutation on

iron metabolism and thus participate in the incomplete penetrance of the disease.

In summary, our results (i) demonstrate that hepcidin mRNAs in the mouse are differentially expressed according to the strain and the gender; (ii) suggest that these genetic factors may play a noticeable role in the phenotypic expression of genetic hemochromatosis. Identification of both hepcidin expression regulators and molecular partners will provide key elements to improve our understanding of HFE-1 hemochromatosis and iron metabolism related diseases.

Note added in proof

During the submission and editorial process, Lou et al. published that Hepc2 gene does not act on iron metabolism like Hepc1 (Blood first edition paper, doi:10.1182/Blood.2003.07.2524.

Acknowledgments

This work was supported by Conseil Régional de Bretagne, European Community Grant QLK1-CT-200100444, Ligue Contre le Cancer Comité d'Ille et Vilaine, and Association Fer et Foie.

References

[1] A. Krause, S. Neitz, H.J. Magert, A. Schulz, W.G. Forssmann, P. Schulz-Knappe, K. Adermann, LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity, FEBS Lett. 480 (2000) 147–150.

[2] C.H. Park, E.V. Valore, A.J. Waring, T. Ganz, Hepcidin, a urinary antimicrobial peptide synthesized in the liver, J. Biol. Chem. 276 (2001) 7806–7810.

[3] C. Pigeon, G. Ilyin, B. Courselaud, P. Leroyer, B. Turlin, P. Brissot, O. Loréal, A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload, J. Biol. Chem. 276 (2001) 7811–7819.

[4] G. Nicolas, M. Bennoun, I. Devaux, C. Beaumont, B. Grandchamp, A. Kahn, S. Vaulont, Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 8780–8785.

[5] G. Ilyin, B. Courselaud, M.B. Troadec, C. Pigeon, M. Alizadeh, P. Leroyer, P. Brissot, O. Loréal, Comparative analysis of mouse hepcidin 1 and 2 genes: evidence for different patterns of expression and co-inducibility during iron overload, FEBS Lett. 542 (2003) 22–26.

[6] R.E. Fleming, W.S. Sly, Hepcidin: a putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 8160–8162.

[7] G. Nicolas, M. Bennoun, A. Porteu, S. Mativet, C. Beaumont, B. Grandchamp, M. Sirito, M. Sawadogo, A. Kahn, S. Vaulont, Severe iron deficiency anemia in transgenic mice expressing liver hepcidin, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 4596–4601.

[8] A. Roetto, G. Papanikolaou, M. Politou, F. Alberti, D. Girelli, J. Christakis, D. Loukopoulos, C. Camaschella, Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis, Nat. Genet. 33 (2003) 21–22.

[9] J.N. Feder, A. Gnirke, W. Thomas, Z. Tsuchihashi, D.A. Ruddy, A. Basava, F. Dormishian, R. Domingo Jr., M.C. Ellis, A. Fullan, L.M. Hinton, N.L. Jones, B.E. Kimmel, G.S. Kronmal, P. Lauer, V.K. Lee, D.B. Loeb, F.A. Mapa, E. McClelland, N.C. Meyer, G.A. Mintier, N. Moeller, T. Moore, E. Morikang, R.R. Wolff, et al., A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis, Nat. Genet. 13 (1996) 399–408.

[10] Y.M. Deugnier, O. Loréal, B. Turlin, D. Guyader, H. Jouanolle, R. Moirand, C. Jacquelinet, P. Brissot, Liver pathology in genetic hemochromatosis: a review of 135 homozygous cases and their bioclinical correlations, Gastroenterology 102 (1992) 2050–2059.

[11] C. Niederau, R. Fischer, A. Sonnenberg, W. Stremmel, H.J. Trampisch, G. Strohmeyer, Survival and causes of death in cirrhotic and in non-cirrhotic patients with primary hemochromatosis, N. Engl. J. Med. 313 (1985) 1256–1262.

[12] C. Niederau, R. Fischer, A. Purschel, W. Stremmel, D. Haussinger, G. Strohmeyer, Long-term survival in patients with hereditary hemochromatosis, Gastroenterology 110 (1996) 1107–1119.

[13] P. Brissot, R. Moirand, A.M. Jouanolle, D. Guyader, J.Y. Le Gall, Y. Deugnier, V. David, A genotypic study of 217 unrelated probands diagnosed as "genetic hemochromatosis" on "classical" phenotypic criteria, J. Hepatol. 30 (1999) 588–593.

[14] J.K. Olynyk, D.J. Cullen, S. Aquilia, E. Rossi, L. Summerville, L.W. Powell, A population-based study of the clinical expression of the hemochromatosis gene, N. Engl. J. Med. 341 (1999) 718–724.

[15] Z.J. Bulaj, R.S. Ajioka, J.D. Phillips, B.A. LaSalle, L.B. Jorde, L.M. Griffen, C.Q. Edwards, J.P. Kushner, Disease-related conditions in relatives of patients with hemochromatosis, N. Engl. J. Med. 343 (2000) 1529–1535.

[16] E. Beutler, V.J. Felitti, J.A. Koziol, N.J. Ho, T. Gelbart, Penetrance of 845G→A (C282Y) HFE hereditary haemochromatosis mutation in the USA, Lancet 359 (2002) 211–218.

[17] E. Beutler, V.J. Felitti, The clinical penetrance of hereditary hemochromatosis, Hepatology 37 (2003) 711.

[18] R.C. Leboeuf, D. Tolson, J.W. Heinecke, Dissociation between tissue iron concentrations and transferrin saturation among inbred mouse strains, J. Lab. Clin. Med. 126 (1995) 128–136.

[19] F. Dupic, S. Fruchon, M. Bensaid, O. Loréal, P. Brissot, N. Borot, M.P. Roth, H. Coppin, Duodenal mRNA expression of iron related genes in response to iron loading and iron deficiency in four strains of mice, Gut 51 (2002) 648–653.

[20] M. de Sousa, R. Reimao, R. Lacerda, P. Hugo, S.H. Kaufmann, G. Porto, Iron overload in beta 2-microglobulin-deficient mice, Immunol. Lett. 39 (1994) 105–111.

[21] M.M. Santos, M. de Sousa, L.H. Rademakers, H. Clevers, J.J. Marx, M.W. Schilham, Iron overload and heart fibrosis in mice deficient for both beta2-microglobulin and Rag1, Am. J. Pathol. 157 (2000) 1883–1892.

[22] T.J. Sproule, E.C. Jazwinska, R.S. Britton, B.R. Bacon, R.E. Fleming, W.S. Sly, D.C. Roopenian, Naturally variant autosomal and sex-linked loci determine the severity of iron overload in beta 2-microglobulin-deficient mice, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5170–5174.

[23] F. Dupic, S. Fruchon, M. Bensaid, N. Borot, M. Radosavljevic, O. Loréal, P. Brissot, S. Gilfillan, S. Bahram, H. Coppin, M.P. Roth, Inactivation of the hemochromatosis gene differentially regulates duodenal expression of iron-related mRNAs between mouse strains, Gastroenterology 122 (2002) 745–751.

[24] K.A. Ahmad, J.R. Ahmann, M.C. Migas, A. Waheed, R.S. Britton, B.R. Bacon, W.S. Sly, R.E. Fleming, Decreased liver hepcidin expression in the hfe knockout mouse, Blood Cells, Mol. Dis. 29 (2002) 361–366.

[25] K.R. Bridle, D.M. Frazer, S.J. Wilkins, J.L. Dixon, D.M. Purdie, D.H. Crawford, V.N. Subramaniam, L.W. Powell, G.J. Anderson, G.A. Ram, Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis, Lancet 361 (2003) 669–673.

[26] S.G. Gehrke, H. Kulaksiz, T. Herrmann, H.D. Riedel, K. Bents, C.

Veltkamp, W. Stremmel, Expression of hepcidin in hereditary hemochromatosis: evidence for a regulation in response to serum transferrin saturation and non-transferrin-bound iron, Blood 102 (2003) 371–376.

[27] G. Nicolas, L. Viatte, D.Q. Lou, M. Bennoun, C. Beaumont, A. Kahn, N.C. Andrews, S. Vaulont, Constitutive hepcidin expression prevents iron overload in a mouse model of hemochromatosis, Nat. Genet. 34 (2003) 97–101.

[28] M. Barry, S. Sherlock, Measurement of liver-iron concentration in needle biopsy specimens, Lancet 2 (1971) 100–103.

[29] B. Courselaud, C. Pigeon, Y. Inoue, J. Inoue, F.J. Gonzalez, P. Leroyer, D. Gilot, K. Boudjema, C. Guguen-Guillouzo, P. Brissot, O. Loréal, G. Ilyin, C/EBPalpha regulates hepatic transcription of hepcidin, an antimicrobial peptide and regulator of iron metabolism. Cross-talk between C/EBP pathway and iron metabolism, J. Biol. Chem. 277 (2002) 41163–41170.

[30] D.A. Weinstein, C.N. Roy, M.D. Fleming, M.F. Loda, J.I. Wolfsdorf, N.C. Andrews, Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease, Blood 100 (2002) 3776–3781.

[31] H. Shike, X. Lauth, M.E. Westerman, V.E. Ostland, J.M. Carlberg, J.C. Van Olst, C. Shimizu, P. Bulet, J.C. Burns, Bass hepcidin is a novel antimicrobial peptide induced by bacterial challenge, Eur. J. Biochem. 269 (2002) 2232–2237.

[32] E. Nemeth, E.V. Valore, M. Territo, G. Schiller, A. Lichtenstein, T. Ganz, Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein, Blood 101 (2003) 2461–2463.

[33] G. Nicolas, C. Chauvet, L. Viatte, J.L. Danan, X. Bigard, I. Devaux, C. Beaumont, A. Kahn, S. Vaulont, The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation, J. Clin. Invest. 110 (2002) 1037–1044.

[34] G. Nicolas, L. Viatte, M. Bennoun, C. Beaumont, A. Kahn, S. Vaulont, Hepcidin, a new iron regulatory peptide, Blood Cells, Mol. Dis. 29 (2002) 327–335.