IRON IS A CRITICAL TRACE ELEMENT involved in nearly all biological functions. While iron is necessary for normal physiological function, too much iron can be quite toxic and lead to cell death (32). Alternatively, too little iron can lead to anemia and many other subsequent biological and behavioral complications (43). Consequently, iron must be highly regulated in all organ systems. Systemic iron homeostasis is maintained by multiple mechanisms, and malfunction in any of these may lead to disease.

Iron deficiency anemia (IDA) is one of the most common, single nutrient-deficiency diseases worldwide. According to a 2001 report from the World Health Organization, IDA affects about 2.5% of the population worldwide. Moreover, an estimated 52% of pregnant women from undeveloped or developing countries, and 20–25% of the world’s infants are afflicted (37, 52). In infancy, iron deficiency has pronounced effects on multiple organ systems and can impair functions ranging from immunity (11, 48) to neurobehavioral development (44, 58). In adulthood, systemic iron deficiency causes fatigue and headache and is linked to heart failure; brain iron deficiency may lead to restless legs syndrome, and neuroleptic malignant syndrome (13, 12, 53, 40).

Dietary iron is absorbed from the intestine into blood circulation, where it is bound to transferrin and transported to different organs. Iron is then transported into target cells by the transferrin receptor (TTR) and divalent metal transporter 1 (6, 10, 19). In cells, iron is sequestered, and redox reactions are facilitated by the transferrin receptor (TfR) and divalent metal transporter 1 (6, 10, 19). Iron is then used for various cellular processes, including energy production, protein synthesis, and signaling pathways.

The present study is a follow-up to our earlier report (29), where seven iron-related parameters were measured in a panel of recombinant inbred (RI) mouse strains that were fed a standard laboratory diet containing adequate iron. The focus of the present study was to elucidate the genetic basis of individual differences in susceptibility to iron deficiency anemia.

The animals used were 22 BXD recombinant strains of mice, along with parental strains, C57BL/6J (B6) and DBA/2J (D2). In this paper, we report the analysis of seven parameters of systemic iron status: hematocrit, hemoglobin, serum iron concentration, transferrin saturation, total iron-binding capacity (TIBC), liver iron, and spleen iron. These seven measures were then subjected to principal components analysis to reveal overt and latent combinations of factors important for iron homeostasis for each dietary condition and sex. The factors identified were then subjected to quantitative trait loci (QTL) analysis to reveal chromosomal regions harboring genes that influence these factors. Recombinant inbred strains, when densely mapped for genetic polymorphisms, are especially well suited for discovery of multiple genes that influence biological and behavioral phenotypes (69). The BXD panel is densely mapped with more than 13,000 markers genome-wide.
MATERIALS AND METHODS
Animals, Treatment, and Housing

Male and female mice from 22 strains plus the parental strains, C57BL/6J and DBA/2J, of the BXD/Ty RI panel were the experimental subjects. All mice came from the Penn State University vivarium. At postnatal day 21, mice were weaned and housed in unisex groups of 2–4/cage. The mice were then randomly assigned to one of two diets, an iron-adequate diet containing 240 μg/g iron (AD, Harlan Teklad, Indianapolis, IN), or an iron-deficient diet containing ~3 μg/g iron (ID; Harlan Teklad). The breeding stock was fed an AD diet. All mice had ad libitum access to distilled water and food. Ambient housing conditions were controlled for temperature (23 ± 2°C) and humidity (40%), and the animals were maintained on a 12:12-h light-dark cycle (lights on at 0600). At 120 days of age, all mice were killed for tissue harvest. The average number per strain × sex × diet group ranged from 10 and 12. Three groups had two animals and three groups had three animals. The total number of mice used was 1,067 (536 males, 531 females). All experimental protocols were conducted in accordance with The National Institutes of Health Animal Care guidelines and were approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

Measures

All mice were weighed and killed by CO2 suffocation between the hours of 0900 and 1200. Plasma iron, hemoglobin, hematocrit, TIBC, transferrin saturation (TfS), and liver iron and spleen iron concentrations were measured using standard methods (17). Hemoglobin values

Fig. 1. Body weights (g) by strain, sex, and diet for mice fed an iron-poor (ID Diet; 3 ppm [Fe]) or an iron-adequate (AD Diet; 240 ppm [Fe]) diet. The animals were fed their respective diets between weaning at postnatal day 21 until 4 mo of age, or 100 days total. Data are expressed as means ± SE.
were determined photometrically by using cyanmethemoglobin standard solution (Sigma Aldrich, St. Louis, MO), and hematocrit values were calculated after centrifugation (13,000 g, 5 min, room temperature) of blood samples in heparinized microcapillary tubes. Serum iron and TIBC were determined photometrically by using cyanmethemoglobin standards.

Table 1. Descriptive statistics for iron measures, by diet

<table>
<thead>
<tr>
<th>Measures</th>
<th>Hct, %</th>
<th>Hb, g/dl</th>
<th>TIBC, µg/dl</th>
<th>TIS, %</th>
<th>Plasma Fe, µg/dl</th>
<th>Liver Fe, µg/g tissue</th>
<th>Spleen Fe, µg/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>45.22</td>
<td>29.65</td>
<td>12.42</td>
<td>6.79</td>
<td>627.83</td>
<td>838.01</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>3.99</td>
<td>9.94</td>
<td>1.69</td>
<td>3.03</td>
<td>212.49</td>
<td>264.45</td>
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<tr>
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<td>6.96</td>
<td>0.67</td>
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<tr>
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<td>57</td>
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<td>17.47</td>
<td>528.6</td>
<td>1406.7</td>
<td>1977.5</td>
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<tr>
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<td>246</td>
<td>256</td>
<td>245</td>
<td>252</td>
<td>239</td>
<td>253</td>
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<tr>
<td>Females</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>mean</td>
<td>45.2</td>
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<td>8.14</td>
<td>595.12</td>
<td>754.2</td>
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<tr>
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<td>7.76</td>
<td>1</td>
<td>159.7</td>
<td>98.26</td>
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</tr>
<tr>
<td>maximum</td>
<td>55</td>
<td>52</td>
<td>21.96</td>
<td>18.11</td>
<td>1478.9</td>
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<td>239</td>
<td>244</td>
<td>235</td>
<td>241</td>
<td>229</td>
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<td>Sexes Combined</td>
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<tr>
<td>mean</td>
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<td>31.89</td>
<td>12.42</td>
<td>7.45</td>
<td>611.84</td>
<td>797</td>
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<tr>
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<td>218.69</td>
<td>242.72</td>
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</tr>
<tr>
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<td>5</td>
<td>6.96</td>
<td>0.67</td>
<td>154.3</td>
<td>98.26</td>
<td></td>
</tr>
<tr>
<td>maximum</td>
<td>57</td>
<td>52</td>
<td>21.96</td>
<td>18.11</td>
<td>1478.9</td>
<td>1451.7</td>
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<td>500</td>
<td>480</td>
<td>493</td>
<td>468</td>
<td>500</td>
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</tbody>
</table>

The ANOVA summary is as follows:

- **Strain**, **Sex**, **Diet**, **Strain×Sex**, **Strain×Diet**, **Sex×Diet**, and **Strain×Sex×Diet**
- **Plasma Fe**, **Liver Fe**, and **Spleen Fe**

**RESULTS**

**Body Weight**

Figure 1 illustrates the effect of an ID on body weight in the animals at 4 mo of age. ANOVA showed that the main effects to identify candidate genes whose variants (alleles) affect the value of the phenotype. QTL analysis was performed using WebQT (www.genenet.org) for each PCA factor. WebQT performs 2,000 or more permutations of the strain data, and significant QTL are defined by the likelihood ratio statistic (LRS) score of correctly ordered data exceeding all other permutations 95% of the time, i.e., the 0.05 alpha level (69, 70). For each QTL, genes within ±5 Mb of the marker were evaluated using the PosMed system (http://omicspace.riken.jp/PosMed/) to identify iron-related genes. The *cis*-regulated, iron-related genes in this area were then placed under consideration when their expression was significantly correlated with the PCA factors and with an absolute value of *r* greater than 0.5 (26). We then searched the literature for articles concerning the genes and those that had been reported as related to iron-regulation were nominated as candidate genes for that factor. The gene expression profiles (GEPs) used to identify *cis* regulation and correlation with the PCA factors (Table 6) were provided by others and published on GeneNetwork.org. The databases include hematopoietic stem cells, liver, and spleen and are described in Table 6 (for example, the link to spleen gene expression data is www.genenet.org/dbdoc/IoP_SPL_RMA0509.html). Genetic correlations between the factors and published phenotypes (using BXD strain means) were performed using the GeneNetwork database.
for strain, sex, and diet were all significant beyond $P < 0.001$ ($F_{20,928} = 41.98; F_{1,928} = 673.32; F_{1,928} = 295.30$, respectively), as were all of the interactions, owing to the large error degrees of freedom. Heritability estimates for body weight under the AD diet and ID diets were 0.40 and 0.25, respectively. Overall, animals fed the ID diet showed lower body weights compared with those treated with the iron-adequate (AD) diet, except for males (strain 32) and for females (strains 6, 42, and 18), which showed weight gain. There were also large differences among the strains in response to the iron-

### Table 3. Narrow sense heritability estimates for the parameters

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>0.22</td>
<td>0.29</td>
<td>0.11</td>
<td>0.34</td>
<td>0.32</td>
<td>0.27</td>
<td>0.23</td>
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<td>0.66</td>
<td>0.16</td>
<td>0.36</td>
<td>0.27</td>
<td>0.51</td>
</tr>
<tr>
<td>AD</td>
<td>0.32</td>
<td>0.16</td>
<td>0.36</td>
<td>0.27</td>
<td>0.23</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>ID</td>
<td>0.32</td>
<td>0.16</td>
<td>0.36</td>
<td>0.27</td>
<td>0.23</td>
<td>0.12</td>
<td>0.18</td>
</tr>
</tbody>
</table>

These estimates were calculated from the ANOVA tables (Figure 2) as the ratio of SSstrain/SStotal, as suggested by Belknap (5).

![Fig. 2. Hemoglobin values (g/dl) by strain, sex, and diet for mice fed an iron-poor (ID Diet; 3-ppm [Fe]) or an iron-adequate (AD Diet; 240-ppm [Fe]) diet. The animals were fed their respective diets between weaning at postnatal day 21 until 4 mo of age, or 100 days total. Data are expressed as means ± SE.](http://ajpregu.physiology.org/)

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deficient diet. We performed a step-wise multiple-regression analysis of each of the seven iron-related parameters on body weight. The only parameter to have a significant impact on body weight was hematocrit with a regression coefficient of 0.218 ($P < 0.05$).

Iron Measures: Summary Statistics

Descriptive statistics for the seven measures, by diet or sex, are presented in Table 1. The ANOVA summaries for the iron parameters are listed in Table 2. The main effect of strain was significant for all seven measures. The main effect of sex was significant for all seven measures, except for TfS. For hematocrit and hemoglobin, overall, the values for males were equal to females on the AD diet, but ID females showed higher values than ID males. For TIBC, males showed higher values than females under both diets. For plasma Fe, AD females showed higher values than AD males, while ID females showed lower values than ID males. For both liver Fe and spleen Fe, females showed higher values than males on both diets. The main effect of diet was also significant for all seven measures. The ID diet increased TIBC and reduced all other measures in both sexes compared with AD diet, except for plasma Fe in males (no change). The strain × diet interaction was significant for all seven measures. The strain × sex interaction was significant for all seven measures except for TfS and plasma Fe. The diet × sex interaction was significant for all seven measures except for TIBC. The strain × diet × sex inter-

Fig. 3. Hematocrit (% packed red blood cells) by strain, sex, and diet for mice fed an iron-poor (ID Diet; 3 ppm [Fe]) or iron-adequate (AD Diet; 240 ppm [Fe]) diet. The animals were fed their respective diets between weaning at postnatal day 21 until 4 mo of age, or 100 days total. Data are expressed as means ± SE.
action was significant for all seven measures. Narrow-sense heritability estimates (from ANOVA $SS_{\text{strain}}/SS_{\text{total}}$) for each measure by diet are listed in Table 3.

Strain Distributions for Iron Measures

We observed continuous distributions of means among strains for each measure in each diet, as well as large strain differences in the degree of change in each measure due to ID diet, shown in Fig. 2 (hemoglobin), Fig. 3 (hematocrit), Fig. 4 (plasma iron), Fig. 5 (transferrin saturation), Fig. 6 (TIBC), Fig. 7 (liver iron), and Fig. 8 (spleen iron).

Genetic Correlations Between Measures

Genetic correlations between strain means are presented in Table 4. For the AD diet, we observed significant correlations (all $P < 0.05$) between hemoglobin and hematocrit (in males and combined sexes), between transferrin saturation and TIBC (in both sexes), and between TIBC and plasma iron (in males and combined sexes) (Table 4). For ID diet, we observed significant correlations (all $P < 0.05$) between hemoglobin and hematocrit, between plasma Fe concentration and transferrin saturation, between spleen Fe concentration and hemoglobin, and between spleen Fe concentration and hematocrit, all in both sexes; also a significant correlation was found ($P < 0.05$) between TIBC and plasma Fe concentration in male and sex-pooled mice (Table 4).

Genetic Correlations Between Sexes by Measures

For the AD diet, the correlation between males and females for hemoglobin was weak ($r = 0.26, P > 0.05$), but robust for...
all the other six measures (hematocrit: 0.59, TIBC: 0.86, TTS: 0.74, plasma Fe: 0.57, liver Fe: 0.62, and spleen Fe: 0.75, all \( P < 0.05 \)). For the ID diet, the correlation between males and females was strong only for TIBC (0.71, \( P < 0.01 \)) and were weak for the other six measures (all \( r < 0.5, P > 0.05 \)).

**Principal Components Analysis**

PCA is a method to show inter-relatedness among multiple measures, including latent associations (obscured by partial correlations). We performed PCA on strain means and not on the raw data for the seven measures. We first combined the data from both sexes by strain to explore for major factors involved in iron homeostasis for each diet. We then further analyzed the data of males and females separately for each diet to identify the major factors. Factor loadings (all eigenvalues >0.5) and the percentage of total phenotype variance accounted for by each factor are summarized in Table 5. Strain distribution graphs of the \( z \) scores for each factor are presented in Fig. 9.

**Quantitative Trait Loci Analysis of the Principal Components**

QTL analysis was performed separately on the PCA factors identified for males and females. Table 6 summarizes this analysis and provides candidate genes identified by PosMed within each locus containing the gene symbol, gene description, chromosomal location, gene expression profiles, and correlation between expression and PCA.

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*Fig. 5. Transferrin saturation (%) by strain, sex, and diet for mice fed an iron-poor (ID Diet; 3 ppm [Fe]) or an iron-adequate (AD Diet; 240 ppm [Fe]) diet. The animals were fed their respective diets between weaning at postnatal day 21 until 4 mo of age, or 100 days total. Data are expressed as means ± SE.*
FACTOR 1. One weak QTL on chromosome 4 at 82.26 Mb (marker: rs6258088, LRS/H11005 7.56) was observed. The bootstrap statistic is 27.7% with a width of about 3.8 Mb. One candidate gene, Ptprd, was nominated for this factor.

FACTOR 2. One suggestive QTL on chromosome 10 at 118.41 Mb (marker: mcv25264026, LRS/H11005 11.94) was observed. The bootstrap statistic is 40% with a width of about 4 Mb. One candidate gene, Mdm1, was nominated for this factor.

FACTOR 3. One suggestive QTL on chromosome 14 at 41.43 Mb (marker: rs6314716, LRS = 12.54) was observed. The bootstrap statistic is 41.1%, and the width is about 2 Mb. No candidate gene was identified for this factor.

AD, females. FACTOR 1. We observed 1 weak QTL on chromosome 9 at 33.19 Mb (marker: rs6406454, LRS = 9.63). The bootstrap statistics is 22.2% with a width of about 2 Mb. No candidate gene was identified for this factor.

FACTOR 2. No suggestive or significant QTL was observed.

FACTOR 3. Two suggestive QTLs were observed, one on chromosome 7 at 95.16 Mb (marker: rs3672782, LRS = 15.07) with bootstrap statistics 55.4% (width around 0.5 Mb), and another one on chromosome 18 at 42.91 Mb (marker: rs13483326, LRS = 11.80) with bootstrap statistics 41.5% (width around 1.8 Mb). Two candidate genes, Picalm and Tcerg1, were identified for this factor.

Fig. 6. Total iron binding capacity (μg/dl) by strain, sex, and diet for mice fed an iron-poor (ID Diet; 3 ppm [Fe]) or an iron-adequate (AD Diet; 240 ppm [Fe]) diet. The animals were fed their respective diets between postnatal day 21 until 4 mo of age, or 100 days total. Data are expressed as means ± SE.
**FACTOR 1.** No suggestive or significant QTL was observed.

**FACTOR 2.** No suggestive or significant QTL was observed.

**FACTOR 1.** We observed one significant QTL, on chromosome 7 at 95.15 Mb (marker: rs3672782, LRS = 16.92) with bootstrap statistic of 56.3% (width around 0.8 Mb), and two suggestive QTL, one on chromosome 17 at 65.34 Mb (marker: rs13483071, LRS = 12.49), and another one on chromosome 18 at 42.91 Mb (marker: rs13483326, LRS = 11.53), with bootstrap statistics 46.1% (width around 0.5 Mb) and 33.25% (width around 3 Mb), respectively. Two candidate genes were identified for this factor: *Skp2* and *Lip1*.

**FACTOR 3.** We observed one suggestive QTL on chromosome 2 at 82.83 Mb (marker: rs13476608, LRS = 10.33). The bootstrap statistic is 45.7% with the width about 1 Mb. One candidate gene was nominated for this factor, *Frzb*.

**DISCUSSION**

This is the first study of its kind on dietary iron deficiency using a systems biology/genetics approach. By feeding animals from a genetic reference population diets from wean-
ing until 4 mo of age, our aim was to model individual differences in susceptibility to the effects of dietary iron deficiency. In fact, some of the strains appeared to meet the criteria for iron-deficient anemia, i.e., increased TfS, and decreased hemaglobin, hematocrit, TIBC, and iron concentration in plasma, liver, and spleen, while other strains appeared to be unaffected by the iron-deficient diet. For plasma iron, females in one strain and males in at least four strains showed dramatic increases under iron deficiency. The extent to which this represented hemolysis, fragile erythrocytes, or leaching of iron from some other compartment is unknown. We do not know what the status of this increased iron is, bound, loosely bound, or unbound; however, as Kell (32) recently pointed out, loosely bound iron poses a problem and may be an etiological factor for cardiovascular and inflammation-related diseases. Moreover, it may contribute, together with environmental toxicants, to neurodegenerative disease, such as Parkinson’s disease (75). One more remarkable finding was that the iron-poor diet produced varying degrees of splenomegaly, an observation previously reported by us (23). Iron-deficient diets also reduced body weight in most strains, and this was related to reduced hematocrits. This indicates that insufficient iron availability in the postnatal period affects overall bodily growth.

The wide variation in each measure across the lines suggests that iron management is a complex trait under the influence of multiple genes and that this genetic effect interacts with dietary iron intake. The large between-strain variations in the response to the ID diet can be used to elucidate mechanisms underlying individual differences in susceptibility to iron-deficiency anemia and other iron deficiency-related disorders in humans (2, 6, 7, 50).

Fig. 8. Spleen [Fe] (μg/g wet tissue weight) by strain, sex, and diet for mice fed an iron-poor (ID Diet; 3 ppm [Fe]) or an iron-adequate (AD Diet; 240 ppm [Fe]) diet. The animals were fed their respective diets between weaning at postnatal day 21 until 4 mo of age, or 100 days total. Data are expressed as means ± SE.
Table 4. Genetic correlations between measures for AD and ID diets

<table>
<thead>
<tr>
<th>Iron Measures</th>
<th>Males</th>
<th>Females</th>
<th>Males + Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb and Hct</td>
<td>$r = 0.91$</td>
<td>n.s.</td>
<td>0.772</td>
</tr>
<tr>
<td>TIBC and TIS</td>
<td>$r = -0.54$</td>
<td>0.59</td>
<td>-0.61</td>
</tr>
<tr>
<td>TIBC and Plasma Fe</td>
<td>$r = 0.69$</td>
<td>n.s.</td>
<td>0.50</td>
</tr>
<tr>
<td>ID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb and Hct</td>
<td>$r = 0.93$</td>
<td>0.85</td>
<td>0.89</td>
</tr>
<tr>
<td>TIBC and Plasma Fe</td>
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<td>n.s.</td>
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</tr>
<tr>
<td>TIS and Plasma Fe</td>
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<td>Hct and Spleen Fe</td>
<td>$r = 0.66$</td>
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</tr>
</tbody>
</table>

These Pearson correlation coefficients were calculated using the strain means. These are genetic correlations because using strain means eliminates much of the environmentally based covariance. Hct, hematocrit; Hb, hemoglobin; TIBC, total iron binding capacity; TIS, transferrin saturation. Except where indicated, $r$ values are significant at $P < 0.05$.

From a systems biology perspective, the ID diet altered PCA factor structures compared with the iron-adequate diet, recruiting at least one of the two iron storage depots (spleen, liver) to the first factor in both sexes. This is consistent with the fact that an iron-poor diet mobilizes iron availability from storage compartments. Exactly what physiological mechanisms account for the changes in the other PCA factor structures remain to be seen, but this observation may provide insight into understanding the pathophysiology of iron deficiency with or without anemia.

Principal Components Analysis and Candidate Genes

PCA has been shown to be useful for identifying apparent and latent composite variables that influence composite traits (29, 60). This is particularly valuable for genetic mapping of synthetic variables involved in biological systems, such as iron homeostasis. During the course of our QTL analysis, we identified 7 candidate genes within the loci detected. Moreover, we propose that polymorphisms in these genes at least partially underlie individual differences in the regulation of iron homeostasis and susceptibility to the effects of an iron-poor diet.

The expression of two genes, *Ptprd* and *Mdm1*, were significantly correlated with PCA factor 1 (hemoglobin and hematocrit) and PCA factor 2 (plasma Fe and TIBC), respectively in male mice fed the AD diet. *Ptprd* encodes for protein tyrosine phosphatase receptor type D, which is a key regulator in brain development, specifically in the process of neurogenesis (54) and migration of cortical interneurons (18). Genome-wide association studies in humans have revealed *PTPRD* as a risk factor for restless legs syndrome (RLS) (57, 74); however, the mechanism accounting for its role in RLS is currently unknown. One of the pathological characteristics of RLS is low iron content in the substantia nigra, and a significant percentage of patients with RLS are responsive to iron treatment (9, 13, 66). The single nucleotide polymorphism (SNP) density near *Ptprd* in BXD RI mice is high (3,476), thereby making it a good candidate underlying individual differences in iron regulation.

*Mdm1* encodes a nuclear protein, transformed mouse 3T3 cell double minute 1, and its involvement in iron homeostasis might be mediated by another gene *Ifng* (interferon gamma), which regulates iron homeostasis in inflammation. Indeed, *Mdm1* has been reported to be in linkage disequilibrium with *Ifng* on chromosome 10 among inbred mouse strains (65). During inflammation, *Ifng* activates macrophages (37) and regulates expression of the genes for TfRs (33), and the hormone hepcidin (20), resulting in alteration of macrophages iron recycling and iron metabolism (33, 61, 62). Depressions in plasma iron and in the ratio of plasma iron to TIBC are common signs of inflammation-related anemia (76). Notably, the expression of *Mdm1* was significantly correlated with these two iron parameters (PCA factor 2 loadings in AD males) in our study. Thus, we nominate *Mdm1* as a candidate gene involved in iron homeostasis and the polymorphism of *Mdm1* might give us a clue to better understand the crosstalk among iron homeostasis, predisposition to iron deficiency, inflammation, and anemia.

*Picalm* (phosphatidylinositol-binding clathrin assembly protein), and *Tcerg1* (transcription elongation regulator 1, also known as CA150) are the two candidate genes whose expression are significantly correlated with PCA factor 3 (loadings: transferrin saturation and liver Fe) in AD female mice. We hypothesize that these two genes are involved in iron homeostasis by regulating clathrin-mediated endocytosis (CME) of iron (transferrin) and the downstream cellular utilization of endocytosed iron. Erythorid cells acquire iron via CME of transferrin (71). *Picalm* is involved in clathrin-mediated endocytosis (CME) (64) and has recently been shown to be crucial in hematopoiesis and iron metabolism (34). Klebig et al. (34) reported that *Picalm* mutations resulted in truncated Picalm protein, which alters transferrin endocytosis and subsequent cellular iron uptake and iron transport from hemoglobin and

![Image](https://via.placeholder.com/150)

Table 5. Principal components analysis of the parameters by diet and sex

<table>
<thead>
<tr>
<th>Measure</th>
<th>AD Diet Males Factor</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Factor</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>AD Diet Females Factor</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Factor</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>AD Diet Sex Combined Factor</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>AD Diet Sex Combined Factor</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>TIs</td>
<td>0.833</td>
<td></td>
<td></td>
<td></td>
<td>0.864</td>
<td></td>
<td></td>
<td></td>
<td>0.865</td>
<td></td>
<td></td>
<td></td>
<td>0.837</td>
<td></td>
<td></td>
<td></td>
<td>0.969</td>
<td></td>
<td></td>
<td></td>
<td>0.787</td>
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<tr>
<td>Plasma Fe</td>
<td>0.983</td>
<td></td>
<td></td>
<td></td>
<td>0.958</td>
<td></td>
<td></td>
<td></td>
<td>0.931</td>
<td></td>
<td></td>
<td></td>
<td>0.641</td>
<td></td>
<td></td>
<td></td>
<td>0.526</td>
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<td></td>
<td></td>
<td>0.526</td>
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<tr>
<td>Hb</td>
<td>0.864</td>
<td>0.89</td>
<td>0.91</td>
<td>0.762</td>
<td>0.931</td>
<td>0.5</td>
<td>0.902</td>
<td>0.891</td>
<td>0.837</td>
<td>0.641</td>
<td>0.526</td>
<td>0.526</td>
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<tr>
<td>HCT</td>
<td>0.861</td>
<td>0.893</td>
<td>0.56</td>
<td>0.727</td>
<td>0.81</td>
<td>0.746</td>
<td>0.881</td>
<td>0.855</td>
<td>0.65</td>
<td>0.922</td>
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<tr>
<td>TIBC</td>
<td>0.628</td>
<td>0.72</td>
<td>0.851</td>
<td>0.868</td>
<td>0.789</td>
<td>0.751</td>
<td>0.567</td>
<td>0.821</td>
<td>0.821</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>Spleen Fe</td>
<td>0.815</td>
<td>0.72</td>
<td>0.851</td>
<td>0.868</td>
<td>0.789</td>
<td>0.751</td>
<td>0.567</td>
<td>0.821</td>
<td>0.821</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>Liver Fe</td>
<td>0.625</td>
<td>0.696</td>
<td>0.681</td>
<td>0.789</td>
<td>0.751</td>
<td>0.567</td>
<td>0.567</td>
<td>0.821</td>
<td>0.821</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>% of Total</td>
<td>33.24</td>
<td>23.74</td>
<td>21.59</td>
<td>39.52</td>
<td>34.16</td>
<td>24.06</td>
<td>36.41</td>
<td>24.68</td>
<td>24.62</td>
<td>34.51</td>
<td>20.67</td>
<td>23.54</td>
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</tbody>
</table>

This analysis was performed on the strain means for each of the measures and the correlation matrix evaluated by varimax rotation.
the liver to other tissues. This is consistent with our PCA/QTL finding that Picalm expression variation among strains contributed to strain variation in transferrin saturation and liver Fe concentration in AD females; moreover, the SNP density of Picalm in BXD mice is 300.

Tcerg1 might be involved in CME of iron (likely by interacting with another protein, Htt). Htt, coding for huntingtin, is the causative gene in Huntington’s disease, and the disruption of iron homeostasis has been well characterized in Huntington’s disease (28, 48). Both in vivo and in vitro experiments show that the WW domain containing protein Tcerg1 can interact with Htt (27). Dysfunction of Tcerg1 causes binding and coaggregation with Htt in striatal and cortical neurons as Huntington’s disease progresses and Tcerg1 overexpression...
can rescue striatal cell death when Htt mutations exist (4). Htt is ubiquitously expressed throughout the body with its exact physiological function unclear. Recent Htt knockout studies in zebrafish showed that Htt deficiency causes a variety of developmental defects, including abnormal iron homeostasis with evidence of reduced hemoglobin production, increased transferrin receptor 1 transcription, and decreased accessibility of endocytosed iron for cellular use (24, 45). Even though there is no evidence so far showing the involvement of Tcerg1 or the interaction between Tcerg1 and Htt in iron homeostasis, nevertheless, we believe that to rule out Tcerg1 at this time may be premature.

Two candidate genes were identified to account for PCA factor 2 (loadings: transferrin saturation and plasma iron concentration) in ID female mice: Skp2 (S-phase kinase-associated protein 2) and Lip1 (lysosomal acid lipase 1). Skp2 is an F-box-containing ubiquitin ligase required for p21 (cyclin-dependent kinase inhibitor 1A) proteolysis, which controls the cell cycle G1/S transition, including during hematopoiesis (3, 56). Moreover, it has recently been reported that iron depletion alters p21 expression and blocks cells at the G1/S interface, subsequently inhibiting hematopoiesis (1, 22, 38). Thus, polymorphisms of Skp2 could alter p21 proteolysis, thereby altering hematopoiesis and causing abnormal iron metabolism as a consequence. Lip1 knockout mice have been shown to develop macrophage malfunction (73), considering the crucial role of macrophages in iron regulation (35, 67); we thus nominate Lip1 as the candidate gene.

The candidate gene identified for factor 3 (loading: hemoglobin) in ID female mice is Frzb, a secreted frizzled-related protein that antagonizes Wnt signaling by regulating Wnt5a activity (15, 41, 42). The coordination between Frzb and Wnt5a has been shown to play an important role in inner ear development (41), and cancer progression (15, 36, 59). No effect of Frzb or Wnt5a on iron homeostasis has yet to be reported. Wnt5a signaling has, however, been shown to be important in erythropoiesis during development (65). Considering the relationship between erythropoiesis and iron regulation (72, 8), we nominated Frzb as the candidate gene.

**Perspectives and Significance**

Forward genetic analysis of complex traits in genetic reference populations of animals has opened the inquiry into polygenic influence on important biological functions. QTL mapping is a valuable resource for elucidating the mechanisms underlying individual differences in iron biology. Recently, McLachlan et al. (46) identified several candidate genes related to strain differences in basal liver iron inbred mice using haplotype type analysis. Iron deficiency and its consequences constitute a major public health problem worldwide. We now report that individual differences in susceptibility to its effects are driven, in part, by genetics, and we have identified candidate genes that may mediate this susceptibility. While our bioinformatics analysis points to specific candidate genes, functional validation of these findings in animals will be needed to pinpoint their involvement in iron regulation. One major implication of this work is the promise of follow-up on human genome-wide association studies. One such study was reported by McLaren et al. (47). Considering that the mouse and human genomes are more than 90% syntenic, what we find in humans can inform the mouse genome and help us to elucidate biological mechanisms involved in individual differences in susceptibility to iron deficiency.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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**Table 6. Candidate genes by principal component**

<table>
<thead>
<tr>
<th>Diet, Sex, PCA Factor</th>
<th>Chr; Location (Mb)</th>
<th>LRS Score</th>
<th>Candidate Gene</th>
<th>Gene Expression Profile Showing cis Regulation</th>
<th>Gene Expression Profile of Correlation with the Factor</th>
<th>Correlation (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate Fe, Males, PCA factor 1</td>
<td>4 (77.85)</td>
<td>7.56</td>
<td>Ptprda&lt;sup&gt;a&lt;/sup&gt;</td>
<td>UNC Agilent G412A Liver LOWESS Stanford (Jan06) Both Sexes, IoP Affy MOE 430v2 Spleen (May09) RMA</td>
<td>Iop Affy MOE 430v2 Spleen (May09) RMA</td>
<td>−0.626</td>
</tr>
<tr>
<td>Adequate Fe, Males, PCA factor 2</td>
<td>10 (117.58)</td>
<td>11.94</td>
<td>Mdm&lt;sub&gt;b&lt;/sub&gt;</td>
<td>IoP Affy MOE 430v2 Spleen (May09) RMA</td>
<td>UMCG Progenitor Cells</td>
<td>−0.613</td>
</tr>
<tr>
<td>Adequate Fe, Males, PCA factor 3</td>
<td>7 (97.28)</td>
<td>15.07</td>
<td>Pical&lt;sub&gt;c&lt;/sub&gt;</td>
<td>UNC Agilent G412A Liver LOWESS Stanford (Jan06) Both Sexes, IoP Affy MOE 430v2 Spleen (May09) RMA</td>
<td>Iop Affy MOE 430v2 Spleen (May09) RMA</td>
<td>−0.66</td>
</tr>
<tr>
<td>Deficient Fe, Females, PCA factor 2</td>
<td>18 (42.67)</td>
<td>11.80</td>
<td>Tcerg&lt;sub&gt;b&lt;/sub&gt;</td>
<td>IoP Affy MOE 430v2 Spleen (May09) RMA</td>
<td>IoP Affy MOE 430v2 Spleen (May09) RMA</td>
<td>0.694</td>
</tr>
<tr>
<td>Deficient Fe, Females, PCA factor 3</td>
<td>15 (9.04)</td>
<td>13.46</td>
<td>Skp2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>IoP Affy MOE 430v2 Spleen (May09) RMA</td>
<td>UMCG Stem Cells ILM6v1.1</td>
<td>−0.584</td>
</tr>
<tr>
<td>Deficient Fe, Females, PCA factor 3</td>
<td>19 (34.56)</td>
<td>12.22</td>
<td>Lip&lt;sub&gt;b&lt;/sub&gt;</td>
<td>IoP Affy MOE 430v2 Spleen (May09) RMA</td>
<td>UNC Agilent G412A1 Liver LOWESS Stanford (Jan06) Females</td>
<td>0.587</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant cis regulation. <sup>b</sup>Suggestive cis regulation. <sup>c</sup>Significant cis regulation. <sup>d</sup>Suggestive cis regulation.
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GENETIC ANALYSIS OF IRON DEFICIENCY

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