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Quantitative Genetic Analysis of Ventral Midbrain and Liver Iron in BXD Recombinant Inbred Mice

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Male and female mice from 15 of the BXD/Ty recombinant inbred strain panel were examined for regional brain and liver iron content. Brain regions included medial prefrontal cortex, nucleus accumbens, caudate-putamen and ventral midbrain. Our focal tissue was the ventral midbrain, containing the ventral tegmentum and substantia nigra. This area contains the perikarya of the dopamine neurons that project to nucleus accumbens and caudate-putamen. Genetic correlations between ventral midbrain and liver iron content were not statistically significant, suggesting that peripheral and central iron regulatory systems are largely independent. Correlations between ventral midbrain iron and iron in the caudate-putamen and nucleus accumbens, but not the prefrontal cortex were moderately high and significant. Ventral midbrain and liver iron contents were subjected to quantitative trait loci analysis to identify associated chromosomal locations. This analysis revealed several suggestive loci for iron content in ventral midbrain but fewer loci for liver. Genetic correlations between ventral midbrain iron and published dopamine functional indices were significant, suggesting a link between ventral midbrain iron status and central dopamine neurobiology. This work shows the value of quantitative genetic analysis in the neurobiology of iron and in showing the close association between ventral midbrain iron and nigrostriatal/mesolimbic dopamine function.

Keywords: Iron; Dopamine; Brain; Genetics; Liver; QTL

INTRODUCTION

Our interest in brain iron regulation stems from extensive work conducted by ourselves (Morse *et al.*, 1999; Erikson *et al.*, 2000; 2001; Beard *et al.*, 2002; 2003) and by others (Youdim, 2001) showing that iron deficiency early in life impairs the functioning of nigrostriatal and mesolimbic dopamine systems. Moreover, we propose that iron concentration in the ventral midbrain is the key to understanding how iron and dopamine are related in development and in neurodegeneration. We have shown that iron deficiency early in life alters the course of expression of dopamine receptors, including the transporter in the caudate-putamen and in the nucleus accumbens. Postweaning iron deficiency results in decreased density in the D_2 receptor, followed by decreased densities in the dopamine transporter and D_1 receptor (Erikson *et al.*, 2000; 2001; Beard *et al.*, 2002). Preweaning iron deficiency produces the same effects, but more persistent for the D_1 receptor and dopamine transporter; however, preweaning iron deficiency causes an increase in D_2 receptor density in the projection areas (Beard *et al.*, 2003). Dopamine-related behaviors are also affected by preweaning iron deficiency and the effects may be permanent (Piñero *et al.*, 2001). The cell bodies, and hence the synthetic machinery for production of presynaptic (D_2) dopamine receptors and the dopamine transporter for the caudate-putamen and nucleus accumbens lie in the ventral midbrain.

The substantia nigra is also a target of the study of neurodegenerative disease, especially Parkinson's disease. In this condition, neuronal cell bodies die and the projection areas from these dopaminergic neurons lose function, with grave consequences for movement and sometimes, affect (Ueckermann *et al.*, 2003). In this instance, the author suggests that iron is a putative neurotoxin. Excess iron accumulation in

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the substantia nigra overwhelms the protective mechanisms and ferrous iron, through the Fenton reaction produces toxic free radicals that destroy the neuronal soma.

It is our intent to show that iron regulation in the ventral midbrain is a complex trait, i.e. involves multiple genes. Early in development, under normal conditions, the substantia nigra contains little iron; however, at a later developmental stage, the substantia nigra begins to accumulate iron and in adulthood becomes the brain nucleus with the highest iron content (Connor *et al.*, 1990). The mechanisms of iron accumulation in the midbrain are largely unknown, however, we assert that genetic analysis from a complex trait perspective will elucidate the relevant genes and mechanisms. In an earlier study, Leboeuf *et al.* (1995) showed wide, genetic-based variability in serum iron among inbred mouse strains and, using genetic correlation between hepatic iron concentration and transferrin saturation, that these two measures were largely unrelated. This demonstrates the utility of genetically-defined animals to show polygenic influence on traits and to examine associations among traits.

Genetic dissection of complex traits using recombinant inbred strains of rats and mice has yielded valuable information about brain-behavior relationships and common genetic links for more than two decades (Blizard and Bailey, 1979). Another technique that can point to specific genes affecting one or more phenotypes is quantitative trait loci (QTL) analysis. In this approach one can index nucleic acid polymorphisms throughout the genome and correlate those polymorphisms with behavioral or biological phenotypes. This approach has proven successful as a first step to identifying polygenes affecting, for example, hippocampal neurobiology (Lassalle *et al.*, 1994), alcohol pharmacology (Rodriguez *et al.*, 1994; Buck *et al.*, 2002) and cocaine pharmacogenetics (Phillips *et al.*, 1998; Jones *et al.*, 1999), dopamine neurochemistry (Jones *et al.*, 1999; Hitzemann *et al.*, 2003) immunology (Pereira *et al.*, 1997; Hsu *et al.*, 2002) haloperidol sensitivity (Kanes *et al.*, 1996) and other phenotypes.

In this paper, we report genetic correlational and QTL analyses of iron content in the ventral midbrain and in liver in 15 strains of the well-known BXD/Ty recombinant inbred (RI) strain panel. This panel was chosen because the parental strains, C57BL/6 and DBA/2 are known to differ in nearly every phenotype measured related to neurobehavioral function and because they are the most densely mapped panel for chromosomal polymorphisms. Our two major hypotheses were that (1) iron accumulation in the ventral midbrain is a complex trait and involves many genes; and (2) iron accumulation in the ventral midbrain is independent of iron accumulation in the liver. Finally, we show that iron concentration in the ventral midbrain is associated with dopamine function.

MATERIALS AND METHODS

Animals

Male and female mice from 15 of the BXD/Ty RI panel were used in this study. All had been reared and maintained under standard laboratory conditions with a constant light–dark cycle (06:00–18:00, on–off), temperature and relative humidity at 21 ± 2°C and 35%, respectively. All were fed Purina rodent diet #5010 (equivalent to Purina diet #5001) containing not less than 4.5% fat and 240 ppm iron. Food and water were available *ad libitum*. The average age of the animals was approximately 120 days with a range of 90–150 days. All strains and both sexes were represented by between 4 and 9 animals, except for RI#29 with two for the males.

Tissue Harvest

All animals were killed by CO_2 suffocation and placed on ice until the brain was removed within 6 h. The brains were dissected into medial prefrontal cortex (PFC), caudate-putamen (CP), nucleus accumbens (NA) and ventral midbrain (VMB) as described by Boone *et al.* (1997). The VMB was dissected by making a 45° from vertical, rostral-caudal, cut from the posterior end of the hypothalamus (facing up). Next, a true vertical cut was made also at the same place. The resulting wedge of tissue was bisected horizontally at the aqueduct and the dorsal portion discarded. The part retained thus, contains the substantia nigra and ventral tegmentum, our areas of primary interest. Additionally, a 200–300 mg piece of the liver was harvested.

Iron Assay

Brain region iron concentrations were measured according to the modified procedures of Erikson *et al.* (1997). Briefly, each brain region was weighed and combined with 200 µl of ultrapure nitric acid (OmiTrace®, EM Science, NXO407-1) in a 0.5 ml polypropylene micro-centrifuge tube. Brain regions were digested for 48 h in a 60°C sand bath and then re-suspended to 400 µl with nanopure water. Each sample was further diluted 1:50 (1:100 for ventral midbrain) with 0.2% ultrapure nitric acid and immediately analyzed for iron by graphite furnace atomic absorption spectrophotometry (Perkin Elmer AAnalyst 600, Perkin Elmer, Norwalk, CT). Standards were prepared by diluting a Perkin

Elmer iron standard (PE#N9300126) in 0.2% ultrapure nitric acid and blanks prepared with digesting and diluting reagents to control for possible contamination. Liver non-heme iron was determined by the standard colorimetric technique described by Bothwell *et al.* (1979), with ferrozine as the color reagent.

Data Analysis

All iron contents by tissue, strain and sex were reduced to means and standard errors of the mean (s.e.m.) for genetic correlational analysis and data presentation. Analysis of variance for a two between-subjects variables (strain and sex) experiment was performed on the raw data followed by estimated omega squared to estimate the proportion of variance (or broad sense heritability) in VMB and liver iron attributable to genetic makeup. Genetic correlations were calculated by Pearson's r using strain means as the "raw" data (Blizard and Bailey, 1979). The means were then submitted to webQTL (Williams *et al.*, 2001) for QTL analysis and for more correlational analysis using other published data available at http://webqtl.org/. We report QTL with point-biserial correlations with $p < 0.01$ or less. We are well aware of the type 1 error rate problem attendant with multiple comparisons in QTL analysis as described by Belknap *et al.* (1996) and with the conservative approach using LOD score criteria advocated by Lander and Bottstein (1989). Our choice to use the p 0.01 level is based on the fact that we had only 15 of the strains to work with, and hence, had limited power (Belknap *et al.*, 1996)

RESULTS

Tissue Iron Contents

Mean tissue iron contents for PFC, CP, NA, VMB and liver are presented in Figs. 1–5, respectively. The continuous distribution of the means for each tissue is consistent with additive genetic variation, i.e. polygenic influence. The distribution of means

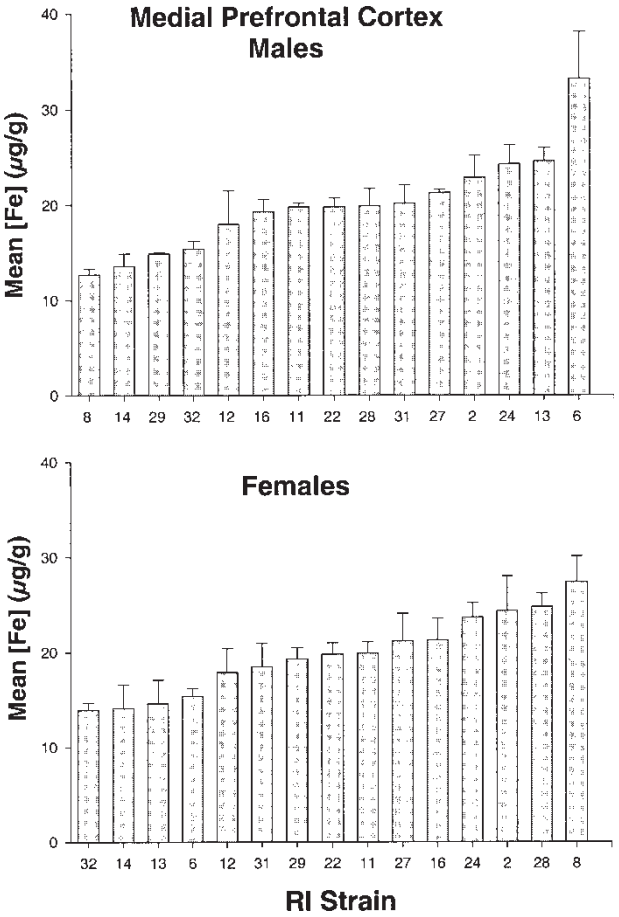

FIGURE 1 Mean iron concentrations (±s.e.m) in the medial prefrontal cortex of male (top panel) and female (bottom panel) BXD/Ty recombinant inbred mice. $N = 4–9$ per strain except for strain 29 male with $n = 2$. Iron concentrations were normalized to tissue wet weights.

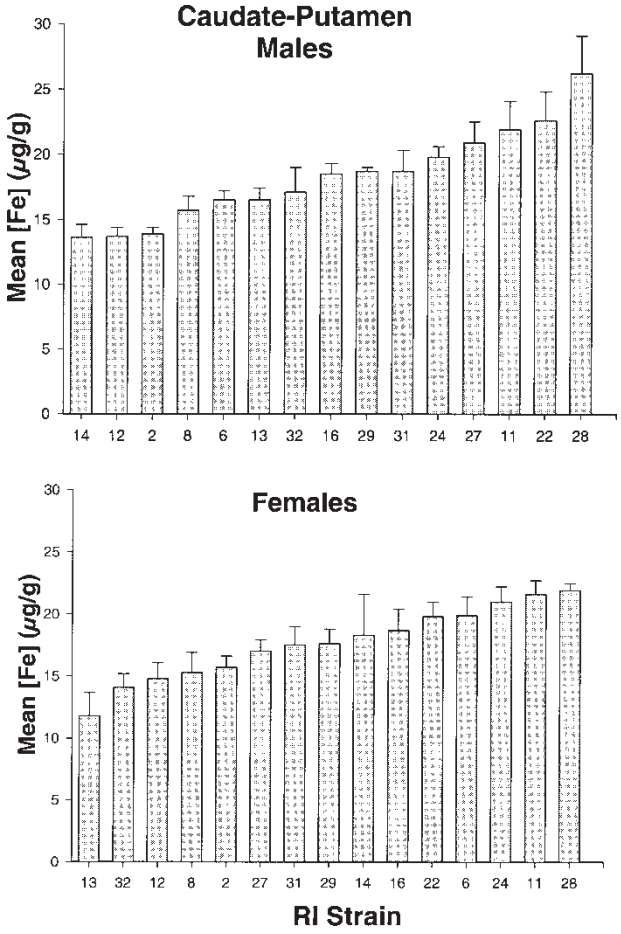

FIGURE 2 Mean iron concentrations (±s.e.m) in the caudate-putamen of male (top panel) and female (bottom panel) BXD/Ty recombinant inbred mice. $N = 4–9$ per strain except for strain 29 male with $n = 2$. Iron concentrations were normalized to tissue wet weights.

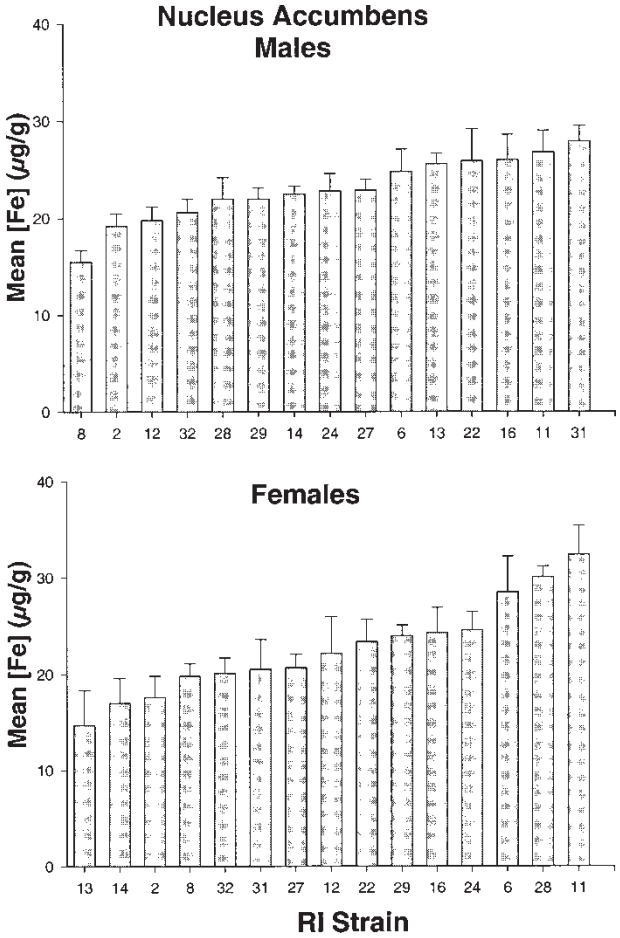

FIGURE 3 Mean iron concentrations (±s.e.m) in the nucleus accumbens of male (top panel) and female (bottom panel) BXD/Ty recombinant inbred mice. $N = 4$–9 per strain except for strain 29 male with $n = 2$. Iron concentrations were normalized to tissue wet weights.

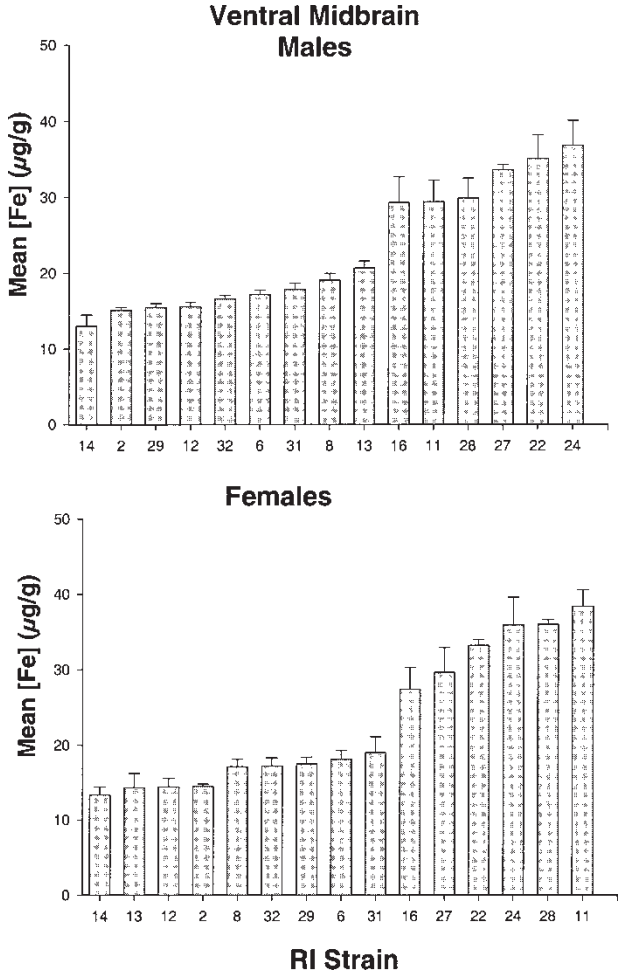

FIGURE 4 Mean iron concentrations (±s.e.m) in the ventral midbrain of male (top panel) and female (bottom panel) BXD/Ty recombinant inbred mice. $N = 4$–9 per strain except for strain 29 male with $n = 2$. Iron concentrations were normalized to tissue wet weights.

for the VMB, however show a discontinuity that would be consistent with one or possibly a few genes exerting a major influence (see below). Analysis of variance revealed that for the PFC, there was a significant effect of strain ($F_{14,186} = 2.73$, $p < 0.001$), no significant effect of sex ($F < 1$) and a significant strain by sex interaction ($F_{14,186} = 5.31$, $p < 0.001$). In the NA, the only significant factor was strain ($F_{14,193} = 6.94$, $p < 0.001$). As with the NA, iron content in the CP varied only by strain ($F_{14,191} = 2.57$, $p < 0.002$). In the VMB, there was a significant effect of strain ($F_{14,184} = 31.56$, $p < 0.001$). The effect of sex was not significant ($F_{1,184} < 1$) and neither was the strain by sex interaction ($F_{14,184} = 1.357$, $p > 0.10$). In fact, there was remarkable consistency between the sexes in VMB iron content. In the liver, iron concentrations were found to vary significantly by strain and sex and there was also a significant strain by sex interaction ($F_{14,182} = 6.38$, $p < 0.001$; $F_{1,182} = 56.57$, $p < 0.001$; $F_{14,182} = 2.30$, $p < 0.01$, respectively). In general, females showed 40% higher concentrations of liver iron than did males (165 vs. 118 $\mu g\, g^{-1}$); therefore, we conducted heritability estimates for the sexes separately.

Genetic Correlational Analysis and Heritability

We observed Pearson correlation coefficients of -0.29 and -0.47 for males and females, respectively, between VMB and liver iron content. While these correlations are suggestive, they are not statistically significant. We also observed robust correlations of 0.74 and 0.61 ($p < 0.05$ each) between VMB and CP iron contents for males and females and 0.53 and 0.62 ($p < 0.05$ each), respectively between VMB and NA iron contents. Correlations between VMB and MPFC for iron content were 0.13 and 0.25 for males and females, respectively and are not statistically significant. Heritability estimates for iron content in the FC, NA, CP and VMB were 0.08, 0.27, 0.09 and 0.66, respectively and in the liver, 0.50 and 0.22, for males and females, respectively.

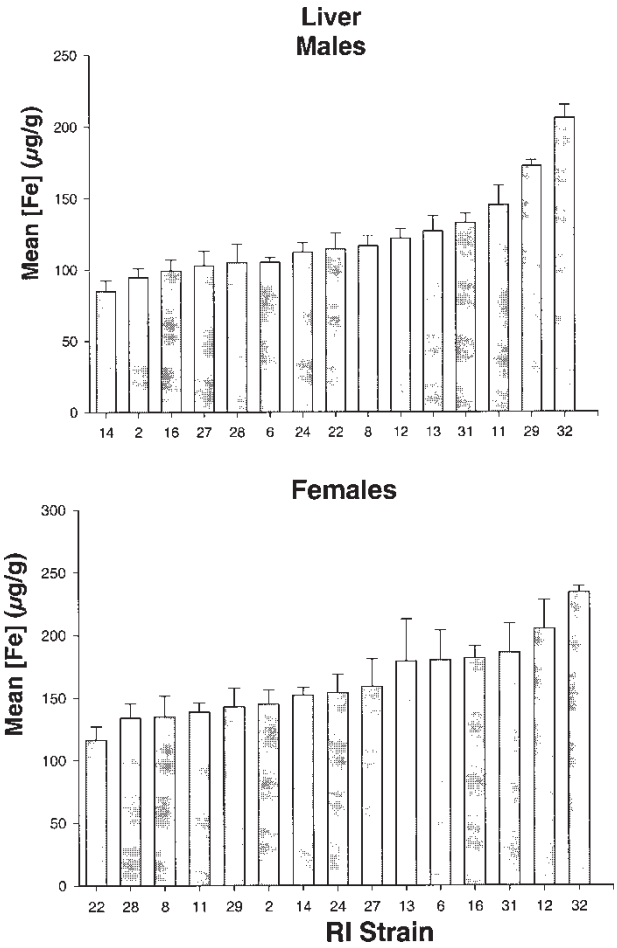

FIGURE 5 Mean iron concentrations (± s.e.m) in the liver of male (top panel) and female (bottom panel) BXD/Ty recombinant inbred mice. $N = 4–9$ per strain except for strain 29 male with $n = 2$. Iron concentrations were normalized to tissue wet weights.

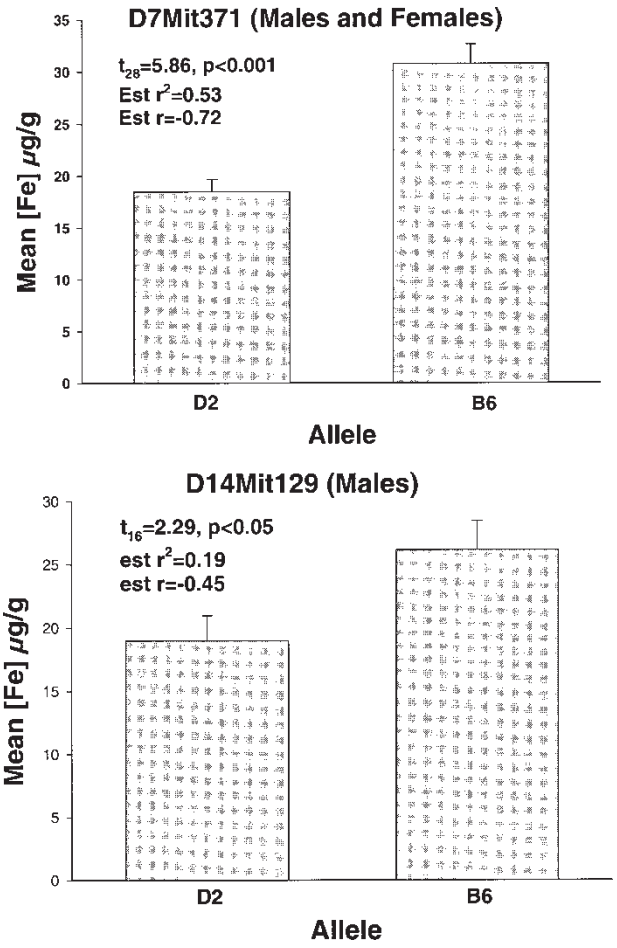

FIGURE 6 Top panel. Haplotype analysis of iron concentration in the ventral midbrain (mean ± s.e.m.) for the marker, D7Mit371, for male and female mice from the BXD/Ty recombinant inbred panel. Segregation is by those strains homozygous for alleles originating from the C57BL/6 (B6) strain and the DBA2 (D2) strain. The bottom panel is a haplotype analysis as above for the D14Mit129 marker, but only for males.

Quantitative trait loci analysis. The results of QTL analysis for VMB iron content are presented in Table I and the results of QTL analysis for liver iron content are presented in Table II. For VMB iron content, we identified 11 regions with associated nucleotide polymorphisms on chromosomes $1, 2, 5, 7, 9, 11, 13, 14, 17$ and 18. All, except for one region on chromosome 13 and one region on chromosome 14 were sex-concordant. The most salient markers for VMB iron content were seen for both sexes on chromosome 7 and for the males, one on chromosome 14. Figure 6 presents a haplotype analysis of the chromosome 7 marker for male and female mice in the top panel and the same for males for the chromosome 14 marker. For mice of both sexes carrying the C57BL/6 allele, VMB iron content was nearly twice that observed in the strains carrying the DBA/2 allele ($t_{28} = 5.86$, $p < 0.001$). For the marker on chromosome 14, males carrying the C57BL/6 allele showed about a 25% greater VMB iron content compared to those carrying the DBA/2 allele ($t_{28} = 2.29$, $p < 0.05$).

For the liver, we identified regions on chromosomes 2, 3, 6, 14 and X, but with only the regions on 2 and X being sex-concordant.

Genetic Correlations between VMB Iron Content and Published Phenotypes in the BXD RI Panel

Table III presents correlations between VMB iron content and other phenotypes measured by others and compiled by Williams *et al.* (2001). In this table, we list only those phenotypes related to dopamine actions. As is shown in the table, VMB iron concentrations correlate highly and negatively with spontaneous activity, (Demarest *et al.*, 1999; Buck *et al.*, 2000; Cunningham, 1995), low-dose cocaine (Phillips *et al.*, 1998). Also correlated with VMB iron concentration are haloperidol-induced catalepsy (Kanes *et al.*, 1996) and quinpirole-related decrease in activity (Buck *et al.*, 2000).

TABLE I QTL analysis of iron content in the ventral midbrain of 15 BXD/Ty recombinant inbred mouse strains

Marker	CHR	MB	r_f	p	r_m	p
D1Mit425	1	157.741	0.63	0.0096	0.66	0.0066
D2Mit5	2	9.402	−0.62	0.0110	−0.63	0.0101
D5Mit79	5	43.013	0.66	0.0059	0.60	0.0166
D7Mit371	7	122.235	−0.76	0.0006	−0.70	0.0026
S07Gnf129.970	7	123.174	−0.66	0.0057	−0.58	0.0210
D7Mit246	7	22.611	−0.63	0.0097	−0.70	0.0027
D9Mit21	9	58.220	−0.68	0.0038	−0.70	0.0029
D9Mit104	9	66.515	−0.68	0.0038	−0.70	0.0029
D11Mit104	11	77.596	−0.70	0.0024	−0.67	0.0051
D11Mit245	11	77.596	−0.70	0.0024	−0.67	0.0051
S11Gnf085.885	11	79.889	−0.70	0.0024	−0.67	0.0051
D11Mit131	11	56.647	−0.59	0.0187	−0.69	0.0036
S11Gnf059.515	11	58.283	−0.59	0.0187	−0.69	0.0036
D11Mit86	11	56.647	−0.59	0.0187	−0.69	0.0036
D11Mit154	11	52.459	−0.59	0.0187	−0.69	0.0036
D11Mit308	11	42.801	−0.59	0.0201	−0.61	0.0147
D11Mit296	11	43.221	−0.59	0.0201	−0.61	0.0147
D13Mit196	13	110.358	−0.73	0.0014	–	–
S13Gnf003.255	13	5.381	−0.69	0.0034	−0.68	0.0038
D14Mit214	14	41.753	–	–	−0.72	0.0015
D14Mit140	14	43.715	–	–	−0.72	0.0015
D14Mit121	14	40.474	–	–	−0.72	0.0015
D14Mit62	14	43.155	–	–	−0.72	0.0015
D14Mit129	14	38.206	–	–	−0.72	0.0015
D17Mit11	17	40.213	0.73	0.0014	0.64	0.0092
D17Mit49	17	44.331	0.63	0.0110	0.61	0.0133
D17Mit136	17	43.257	0.63	0.0110	0.61	0.0133
D17Mit139	17	51.712	0.56	0.0289	0.59	0.0199
D17Mit20	17	56.174	0.56	0.0289	0.57	0.0232
D17Mit89	17	62.749	0.59	0.0177	0.62	0.0122
D18Mit31	18	11.064	0.58	0.0209	0.65	0.0075
S18Gnf008.065	18	10.907	0.58	0.0209	0.65	0.0075

CHR, chromosome number; MB, distance from centromere in millions of base pairs (2 MB ≈ 1cMorgan); r_f, point-biserial correlation coefficient between the marker (B6 = 0, D2 = 1) and iron content for females; r_m, r_{pb} for males.

DISCUSSION

The role of iron in the brain has been the subject of study for a number of years. Iron deficiency during development alters the course of neurobehavioral development (Erikson *et al.*, 2000; 2001; Piñero *et al.*, 2001; Beard *et al.*, 2002; 2003) and iron deficiency in late adulthood has been implicated in movement disorders and neuroleptic malignant syndrome (Lee, 1998; Allen *et al.*, 2001). Moreover, iron overload in adulthood has been shown to participate in neurodegenerative disorders (Faucheux *et al.*, 2002; Sipe *et al.*, 2002) and diseases of excess iron in tissues, for example, hemochromatosis have associated neurological and psychiatric disorders (Cutler, 1994; Feifel and Young, 1997). It is evident therefore that proper iron regulation is critical for normal CNS development and functioning. Indeed, there are many proteins that regulate iron availability, transport, packaging, etc. and in turn, there are proteins whose expression is dependent on iron (Siddappa *et al.*, 2003). The role of genetics in the management of iron to date has been examined largely through the use of genetically altered animals, involving amplified (for example, Saleh *et al.*, 2003) and nullified genes (for example, Patel *et al.*, 2002; Grabill *et al.*, 2003). Also, these single-gene models have been directed mostly toward diseases of iron overload, namely hemochromatosis and hemosiderosis. Others have revealed genes for proteins involved in iron transport and packaging (Roy and Andrews, 2001). Despite these advances, however, there remain iron-related syndromes that are

TABLE II QTL analysis of liver iron content in 15 BXD/Ty recombinant inbred mouse strains. Columns 4 and 6 contain the point-biserial correlation coefficients for males and females, respectively, with the p values in adjacent columns

Marker	CHR	MB	r_f	p	r_m	p
D2Mit5	2	9.402	.61	.02	.51	.05
D2Mit412	2	163.258	.70	.003	–	–
D2Mit51	2	163.921	.70	.003	–	–
D2Mit411	2	160.468	.67	.007	–	–
D3Mit12	3	100.566	−.70	.003	–	–
D3Mit189	3	101.078	−.63	.009	–	–
D6Mit301	6	136.396	–	–	.71	.003
D6Mit374	6	139.296	–	–	.71	.003
D6Mit13	6	132.918	–	–	.69	.004
D6Mit194	6	132.000	–	–	.69	.004
S06Gnf140.060	6	140.458	–	–	.68	.005
D6Mit254	6	131.080	–	–	.66	.007
D14Mit185	14	108.241	−.65	.008	–	–
DXMit68	X	38.941	.67	.005	.41	.02
DXMit144	X	47.122	.67	.005	.41	.02

TABLE III Genetic correlational analysis of VMB iron content and published phenotypes using the BXD/Ty recombinant inbred mouse strains

PHENOTYPE	*r*	*p*	Reference
Locomotor activity following saline injection	−.79	.0002	Buck *et al.*, 2000
Quinpirole-related decrease in locomotion	.75	.0011	Buck *et al.*, 2000
Locomotor activity	−.74	.0025	Cunningham, 1995
Locomotor activity following saline injection	−.71	.0032	Demarest *et al.*, 1999
Cocaine-induced hyperlocomotion	−.60	.0156	Phillips *et al.*, 1998
Haloperidol-induced catalepsy	−.51	.0487	Kanes *et al.*, 1996
Dopamine D2 receptor mRNA	.68	.0041	webQTL.org

idiopathic and not yet tied to specific genetic or environmental causes.

The results of our regional iron analysis in brain and in the liver show that iron concentration in each of the tissues is continuously distributed, which is in agreement with polygenetic influence on each. Of all of the tissues examined, the VMB showed the greatest impact of strain on iron concentration. The VMB, however showed a discontinuity, related to the marker on chromosome 7 and observed in both sexes. Genetic correlational analysis of iron contents show that VMB iron content correlates highly with the iron content in its projection areas, caudate-putamen and nucleus accumbens, but that the correlation with iron content in the PFC does not differ significantly from zero. Also, the correlation between liver iron and VMB iron was low and not significant, suggesting that iron regulation in the liver and in the VMB are largely independent. This point is important because in men and women with restless legs syndrome, the clinical picture is of normal hematocrit/hemoglobin, but magnetic resonance imaging and postmortem examination show a deficiency of iron in the substantia nigra. Thus, peripheral iron status under otherwise normal conditions may not be informative of iron status in the VMB. Of course, this could change under conditions of dietary iron deficiency or in the case of iron overload.

The focus of our research is on iron management in the VMB because this region, containing both the substantia nigra and ventral tegmentum contains the cell nuclei that produce the dopamine autoreceptors and transporters for the corpus striatum and mesolimbic projection areas. Recent work from our laboratory (Beard *et al.*, 2003) and earlier work by others (Youdim and Ben Shachar, 1987) has shown conclusively that iron deficiency during development alters the expression of dopamine receptors and the transporter in the caudate-putamen and nucleus accumbens in rats. Because the nuclei that contain the synthetic machinery for presynaptic receptors/transporter for these areas reside in the ventral midbrain, we propose that the link between iron and dopamine receptor expression in the projection areas can be tied directly to iron regulation in the ventral midbrain. This hypothesis is supported by the correlational evidence presented in Table III, especially the association between VMB iron status and dopamine pharmacology and expression of dopamine D_2 receptor mRNA (see below).

What can the QTL analysis tell us about possible candidate genes affecting VMB iron concentration? Our most interesting marker, D7Mit371 "explains" 53% of the variance in VMB iron among the RI strains and it is clear that there other QTL involved in smaller-scale differences as well. In a search for plausible genes nearby, *th*, tyrosine hydroxylase was found at 133.327 MB and within 12 MB of the marker. This is of interest, because iron is known to be a cofactor for this enzyme (Fitzpatrick, 1989), the rate-limiting enzyme for catecholamine synthesis. All of the known "housekeeping" protein genes, i.e. for ferritin, iron response proteins iron response elements, transferrin and transferrin receptor are located on different chromosomes in the mouse. There are two possibilities to explain this apparent paradox. The first is that the majority of genes have yet to be identified, despite the fact that the mouse genome sequence is now complete. Most of the genes are anonymous at this time and it is possible that there is an important gene/protein that regulates iron in the VMB that has yet to be named and characterized. The other possibility is that the QTL is near a gene that regulates one or more genes involved in management of iron in the VMB, i.e. the influence, while large, is indirect. As Kanes *et al.* (1996) asserted, much of the business of managing complex traits such as expression of neurotransmitter receptors is attributable to genetic actions at locations other than the structural genes for the receptors.

Chromosome 11 is interesting because the QTL identified are near the genes encoding the GABA-A receptor subunit alpha 1 the GABA-A receptor subunit gamma 2, and the adrenergic receptor, alpha 1b receptor. The QTL identified on chromosome 9 is interesting because it is near the gene encoding the dopamine D_2 receptor and near the QTL identified by Buck *et al*. (2000) that affects quinpirole sensitivity.

More evidence that VMB iron concentration is related to dopamine function comes from

the associated phenotypes listed in Table III. Sensitivity to the actions of quinpirole and haloperidol activities are related to VMB iron. Quinpirole is a D_2 receptor agonist while haloperidol is a D_2 receptor blocking agent. Williams *et al.* (2001) also performed a microarray analysis of mRNA from the brains of the BXD RI panel. When VMB iron was matched with the database, UTHSC Brain mRNA U74Av2, correlations of 0.57 and 0.68 with the D_2 receptor were observed for males and females, respectively. The correlation for the males was significant and is important because all of the mRNA used in the database was from females.

QTL analysis of liver iron presents a very different picture. We obtained far fewer QTL for this measure and there was very little overlap between the sexes. For the females, the markers on chromosome 2 at 160 + MB are close to the ferritin light chain gene. No obvious candidates were identified on the other chromosomes.

Our findings of consistent and significant negative correlations between VMB iron concentration and spontaneous activity merits discussion. People who have restless legs syndrome are also deficient in iron in the substantia nigra (Allen *et al.*, 2001). Iron deficiency, we propose, compromises the activity of D_2 receptors (probably presynaptic) in the corpus striatum, and this in turn increases spontaneous and "unwanted" movements. Our earlier work in rats shows that severe iron deficiency during development also impairs D_2 receptor activity and causes markedly decreased spontaneous activity (Piñero *et al.*, 2001; Beard *et al.*, 2002; 2003). In the case of severe iron deficiency, in addition to the D_2 receptors being affected, the dopamine transporter and D_1 receptors are affected as well (Beard *et al.*, 2003). Thus, from a developmental perspective, we believe that iron deficiency in the brain can produce quite different effects, depending upon the developmental timing.

The results from this study suggest that iron regulation in the VMB is a complex trait, i.e. it is influenced by several genes. Moreover, under normal conditions, this regulation is largely independent from iron regulation in the liver. Genetic correlational analysis shows that iron concentration in the VMB is highly related to iron concentration in its immediate projection areas, caudate-putamen and nucleus accumbens. QTL analysis shows that among the many possible candidates, there is remarkable concordance between males and females, a condition that is not seen in the liver. Finally, and most importantly, genetic correlational analysis with published dopamine-related phenotypes and with mRNA expression data, shows that VMB iron and dopamine function are closely related. We anticipate that our future work will show the genetic-based mechanisms that regulate iron in the VMB.

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