Genetic Analysis Reveals Polygenic Influences on Iron, Copper, and Zinc in Mouse Hippocampus With Neurobiological Implications

Leslie C. Jones,[1] John L. Beard,[2] and Byron C. Jones[1,3]*

ABSTRACT: Fe, Cu, and Zn are of widespread neurobiological importance, but must be regulated closely as too much or too little of these metals can have adverse effects on brain function. Recent evidence from nutritional models notes that the hippocampus is particularly vulnerable to Fe and Zn deficiencies. We recently performed a quantitative trait loci (QTL) analysis as a preliminary step in identifying genes that contribute to natural variation in hippocampal Fe, Cu, and Zn content. We used ICP-MS to measure the concentrations of these metals in 120-day-old mice from 30 strains of the BXD/TY panel. The BXD/Ty recombinant inbred strain panel is well-suited for complex trait analysis, as all strains are genotyped with a dense marker set and have been phenotyped extensively for neurobehavioral traits and hippocampal gene expression. We observed a wide-range of hippocampal Fe, Cu, and Zn concentrations across the BXD strains. These concentrations were related to systemic Fe status, but not to Fe, Cu, and Zn elsewhere in the brain. The three metals also showed strong covariance, suggestive of overlap in their regulatory pathways. We identified two QTL, on chromosomes 14 and 9, most strongly associated with Cu but also suggestively associated with Fe (chr. 14) and Zn (chr. 9). We also performed genetic correlational analyses with existing data on these strains and revealed associations with cognitive, anxiety-related, and alcohol-related phenotypes. Covariance of these metals with gene expression is also discussed. This work shows that hippocampal Fe, Cu, and Zn are under polygenic influence and that trace metal regulation is associated with hippocampus-related behaviors. Future work will elucidate the genes underlying the two QTL identified, to aid in identifying homologous genetic variants in human populations, which may underlie altered trace metal homeostasis and related neurological disease. © **2008 Wiley-Liss, Inc.**

KEY WORDS: mice; recombinant inbred; BXD; trace metals; QTL

INTRODUCTION

Fe, Cu, and Zn in the brain are of widespread neurobiological importance as they contribute to a diverse set of biochemical processes, including energy metabolism (Fe, Cu), antioxidant defense (Cu, Zn), myelination (Fe), DNA synthesis (Fe, Zn), and neurotransmitter synthesis (Fe, Cu) (Linder and Hazegh-Azam, 1996; Beard, 2003; Frederickson et al., 2005). Deficiencies of these metals are thus detrimental to brain function; yet, they must be regulated strictly because they are toxic when in excess (Levenson, 2005). Indeed, several neurodegenerative disorders are associated with Fe, Cu, and Zn imbalances (Choi et al., 2006; Gaggelli et al., 2006; Schenk et al., 2006; Patrick, 2007; Shcherbatykh and Carpenter, 2007; Smith and Lee, 2007).

Recent evidence notes that the hippocampus is particularly vulnerable to developmental Fe deficiency (ID). New evidence exposes wide and lasting changes in hippocampal gene expression in response to perinatal ID (Carlson et al., 2007), as well as specific hippocampus-related cognitive deficits (Schmidt et al., 2007). The effects of Zn deficiency on the hippocampus were also recently highlighted in a study revealing associated impaired calcium regulation (Takeda et al., 2007, 2008). These new findings complement previous observations that the hippocampus appears to be more sensitive than other brain regions, including earlier and greater Fe and Zn losses in response to Fe and Zn deficient diets (Erikson et al., 1997; Takeda et al., 2001) and a wide range of functional indices (Hunt and Idso, 1995; de Deungria et al., 2000; Rao et al., 2003; McEchron and Paronish, 2005; McEchron et al., 2005). The hippocampus also has unique regulatory demands for these metals as each contributes to synaptic plasticity (Doroulee et al., 1997; Leiva et al., 2003; Goldschmith et al., 2005; Jorgenson et al., 2005; Izumi et al., 2006) and Cu and Zn are neuromodulators here as well (Frederickson et al., 2005; Schlief et al., 2006).

Taken together, these findings highlight the importance of local, homeostatic regulation of these metals in the hippocampus; yet, the local regulatory mechanisms are largely unknown. In particular, genetic-based variation in these mechanisms is unexplored. While several genetic disorders of Fe and Cu homeostasis with profound effects on brain function are now described (Kodama et al., 1999; Portala et al., 2001; Pietrangelo, 2006), we still know little about natural variation in hippocampal trace metal homeostasis, its genetic underpinnings, and its biobehavioral significance.

Regional regulation of these metals is likely complex, involving interactions between Fe, Cu, and Zn regula-

[1] **Intercollege Graduate Degree Program in Neuroscience, The Huck Life Sciences Institute, The Pennsylvania State University, University Park, Pennsylvania;** [2] **Department of Nutrition, The Pennsylvania State University, University Park, Pennsylvania;** [3] **Department of Biobehavioral Health, The Pennsylvania State University, University Park, Pennsylvania**

Grant sponsor: USPHS; Grant number: AG-021190; Grant sponsor: Pennsylvania State Tobacco Settlement Grant.

*Correspondence to: Byron C. Jones, 315 E. HHD Building, University Park, PA 16802-6508, USA. E-mail: bcj1@psu.edu

Accepted for publication 23 November 2007
DOI 10.1002/hipo.20399
Published online in Wiley InterScience (www.interscience.wiley.com).

tory networks, local cell signaling pathways, and environmental perturbations. Thus, variation likely involves multiple genes. Genetic-based individual differences in neurobiological phenotypes are most easily studied in genetically defined animals, usually inbred or selected lines of mice or rats. For the analysis of complex traits, recombinant inbred (RI) mice are highly advantageous (Belknap, 1998). The BXD/TyJ RI strains (Williams et al., 2001) have been in existence for nearly three decades and were derived from the inbred C57BL/6J and DBA/2J strains, which differ in many phenotypes. Each strain consists of genetically identical and, therefore, genetically replicable mice. Thus, these animals are ideal for conducting genetic correlational analysis among phenotypes even if the measures come from different laboratories. Accordingly, these strains have been phenotyped extensively, including behavioral, neurochemical, immunological, and physiological parameters. In addition, they are profiled for gene expression in the whole-brain and hippocampus. Most importantly, these strains are fully genotyped and densely mapped to facilitate quantitative trait loci (QTL) analysis, a preliminary step in identifying the polymorphic genes that contribute to variance in complex phenotypes (Belknap, 1998; Williams et al., 2001).

We have previously phenotyped these strains for brain Fe, Cu, and Zn content in several regions including the hippocampus, and reported wide, genetic-based variation in all measures (Morse et al., 1999; Jones et al., 2003, 2006). This was expected, as their parental strains showed differences in brain Fe content and Fe loss during deficiency (Morse et al., 1999). QTL analyses of Fe, Cu, and Zn concentrations showed that the concentrations of these metals are influenced by multiple loci, some that are common to multiple metals and brain regions, and some that are distinct for each metal and/or region (Jones et al., 2003, 2006).

In the current investigation, we extend our genetic analysis to the hippocampus. We have measured Fe, Cu, and Zn in the hippocampus of 28 BXD/TyJ RI strains as well as their C57BL/6J (B6) and DBA/2J (D2) parental strains. Here, we report significant, genetic-based variability in the concentrations of these metals and several associated QTL. We also explore correlations with other neurobehavioral traits and with the expression of related genes. This work not only allows for comparison of hippocampal trace metal regulation with that of other brain regions, but also provides a preliminary genetic analysis that supports future work to elucidate the genetic polymorphisms mediating natural variation in hippocampal trace metal regulation. The intimate involvement of Fe, Cu, and Zn in hippocampal function and pathology demands a better understanding of their genetic regulation.

MATERIALS AND METHODS

Animals

Eight hundred-five male and female mice from 28 BXD/TyJ RI strains and their parental strains, C57BL/6J, DBA/2J were analyzed for hippocampal Fe, Cu, and Zn content. Each strain was represented by an average of 13 mice per sex, with a range of 7–23 mice. An exception was strain 15, a poor breeding line, which was only represented by 3 male and 2 female mice. All mice were reared and maintained under a constant light-dark cycle (06:00–18:00, on–off), ambient temperature (23°C ± 2°C), and relative humidity (40%). In most cases, two to three mice were housed per cage, with a maximum of three males or five females. Food and water were available ad libitum. Experimental mice were fed Purina rodent diet #5,010, a standard laboratory diet reportedly containing approximately 240 ppm Fe, 18 ppm Cu, and 124 ppm Zn. All experimental protocols were conducted in accordance with the National Institutes of Health Animal Care guidelines and were approved by the Penn State Institutional Animal Care and Use Committee.

Tissue Harvest

At 120 ± 3 days of age, all mice were sacrificed by CO_2 suffocation. Blood samples were collected immediately via cardiac puncture for subsequent analysis of plasma Fe, hematocrit, hemoglobin, and total Fe binding capacity (TIBC) (reported in Jones et al., 2007). Within 5 min of death, the brains were dissected on ice to yield the hippocampus.

Tissue Preparation for ICP-MS

We used a standard digestion protocol in our lab modified from a wet-ashing procedure by Clegg et al. (1981). Hippocampal tissues were placed in 1.5 ml polypropylene centrifuge tubes containing 400 µl ultrapure nitric acid (J.T. Baker Ultrex II, Mallinckrodt Baker, Phillipsburg, NJ). They were then heated to 80°C on a heat block for at least 24 h. After cooling, 400 µl nanopure water was added to each tube. A 100 µl aliquot of digested sample was then added to 1.4 ml nanopure water in acid-washed autosample tubes.

ICP-MS Analysis

Analysis of Fe, Cu, and Zn was performed using a Finnigan ELEMENT High Resolution ICP-MS (Thermo Scientific, Inc., Waltham, MA). Quality control was performed each day of the analysis and included controls used to verify calibration curve accuracy. Between each sample, the siphon was washed with MilliQ water and this wash was monitored for contamination. Method detection limits (3*SD of the blanks/slope of the isotope) were 9 ppb, 9 ppb, and 119 ppb for Fe, Cu, and Zn, respectively. The relative standard deviation (RSD) of same-day replicates ($N = 6$) was 3% for Fe, 4% for Cu, and 13% for Zn. The RSD for between-day replicates ($N = 4$) was 1, 3, and 2% for Fe, Cu, and Zn.

Data Analysis

ICP-MS values for Fe, Cu, and Zn were normalized to hippocampal weight (µg/g). Data reported are mean tissue concentrations (±s.e.m.) by strain and sex. Outliers, defined as values

exceeding two standard deviations from the mean, were removed. A two-factor analysis of variance (ANOVA) was performed for each metal with strain and sex as between-subjects variables. Subsequently, strain means were used as expected concentrations for each strain to describe the variation in hippocampal metal content across the BXD panel. Strain means were also used for the correlational and QTL analyses. Pair wise association between sexes and between metals in hippocampus was measured by Pearson's r. Additional correlational analyses, described later, were performed using a public BXD phenotype database and a BXD gene expression database.

QTL Analysis

QTL analysis involves measuring phenotypic variation in individuals or RI strains, then associating phenotypic differences with polymorphic regions of the genome. QTL Analysis was performed using WebQTL (http://www.genenetwork.org), an online neuroinformatics resource featuring tools for QTL analysis for genetic reference populations (Wang et al., 2003). Briefly, QTL analysis of a phenotypic trait with WebQTL involves quantifying the trait in a number of BXD strains, then using an algorithm that scans 3,795 polymorphic markers across the genome for associations with the strain mean values. Associations are reported as point-biserial correlations with probabilities as likelihood of the odds (LOD) scores for each marker. The LOD score at any locus represents the likelihood that the genotype at that variation in the locus is associated with the phenotypic variation of the trait of interest. The statistical methods for calculating LOD scores on WebQTL include 1,000 permutations of the trait data. A stringent genome-wide significance threshold of $P < 0.05$ is determined per scan, dependent on the results of the permutation analysis. A suggestive threshold ($P < 0.67$) is also determined to include QTL of smaller effect size because of low power to detect such QTL in analyses of low numbers of strains. Significant and suggestive QTL can then be validated with follow-up replications in other crosses.

BXD Phenotype and Gene Expression Database Analysis

Genenetwork also provides access to a BXD phenotype database and multiple gene expression databases that allow correlational analysis between the trait of interest and other traits, including gene transcript abundance. The phenotype database contains data on over 700 traits previously quantified in the BXD strain panel. Likewise, the gene expression databases contain data on gene expression in the BXD strain panel, based on the results of microarray analyses of basal gene expression in a variety of tissues, including hippocampus. These databases report transcript abundance for any given gene across the BXD strains and illustrate in many cases, wide, genetic-based differences in expression (Chesler et al., 2005). The hippocampus expression database (Hippocampus Consortium M430v2 (June 06) was our database of choice for this study and we chose the RMA method of normalization (Irizarri et al., 2003).

RESULTS

Hippocampal Fe, Cu, and Zn Concentrations

The distribution of mean Fe, Cu, and Zn concentrations across the panel is shown in Figures 1a–c and 2a–c. Among the strains, the range of variation in the mean hippocampal concentrations of these metals was 3-fold for Cu (2.8–10.3 μgCu/g), approximately 2-fold for Fe (11.0–19.3 μgFe/g), and 1.5-fold for Zn (15.1–24.9 μgZn/g). The two-way ANOVA revealed a main effect of strain on Fe ($F_{29/761} = 8.881$, $P < 0.001$), Cu ($F_{29/765} = 10.401$, $P < 0.001$), and Zn ($F_{29/767} = 5.272$, $P < 0.001$) concentrations. There was no main effect of sex or for the interaction of sex by strain for any metal.

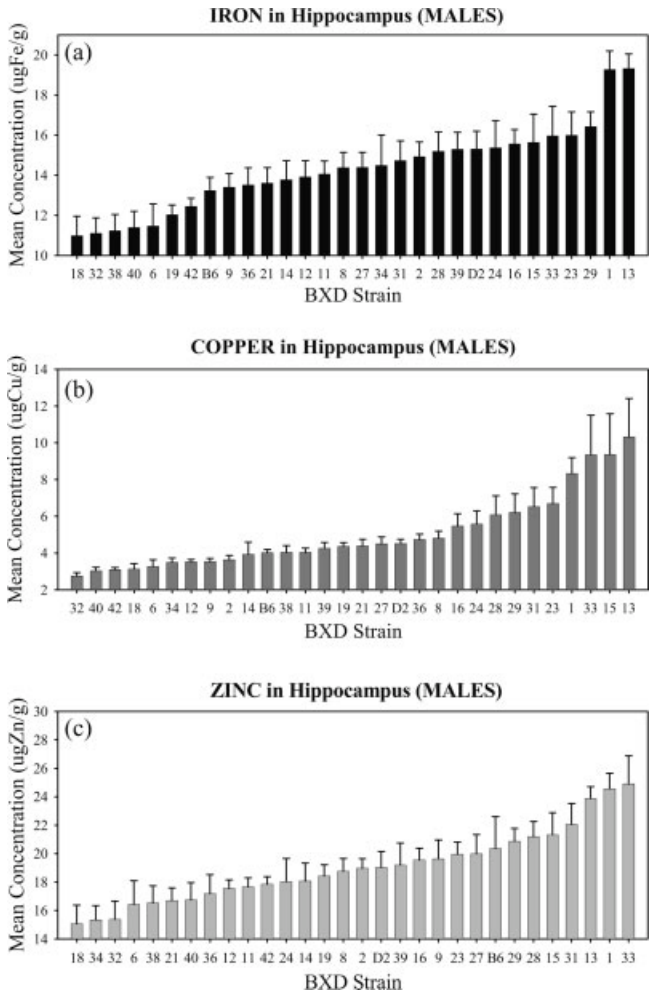

FIGURE 1. Hippocampal iron (a), copper (b), and zinc (c) concentrations (mean ± s.e.m.) for male mice from 28 BXD strains and their C57BL/6J (B6) and DBA/2J (D2) progenitors.

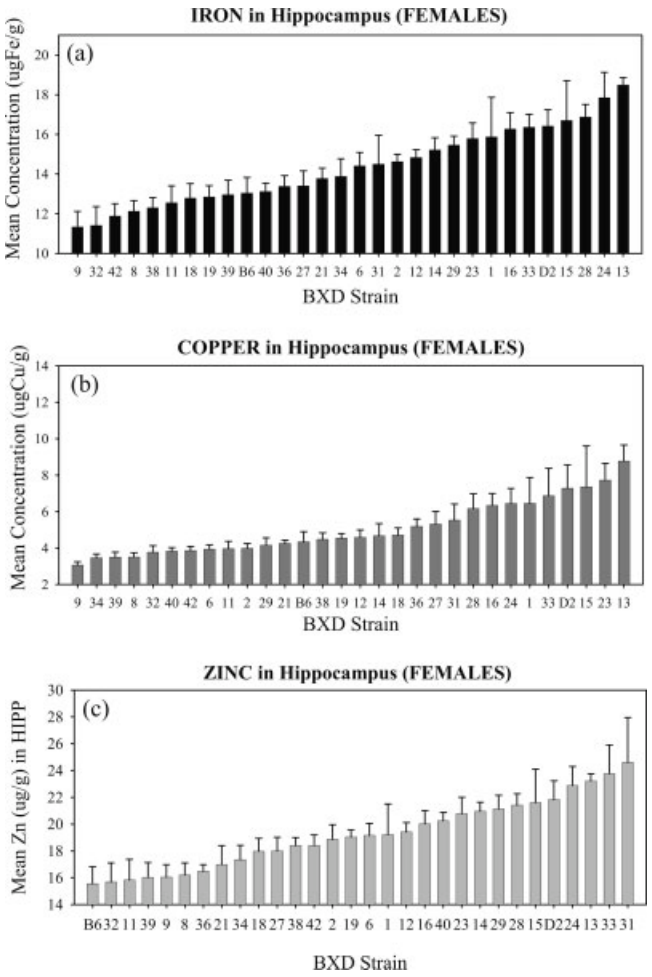

FIGURE 2. Hippocampal iron (a), copper (b), and zinc (c) concentrations (mean ± s.e.m.) for female mice from 28 BXD strains and their C57BL/6J (B6) and DBA/2J (D2) progenitors.

Narrow-sense heritability estimates based on SS_{Strain}/SS_{Total} for Fe, Cu, and Zn were 0.25, 0.29, and 0.15, respectively.

Genetic Correlations Between Metals by Sex

Correlational analysis showed that hippocampal Fe, Cu and Zn concentrations were highly associated. For males, Cu and Zn were most strongly correlated ($r = 0.85$, $P < 0.001$), followed by Cu and Fe ($r = 0.80$, $P < 0.001$) and Zn and Fe ($r = 0.80$, $P < 0.001$). For females, Cu and Fe were highly correlated ($r = 0.83$, $P < 0.001$) as were Zn and Fe ($r = 0.80$, $P < 0.001$) and Cu and Zn ($r = 0.71$, $P < 0.001$).

Genetic Correlations Between Sexes by Metal

Significant correlations were also observed for each metal between the sexes. For Fe and Cu, male and female concentrations were strongly correlated ($r = 0.73$ ($P < 0.001$) and $r = 0.81$ ($P < 0.001$), respectively). Zn concentrations in males and females were more modestly associated ($r = 0.54$, $P < 0.01$). Importantly, hippocampal Fe, Cu, and Zn concentrations were not associated with body weight.

Genetic Correlations Between HIPP Fe, Cu, and Zn Concentrations and Those in Other Brain Regions

We used resources at http://genenetwork.org, including the BXD phenotype database, to explore the possible relationships between hippocampal trace metal content and other neurobiological and neurobehavioral phenotypes. Correlational analysis of the six traits of interest and 700+ traits previously quantified in the BXD Panel revealed several associations not previously suggested in the published literature. These results are summarized in Table 2, which lists all phenotypes significantly correlated with two or more traits. All six traits, i.e., Fe, Cu, and Zn in male and female mice, were positively associated with hematological measures from our previous study of systemic Fe regulation in the BXDs (Jones et al., 2007). Hematological measures included hemoglobin, hematocrit, and total Fe binding capacity. In contrast, the hippocampal content of these metals was largely unrelated to that in other brain regions, including the medial prefrontal cortex (PFC), ventral midbrain (VMB), nucleus accumbens (NA), and caudate-putamen (CP) (Jones et al., 2003, 2006). The only exception was Cu in the CP in female mice, which was correlated with HIPP Cu and Fe in both male and female mice (see Table 2). This correlation was exclusive to Cu and to female mice; significant correlations were not found with CP Cu in male mice or Fe and Zn in the CP.

Genetic Correlations Between HIPP Fe, Cu, and Zn and Neurobehavioral Phenotypes

Several neurobehavioral phenotypes were related to HIPP metals. HIPP Fe, Cu, and Zn were positively associated with latency to locate the platform on day 3 of training in the Morris water maze (a hippocampus-dependent learning and memory paradigm), such that strains with relatively low HIPP Fe, Cu, and Zn content had better learning and memory performance (Milhaud et al., 2002). Strains with relatively low HIPP Zn were also recently shown to exhibit less exploratory behavior in an anxiety-related paradigm. This was reflected in a positive correlation between HIPP Zn in male and female mice and time spent in the open arms of the elevated plus maze (Holmes A, Unpublished findings; publicly available at http://www.genenetwork.org. Cited with permission of author). Finally, strains with relatively low HIPP Zn and Fe in female mice also tended to show higher alcohol acceptance in the study by Crabbe et al (1983). We also found it worth noting that some correlations that were expected to be found were not observed. For instance, in Jones et al. (2006) we reported that Zn in the PFC and NA was negatively correlated with seizure susceptibility in the BXD panel (McCall and Frierson, 1981; Plomin et al., 1991). Because of the involvement of the hippocampus in seizure activity, the neuromodulatory roles Zn plays

TABLE 1.

QTL Analysis of Hippocampal Cu and Zn

Phenotype	Chr.	Mb	Marker	LRS	LOD	Add. eff.
Zn, males	9	30.9	rs13480122	12.297	2.67	−1.670
		40.2	rs6240566	12.603	2.73	−1.819
		50.9	rs13480191	11.704	2.53	−1.637
Cu, males	9	30.9	rs13480122	12.491	2.71	−1.320
		40.2	rs6240566	15.621	3.39	−1.552
		50.9	rs13480191	12.688	2.75	−1.329
	14	38.7	rs6197032	10.872	2.36	−1.169
		40.9	rs13482156	13.637	2.96	−1.307
		47.2	rs4135454	13.637	2.96	−1.307
Cu, females	14	38.06	rs13482141	13.623	2.96	−0.907
		38.7	rs6197032	21.634*	4.69*	−1.064
		40.9	rs13482156	19.307*	4.19*	−1.045
		47.2	rs4135454	19.307*	4.19*	−1.045
		67.7	rs3089069	10.348	2.24	−0.813
Fe, females	14	38.7	rs6197032	11.957	2.59	−1.133
		39.3	CEL-14_36454776	11.957	2.59	−1.133

This table presents the locations of at least two QTL (on chromosomes 14 and 9) associated with hippocampal Cu and/or Zn concentrations. Also shown are the LRS and LOD scores for these QTL at peak and flanking genetic markers. Chr, chromosome; Mb, megabases from centromere; LRS, likelihood ratio statistic; LOD, likelihood of odds ratio; Add Eff, additive effect of QTL. Negative numbers indicate C57BL/6 as increasing allele.
*Significant.

in this region with NMDA receptors (Izumi et al., 2006), and the association of Zn with epilepsy (Hirate et al., 2002; Flynn et al., 2007), we expected to observe similar correlations, but did not.

QTL Related to HIPP Fe, Cu, and Zn

QTL analysis revealed two loci of interest on chromosomes 14 and 9, which were most robustly associated with Cu. Specific markers, including flanking and peak markers, are reported in Table 1 for these QTL. The most robust QTL, near the proximal end of chromosome 14 (38.7 Mb, marker rs6197032), was significantly associated with hippocampal Cu in female mice. Its peak LOD score was 4.69 for Cu in females at 38.7 Mb (marker rs6197032); however, the QTL was significantly associated with markers throughout an interval spanning 38.7 to 47.2 Mb. This chromosome 14 QTL was also associated with hippocampal Cu in male mice, though for males the LOD score for marker rs6197032 was only suggestive (2.36) and the QTL instead peaked between 40.9 and 47.2 Mb (LOD = 2.96; markers rs13482156 and rs4135454). Notably, this QTL was also weakly associated with hippocampal Fe concentrations in females (see Table 1). It is yet unclear whether overlapping QTL for any two of these three metals reflects coregulation of the metals or covariance among metals in hippocampal concentrations. The second QTL is located on chromosome 9 at 40.2 Mb and is associated with both Cu and Zn. This QTL only appeared in male mice. Although not significant, the Cu/Zn QTL is suggestive with a peak LOD of 3.39 for Cu and 2.73 for Zn (marker rs6240566).

Associations of the Two HIPP QTL with Fe, Cu, and Zn in Other Brain Regions

To investigate the involvement of the HIPP Cu-Zn- and HIPP Cu-Fe-related QTL with Fe, Cu, and Zn in other brain regions, we reviewed the QTL previously associated with Fe, Cu, and Zn in the PFC, NA, CP, and VMB (Jones et al., 2003, 2006). Surprisingly we found several interesting associations. Two markers for the chromosome 14 QTL, rs13482156 at 40.9 Mb and rs4135454 at 47.2 Mb, were equally associated with Zn in the PFC of female mice (LOD = 2.7) and Fe in the VMB and CP of male mice (LOD = 2.4, LOD = 1.38, respectively). The peak marker for the chromosome 9 QTL, rs6240566 at 40.2 Mb, was weakly associated with Cu in the NA of male mice (LOD = 1.3). In addition, a nearby QTL, marker rs6206488 at 54.7 Mb, was associated with Fe in the CP of male mice and Zn in the PFC of female mice (LOD = 2.30, LOD = 2.48, respectively). We note that these are very weak LOD scores; these associations are only worth consideration due to the common relationship with HIPP metals and due to the low number of strains in the previous analyses, which lowered the power to detect existing QTL of small effect size.

Genetic Correlations Between HIPP Fe, Cu, and Zn and Hippocampal Gene Expression

We also used the hippocampal gene expression database in Genenetwork, to search for covariance between basal gene expression in the BXD strains and HIPP Fe, Cu, and/or Zn. The hippocampal expression of several genes was related to all three metals. These included tripartite motif protein 16 (*trim16*), a gene involved in the immune response, preimplantation protein 3 (*Prei3*), which encodes a Zn-binding protein involved in development, heme-binding protein 2, and δ-aminolevulinic acid dehydratase (*alad*), which encodes a Zn-binding enzyme in the heme and porphyrin biosynthetic pathways (see Table 2 for correlation coefficients). More gene expression correlations were found with only one metal or with a combination of two metals; a summary of related gene expression is listed in Table 3. Many of these genes are regulated by QTL on chromosomes other than 9 and 14, thus, if their correlation with HIPP Fe, Cu, and Zn reflects a functional relationship, it is likely to reflect an effect of, not a cause of, variance in these concentrations. Of special interest, however, were two genes located within the chromosome 9 QTL interval, including leucine rich repeat containin 35 (*Lrrc35,* Chr.9@42.2 Mb) and a Riken cDNA gene (*D60033011Rik*, Chr. 9@43.0 Mb), both of which had expression that was negatively related to HIPP Fe, Cu, and Zn in male mice. The Riken gene is especially interesting as a candidate gene for this QTL, as it is highly *cis*-regulated. Concerning the chromosome 14 QTL, there was also a candidate gene based on its *cis*-regulation and location within the QTL interval. Sterile alpha motif domain containing 4

TABLE 2.

Correlational Analysis of Hippocampal Fe, Cu, and Zn in Relation to Other BXD Phenotypes

Phenotypic trait	Reference	*N*	Fe		Cu		Zn	
			Males	Females	Males	Females	Males	Females
Hemoglobin	Jones et al., 2007	30	0.77***	0.73***	0.59***	0.60***	0.52***	0.54**
Hematocrit	Jones et al., 2007	30	0.70***	0.55***	0.56***	0.54***	0.56***	–
Total Fe binding capacity	Jones et al., 2007	30	0.53***	0.52**	0.58***	0.46***	0.40*	0.51**
Morris water maze log latency 3	Milhaud et al., 2002	21	0.45*	0.56**	0.62**	0.53*	0.47*	0.68***
Striatal Cu females	Jones et al., 2006	14	0.66**	0.64**	0.67**	0.54*	–	–
Ethanol acceptance	Crabbe et al., 1983	16	–	−0.68**	–	–	–	−0.69**
% time in open arms in elevated plus maze	Holmes and Yang, 2006	16	–	–	–	–	0.64*	0.67**

This table shows correlations that were revealed in an analysis that compared hippocampal Fe, Cu, and Zn data with over 700 phenotypes previously quantified in the BXD panel. Pearson's *R* correlation coefficients are shown. Criteria for inclusion in this table were (a) traits correlated with at least one metal whose *R*-values were higher than 0.6 or (b) traits correlated with more than one metal. All values are statistically significant at $^{*}P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.001$, however, these values have not been corrected for multiple comparisons. *N*, number of strains being compared. A (–) symbol means that either no correlation or only a weak correlation was present between two variables.

(*Samd4,* Chr. 14@46.0 Mb) was positively associated with Fe, Cu, and Zn in females and Cu in males.

DISCUSSION

Fe, Cu, and Zn play diverse and important roles in hippocampal function, but when these metals are deficient or in excess, they are associated with the impairment of several neurobehavioral processes and neurodegenerative disorders. Elucidating the genetic mechanisms by which these metals are managed in this region is one approach to understanding how homeostasis is maintained here and how these metals may become mismanaged in certain individuals. Systems genetic analysis using RI murine strains is an efficient way to explore the polygenic influences on complex neurobiological phenotypes. This involves exploiting natural variation in a biological or behavioral phenotype to reveal related genetic loci, gene expression, and phenotypes. In this study, we demonstrated significant, genetic-based differences in hippocampal Fe, Cu, and Zn content in the BXD/Ty RI panel, and then subjected these differences to systems genetic analyses. We observed two significant QTL, a number of related genes, and several related neurobehavioral phenotypes. In addition, because we measured multiple phenotypes (Fe, Cu, and Zn in males and females), we were able to gain insight into the inter-relatedness of these three metals and their unique regulation in the hippocampus.

Natural Variation in HIPP Fe, Cu, and Zn Compared to Nutritional Deficiencies

We observed a range of natural variation in these metals that was 3-fold for Cu, approaching 2-fold for iron, and 1.5-fold for zinc. Interestingly, these ranges are similar to those observed during dietary deprivation studies, although differences in timing, diet, and length of deprivation make these studies difficult to compare directly. Brain Cu losses can be quite severe; in SV/129 mice, Cu was reduced by approximately 80% in the brain following Cu deficiency from gestation to the first 6 to 8 weeks of life (Zucconi et al., 2007). Similarly, significant Fe losses occur; Fe was reduced by 57% in the developing rat hippocampus when weanlings were fed an iron-deficient diet for 2 weeks, although other regions of the brain are resistant to iron loss (Erikson et al., 1997; Morse et al., 1999). Compared to Fe and Cu, the hippocampus has been shown to be more resistant to Zn loss. No losses were observed after 4 weeks of a zinc-deficient diet, while 12 weeks reduced hippocampal zinc by approximately 1/3 in adult rats (Takeda et al., 2001, 2005) Considering these similarities, we note several points in light of the following discussion. It is with caution that we place strains with genetically-based "high" or "low" metal concentrations in the same context as mice with excessive or deficient Fe, Cu, or Zn resulting from dietary manipulations. These nutritional models can be accompanied by the confounding factors of malnutrition-induced stress and metal–metal interactions. Moreover, assigning the terms "low" and "high" to the strains in our analysis may be misleading, as these terms are based on relative comparisons and it is yet to be determined what the optimal concentrations of Fe, Cu, and Zn are in the hippocampus.

Hippocampal Fe, Cu, and Zn Regulation in Relation to Behavior

Genetic correlational analysis showed that hippocampal Zn is positively correlated with exploratory behavior on the elevated plus maze (Holmes and Yang, unpublished observations). This correlation supports existing experimental evidence that hippocampal Zn is functionally related to anxiety-like phenotypes. These include the findings from two dietary manipulation studies, in which Zn deficiency led to increased anxiety-

TABLE 3.

A Summary of Genes Whose Expression Is Related to One or More HIPP Metals, Grouped by the Chromosome on Which the Peak QTL that Regulates Their Expression Is Located

Gene network ID	Exp	Gene symbol	Gene description	Chr	Mb	Peak QTL Chr.	Peak QTL LOD	Fe M	Cu M	Zn M	Fe F	Cu F	Zn F
1423168_at	11.49	Prei3	Preimplantation protein 3	1	55.1	1	2.8	0.50	0.60	–	0.51	0.66	0.51
1419407_at	7.08	Hc	Hemolytic complement	2	34.8	3	2.1	–	–	–	−0.61	−0.50	−0.56
1424877_a_at	9.54	Alad	Aminolevulinate,delta-, dehydratase	4	62.0	4	27.8	0.47	0.44	–	0.61	0.59	0.42
1452362_at	7.94	Trim16	Tripartite motif protein 16	11	62.7	4	1.95	−0.52	−0.56	−0.47	−0.59	−0.62	−0.55
1434330_at	10.41	Lrrc35	Leucine rich repeat containing 35	9	42.2	4	3.2	0.52	0.56	0.62	–	–	–
1443458_at	7.73	D60033011Rik	Riken cDNA D6300333011 gene	9	43.0	9	9.5	−0.58	−0.53	−0.60	–	–	–
1437396_at	7.72	Creb3l2	cAMP resp. elem. binding protein 3-like 2	6	37.3	9	1.7	−0.66	−0.62	–	−0.58	−0.58	–
1454317_at	7.76	Adam1b	A disintegrin and metalloproteinase domain 1b	5	121.8	9	4.5	−0.52	–	–	–	−0.51	–
1437396_at	7.72	Creb3l2	cAMP resp. elem. binding protein 3-like 2	6	37.3	9	1.7	−0.66	−0.62	–	−0.58	−0.58	–
1444154_at	7.88	Ndufa11	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 11	17	56.4	9	2.1	−0.58	–	–	−0.52	–	–
1425762_a_at	8.91	Rxra	Retinoid X receptor alpha	2	27.6	12	3.6	−0.59	–	–	–	–	–
1448243_at	12.50	Napa	*N*-ethylmaleimide sensitive fusion protein attachment protein alpha	7	15.3	12	3.1	−0.50	−0.55	–	−0.47	–	–
1439084_at	8.25	Cxcl12	Chemokine (C-X-C motif) ligand 12	6	117.1	12	3.0	−0.49	–	–	−0.57	–	–
1456293_s_at	10.63	Ccnh	Cyclin H	13	85.7	13	5.2	0.51	–	–	0.62	–	–
1436356_at	8.26	Samd4	Sterile alpha motif domain containing 4	14	46.0	14	4.2	–	0.55	–	0.51	0.64	0.64
1446415_at	7.46	Hebp2	Heme binding protein 2	10	18.2	16	2.8	–	−0.60	–	−0.68	−0.59	−0.59
1454984_at	10.53	Lifr	Lifr	15	7.1	17	1.5	–	–	–	0.62	0.64	–
1440603_at	7.52	Asb18	Ankyrin repeat SOCS box-containing 18	1	91.8	18	3.6	–	–	–	−0.60	−0.57	−0.67
1426425_at	10.75	Sugt1	SGT1, suppressor of G2 allele of SKP1 (S. cerevisiae)	14	78.3	19	2.3	0.51	0.57	0.51	–	0.60	–
1416595_at	10.02	Mrps22	Mitochondrial ribosomal protein S22	9	98.4	19	3.4	0.53	0.54	–	0.60	0.56	–
1452158_at	8.63	Eprs	Glutamyl-prolyl-tRNA synthetase	1	187.1	19	2.3	–	–	–	0.65	–	0.56
1449309_at	6.72	Cyp8b1	Cytochrome P450,family 8,subfamily b,polypeptide 1	9	121.8	X	2.1	−0.58	–	–	−0.64	−0.61	−0.59
1422494_s_at	8.53	Ccrk	Cell cycle related kinase	13	64.5	X	3.7	−0.56	−0.57	–	−0.55	−0.53	–
1434248_at	8.17	Prkch	Protein kinase C, eta	12	74.7			0.60	0.52	–	–	–	–
1420393_at	6.94	Nos2	Nitric oxide synthase 2, inducible, macrophage (inflammation mediator)	11	78.8	X	11.9	−0.46	–	–	−0.54	–	–
1422783_a_at	8.84	Krt2-6a	Keratin complex 2, basic, gene 6a	15	101.5	X	13.8	−0.55	–	–	–	–	–
1424162_at	7.47	Trim29	Tripartite motif protein 29	9	43.1	X	13.3	−0.52	–	–	–	–	–

Gene expression data is from the Hippocampus RMA database from the June, 2006 series. All data available at http://genenetwork.org. Exp, expression level of the transcipt on a logarithmic scale where 8 is mean expression and an increase of one equals a two-fold increase in expression; Chr, chromosomal location of transcript; Mb, megabases from centromere; Peak QTL Chr, chromosome containing the peak eQTL for each transcript; Peak QTL LOD, likelihood that the eQTL reflects a true association; M, male mice; F, female mice.

like behaviors in rodents (Chu et al., 2003; Takeda et al., 2007, 2008).

We also found that hippocampal Fe, Cu, and Zn are related to performance in the Morris Water Maze, a learning and memory task, supporting involvement of these metals in cognition (Milhaud et al., 2002). An association between metals and cognition is also not new; Fe and Zn deficiencies in humans lead to cognitive impairment that can be irreversible (Groner et al., 1986; Bruner et al., 1996; Siddappa et al., 2004; Beard et al., 2005; Sen and Kanani, 2006; Murray-Kolb and Beard, 2007). Likewise, cognitive impairment has been observed in Fe deficient rodents, particularly as demonstrated by poor performance in the Morris Water Maze (MWM) (Felt and Lozoff, 1996; Kwik-Uribe et al., 2000; Felt et al., 2006). Zn deficiency in rodents and monkeys has also been shown to adversely affect learning and memory (Sandstead et al., 1977; Halas et al., 1983, 1986; Golub et al., 1994, 1995; Takeda et al., 2000), with some exceptions (Chu et al., 2003). Yet, while evidence exists for the involvement of these metals in cognition, it is difficult to interpret the direction of our observed correlation, which showed that strains with lower HIPP Fe, Cu, and Zn perform better in the MWM. This seeming inconsistency with experimental results may reflect several points. One point, discussed earlier, is that comparison of natural variation in these metals with dietary deficiencies has several potential pitfalls. Another important point is that these results are correlational in nature and therefore the higher Fe, Cu, and Zn levels could be a consequence, rather than a cause of, altered neurobehavioral functioning. In fact, such correlations may reflect genetic linkage and no direct relationship at all. Thus, the exact significance of these findings awaits further study; nevertheless, these findings may be of heuristic interest within the context of metals and neurobehavioral phenotypes.

Hippocampal Fe, Cu, and Zn Regulation in Relation to Gene Expression

We also revealed several correlations between Fe, Cu, and/or Zn and gene expression, summarized in Table 3 (also available at http://genenetwork.org). Few of the structural genes for correlated transcripts were located in or regulated by the chromosome 9 or 14 QTL, therefore most are not considered to be candidate genes; yet, they could belong to networks of genes that are coexpressed as hippocampal metal concentrations vary. In fact, many of the genes in Table 3 covary and/or share regulatory expression QTL (see Table 3 for eQTL and http://genenetwork.org for correlations between transcripts).

Of the genes for which expression was related to all three metals in the hippocampus, *alad* is the most functionally relevant to metal homeostasis. *Alad* encodes a Zn-binding enzyme involved in heme and porphyrin biosynthetic pathways. The enzyme, ALAD, has been shown to indicate Fe, Cu, and Zn concentrations in the blood (Thompson et al., 1977). Notably, in addition to its relation to hippocampal Fe, Cu, and Zn, its expression was also shown to be related to Zn in the PFC and NA, and Cu in the VMB (Jones et al., 2006), thus *alad* may be involved in whole-brain metal homeostasis. Interestingly, *alad* is polymorphic in humans and some studies have suggested that these polymorphisms alter susceptibility to lead toxicity (Kaleda et al., 2001)

Two other genes of interest in our correlational analysis were chemokine ligand 12 (*Cxcl12*) and *N*-ethylmaleimide sensitive fusion protein attachment protein alpha (*Napa*), which were both negatively related to HIPP Fe concentrations (see Table 3). These findings support recent observations by Carlson et al. (2007), who reported that these genes were found to be upregulated during Fe deficiency. Neither of these genes is located near or regulated by the chromosome 9 and 14 QTLs we identified, but their covariance with hippocampal Fe may reflect a relationship with biological importance. In fact, *Cxcl12* is involved in nigrostriatal dopamine regulation and is localized in dopaminergic neurons in the striatum and VMB (Banisadr et al., 2003; Skrzydelski et al., 2007). This is of special interest, as a relationship between iron and dopamine regulation is well-established (Unger et al., 2007).

Hippocampal Fe, Cu, and Zn Regulation in a Systemic Context

HIPP Fe, Cu, and Zn concentrations are positively correlated with hematocrit, hemoglobin, and total Fe binding capacity (TIBC) (Jones et al., 2007). This finding reflects the uniqueness of HIPP Fe, Cu, and Zn regulation, as these metals in other brain regions have been shown to be largely unrelated to systemic Fe status (Jones et al., 2003). This correlation may reflect the specific vulnerability of this region to systemic Fe and Zn deficiencies. In a study of early Zn deficiency in rats, Takeda et al. (2001) found that 4 weeks of Zn deprivation caused hippocampal Zn depletion despite the fact that Zn status elsewhere in the brain was unchanged. Similarly, Erikson et al. (1997) showed that during Fe deficiency, the hippocampus and cortex were the only brain regions of eight studied to lose significant amounts of Fe (57 and 67% of control, respectively), and that the hippocampus was the only region with a significant increase in transferrin. In addition, several other Fe regulatory proteins have been shown to be altered in the hippocampus and cortex of Fe deficient rat pups while normal in the striatum and other brain regions (Siddappa et al., 2003). In rats, a specific pattern of reduced energy metabolism occurs during Fe deficiency, wherein cytochrome c oxidase is reduced in the hippocampus and cingulate cortex–including six of seven regions known to be involved in explicit memory–while normal in other regions (de Deungria et al., 2000). In parallel, the cognitive behavioral effects of Fe deficiency appear to be unique to hippocampal dysfunction (McEchron et al., 2005; Schmidt et al., 2007). Thus, nutritional models and now our genetic analyses show that hippocampal Fe and Zn regulation are tied to that in the periphery. Similar nutritional studies on Cu have not been performed to our knowledge.

Hippocampal Fe, Cu, and Zn Regulation in a Whole-Brain Context

While hippocampal Fe, Cu, and Zn concentrations are correlated with systemic iron status, they are largely unrelated to

their concentrations elsewhere in the brain, supporting evidence for local regulation (Jones et al., 2003, 2006, 2007). An exception was striatal Cu in female mice, which was positively correlated with HIPP Fe and Cu (Jones et al., 2006). This could represent common regulatory influences on Cu in these two regions; however, evidence for this is lacking, as only one suggestive QTL for striatal Cu in female mice was identified and its location was on chromosome 12, not chromosomes 9 or 14 as are the QTL for HIPP Cu (Jones et al., 2006). Also, no correlation was observed for striatal Cu in male mice. Although the correlations with striatal Cu in female mice are moderately strong, their functional significance is not readily apparent. On the other hand, the general lack of correlation between metals in the hippocampus and those in other brain regions is not surprising, as Fe, Cu, and Zn are regionally-distributed in the brain (Tarohda et al., 2004; Becker et al., 2005; Jones et al., 2003, 2006; Yoo et al., 2007).

Given the evidence for local regulation, we expected the HIPP-related QTLs we identified to be unique to this region. To the contrary, the HIPP-related QTLs on chromomes 9 and 14 were indeed associated (albeit weakly) with these metals in several other regions. The weakness of these associations reflects an increased likelihood that they were spurious associations, a result of high Type I error risk; yet, the overlap of these QTL could reflect functional influences of these two loci on Fe, Cu, and Zn in areas outside of the hippocampus. Regional concentrations of these metals are likely to be influenced by many polymorphic genes with a range of effect sizes. Such genes could be specifically expressed in one or two regions or expressed throughout the brain. In contrast to our current findings, our previous work showed modest correlations between some brain regions in the concentrations of these metals (Jones et al., 2003, 2006). Thus, while local influences likely drive the regional specificity in concentrations, the moderate covariance between regions we observed suggested that to some extent, general influences on Fe, Cu, and Zn concentrations exist in the brain. The LOD scores of the two QTLs we have currently identified reflect that they are most robustly associated with HIPP Cu; yet, it is possible that the chromosome 14 QTL has a general influence on Fe, Cu, and Zn throughout the brain and that the chromosome 9 QTL has influence on NA Cu in male mice in addition to its influence on HIPP Cu and Zn.

Another point to consider, as concerns shared associations with the chromosome 9 and 14 QTLs, is coregulation. One of our notable observations was that hippocampal Fe, Cu, and Zn concentrations were highly interrelated. This finding is consistent with similar findings in other brain regions and raises the possibility that these metals are coregulated to some extent, i.e., influenced by common genetic loci (Jones et al., 2003, 2006). Indeed, our results were consistent with this. While the two QTL we identified were most strongly related to HIPP Cu; they were additionally associated with hippocampal Fe (chr. 14) and Zn (chr. 9) in the hippocampus and, in the case of the chromosome 14 QTL, throughout the brain. This suggests nonspecific influences on these metals. At the same time, the association of these QTL with Fe and Zn was not as strong as their association with Cu. Thus, the sharing of the QTLs could alternatively reflect an indirect relationship as a product of covariance between the metals. Nonetheless, we found this worthy of note in light of the implications that would follow identification of polymorphic QTL that influence Fe, Cu, and Zn; coregulation would necessitate a systems perspective in studying the roles of these metals in neurological disease and the effects of nutritional deficiency.

Overlap Between HIPP Cu-Related QTL and QTL for Alzheimer's and Prion-Related Disease Phenotypes

The most remarkable feature of the Cu-related QTL on chromosomes 9 and 14 is that their locations coincide with two QTL previously reported to be associated with a separate-but plausibly related-phenotype (Krezowski et al., 2004). Krezowski et al. (2004) investigated an unexplained effect in a mouse model of Alzheimer's disease, in which overexpression of amyloid precursor protein (APP) in the FVB/NCr inbred strain led to a high percentage of premature deaths compared to overexpression of this protein in other inbred strains. The cause of death in these mice remains unknown but it was not associated with amyloid beta levels. Other strains varied in their susceptibility to the lethality effect of APP overexpression; for example, creating an F1 generation from FVB/NCr and 129S6 backgrounds was protective, but a cross between FVB/NCr and the B6 background produced F1 mice with susceptibility to the lethality effect (Carlson et al., 1997). By performing a linkage analysis using crosses between Tg(APP) FVB/NCr and 129S6 strains, the researchers determined that QTL on chromosomes 9 (~42.2 Mb) and 14 (~24.9 Mb) were associated with age at death in the APP overexpressing mice. The authors found no obvious candidate genes but discussed the possibility that disruption of Cu homeostasis and/or Cu/Zn superoxide dismutase (SOD1) pathways could lead to reduced resistance against oxidative stress and result in neurodegeneration. In fact, overexpression of SOD1 has been shown to protect against the APP-overexpression lethality (Carlson et al., 1997). Furthermore, disrupted Cu homeostasis has been implicated in lethality by the findings that Tg(APP) mice have lower Cu concentrations in the brain than controls and that increasing brain Cu protects against early death in Tg(APP) mice (White et al., 1999; Bayer et al., 2003; Phinney et al., 2003). In this study we reported that B6 mice, which are among the strains susceptible to APP-related lethality, are relatively low in hippocampal Cu compared to their D2 counterparts. All of these findings point to an involvement of reduced Cu levels and SOD1 activity in the lethal effects of APP overexpression in certain inbred strains.

A link between Cu homeostasis, SOD1 activity, and APP processing was recently reported as concerns a Cu-binding proteinase, β-site APP-cleaving enzyme 1 (BACE1) (Dingwall, 2007). BACE1 is involved with APP cleavage into β-amyloid as well as the regulation of SOD1 activity, via interactions with the Cu chaperone for SOD1, CCS (Angeletti et al., 2005). Interestingly, human polymorphisms in BACE1 are associated

with Alzheimer's disease (Clarimón et al., 2003; Kan et al., 2005). It was then much to our surprise to find that the gene for BACE1 is located at 45.6 Mb on chromosome 9, thus is located within the QTL interval identified for both Cu and APP-related premature death susceptibility. BACE1 therefore provides an exciting candidate gene for future investigation. Other candidate genes include a *cis*-regulated Riken gene of unknown function at 43.0 Mb and Lrrc35 at 42.2 Mb, both of which have expression negatively related to HIPP Fe, Cu, and Zn in male mice.

As a side note, as Krezowski et al. (1989) discussed, the region of the chromosome 9 QTL is also significantly associated with incubation time for scrapie, a prion-related disease. We found this worthy of mention because scrapie and other prion-related neurodegenerative diseases are also related to Cu homeostasis, in that prions are Cu-binding proteins. The role Cu plays in prion function, however, is not yet clear (Leach et al., 2006).

Although the chromosome 9 QTL for Cu (40.2 Mb) is located in nearly the same location as the QTL for APP-related lethality (∼42.2 Mb), the chromosome 14 QTL for Cu (∼38.7 Mb) is located slightly more distal than the peak of the APP-related QTL on 14 (∼24.9 Mb). The upper flanking marker for that QTL is at 60.3 Mb, showing overlap with our QTL. Genes nearby the QTL on chromosome 14 include the highly polymorphic neuregulin 3 with 649 SNPs (37.2 Mb), *grid1*, a glutamate receptor with 1,048 SNPs (33.6 Mb), and a calcium-dependent voltage gated channel, *cacna2d3*, which is located at 27.7 Mb. Each of these genes is important for hippocampal function but none have related expression or are particularly striking as candidate genes based on function. Many other genes also lie within the large interval of this QTL. The only gene we identified that was *cis*-regulated, related to HIPP Fe, Cu, and Zn, and positioned near the chromosome 14 QTL was *Samd4*. This gene is therefore a candidate but has no known connection with Cu regulation. Thus, while the chromosome 14 QTL was highly significant in both our study and the study of Krezowski et al. (1989), suggesting it has a large effect on our traits, future work is necessary to identify candidate genes within the interval.

Because of the functional relevance of Cu in APP activity, it seems unlikely that both of the QTL we identified for Cu overlap with the QTL for APP-over expression-related lethality by coincidence alone. On the basis of these observations, we hypothesize that the QTL on chromosomes 9 and possibly 14 indirectly influence susceptibility to the lethal effects of APP via alterations in Cu homeostasis. The involvement of BACE1 is not ruled out. If our hypothesis is correct, the fact that these QTL have been identified using both inbred crosses and a RI strain panel would point to a robust genetic effect. Krezowski et al. (1989) suggest that, although lethality in transgenic mice has little direct relevance to Alzheimer's Disease in humans, the genetic pathways involved in this severe response to APP over expression may be similar to human pathways involved in susceptibility to the neurodegenerative effects of this disease. If altered Cu homeostasis underlies the lethality observed in Tg(APP) FVB/NCr mice, it follows, according to their suggestion, that this would support disrupted Cu homeostasis as a factor in Alzheimer's disease etiology. The link between Cu homeostasis, APP processing, and Alzheimer's disease was discussed recently by Bayer and Muthaup (2005), and a role for Cu and SOD1 activity in this and other neurodegenerative diseases has been previously suggested (see Donnelly et al., 2007).

CONCLUSIONS

This study provides compelling new evidence for the link between neurobiological functioning and Fe, Cu, and Zn homeostasis. The approach is new, i.e., by its focus on individual differences in these metals, the underlying genetic architecture and how they relate to functional measures. Although cognitive and affective behaviors are affected by dietary Fe, Cu, and Zn deprivation or overload, this is the first study to report that naturally occurring variation in these metals in the hippocampus is relevant to behavior. This study also identified two QTL for Cu homeostasis in the hippocampus that should open up discussion about Cu's possible involvement in susceptibility to APP-related death in transgenic mice and/or the incubation time of prion-related diseases. Of course, QTL analysis implies that the genes in the interval be identified and although progress to date has been slow in this aspect, nevertheless, new techniques including SNP and haplotype analysis (Mehrian-Shai and Reichardt, 2004) together with gene expression (Jansen and Nap, 2001; Hitzemann et al., 2004) and multipoint strain comparisons (Churchill et al., 2004; Shirley et al., 2004) are speeding up the progress.

Acknowledgments

We would like to thank John Kittleson for his technical expertise and assistance with data collection. We would also like to acknowledge those involved in developing and maintaining GeneNetwork (http://www.genenetwork.org), which provided invaluable access to vast genomic resources for the analysis of our data.

REFERENCES

Angeletti B, Waldron KJ, Freeman KB, Bawagan H, Hussain I, Miller CC, Lau KF, Tennant ME, Dennison C, Robinson NJ, Dingwall C. 2005. BACE1 cytoplasmic domain interacts with the Cu chaperone for superoxide dismutase-1 and binds Cu. J Biol Chem 280:17930–17937.

Banisadr G, Skrzydelski D, Kitabgi P, Rostene W, Parsadaniantz SM. 2003. Highly regionalized distribution of stromal cell-derived factor-1/CXCL12 in adult rat brain: Constitutive expresssion in chollinergic, dopaminergic and vasopressinergic neurons. Eur J Neurosci 18:1593–1606.

Bayer TA, Muthaup G. 2005. Involvement of amyloid β precursor protein (AbetaPP) modulated Cu homeostasis in Alzheimer's disease. J Alzheimer's Dis 8:201–206.

Bayer TA, Schäfer S, Simons A, Kemmling A, Kamer T, Tepest R, Eckert A, Schüssel K, Eikenberg O, Sturchler-Pierrat C, Abramowski D, Staufenbiel M, Multhaup G. 2003. Dietary Cu stabilizes brain superoxide dismutase 1 activity and reduces amyloid Aβ production in APP23 transgenic mice. Proc Natl Acad Sci 100: 14187–14192.

Belknap JK. 1998. Effect of within-strain sample size on QTL detection and mapping using recombinant inbred mouse strains. Behav Genet 28:29–38.

Beard JL, Hendricks MK, Perez EM, Murray-Kolb LE, Berg A, Vernon-Feagans L, Irlam J, Isaacs W, Sive A, Tomlinson M. 2005. Maternal Fe deficiency anemia affects postpartum emotions and cognition. J Nutr 135:267–272.

Becker JS, Zoriy MV, Pickhardt C, Palomero-Gallagher N, Zilles K. 2005. Imaging of Cu, Zn, and other elements in thin section of human brain samples (hippocampus) by laser-ablation inductively-coupled plasma mass spectrometry. Anal Chem 77:3208–3216.

Brown CE, Dyck RH. 2004. Distribution of Zincergic neurons in the mouse forebrain. J Compar Neurol 479:156–167.

Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. 1996. Randomised study of cognitive effects of Fe supplementation in non-anemic Fe-deficient adolescent girls. Lancet 348:992–996.

Carlson GA, Borchelt DR, Dake A, Turner S, Danielson V, Coffin JD, Eckman C, Meiners J, Nilsen SP, Younkin SG, Hsiao KK. 1997. Genetic modification of the phenotypes produced by amyloid precursor protein overexpression in transgenic mice. Hum Mol Genet 6:1951–1959.

Carlson ES, Stead JDH, Neal CR, Petryk A, Georgieff MK. 2007. Pernatal iron deficiency results in altered developmental expression of genes mediating energy metabolism and neuronal morphogenesis in hippocampus. Hippocampus 17:679–691.

Chesler EJ, Lu L, Shou S, Qu Y, Gu J, Wang J, Hsu HC, Mountz JD, Baldwin N, Langston MA, Threadgill DW, Manly KF, Williams RW. 2005. Genetic dissection of gene expression reveals polygenic and pleiotropic networks modulating brain structure and function. Nat Genet 37:233–242.

Choi CJ, Kanthasamy A, Anantharam V, Kanthasamy AG. 2006. Interaction of metals with prion protein: Possible role of divalent cations in the pathogenesis of prion diseases. Neurotoxicology 27: 777–787.

Chu Y, Mouat MF, Harris RB, Coffield JA, Grider A. 2003. Water maze performance and changes in serum corticosterone levels in Zn-deprived and pair-fed rats. Physiol Behav 78:569–578.

Churchill GA, Airey DC, Allayee H, Angel JM, Attie AD, Beatty J, Beavis WD, Belknap JK, Bennett B, Berrettini W, Bleich A, Bogue M, Broman KW, Buck KJ, Buckler E, Burmeister M, Chesler EJ, Cheverud JM, Clapcote S, Cook MN, Cox RD, Crabbe JC, Crusio WE, Darvasi A, Deschepper CF, Doerge RW, Farber CR, Forejt J, Gaile D, Garlow SJ, Geiger H, Gershenfeld H, Gordon T, Gu J, Gu W, de Haan G, Hayes NL, Heller C, Himmelbauer H, Hitzemann R, Hunter K, Hsu HC, Iraqi FA, Ivandic B, Jacob HJ, Jansen RC, Jepsen KJ, Johnson DK, Johnson TE, Kempermann G, Kendziorski C, Kotb M, Kooy RF, Llamas B, Lammert F, Lassalle JM, Lowenstein PR, Lu L, Lusis A, Manly KF, Marcucio R, Matthews D, Medrano JF, Miller DR, Mittleman G, Mock BA, Mogil JS, Montagutelli X, Morahan G, Morris DG, Mott R, Nadeau JH, Nagase H, Nowakowski RS, O'Hara BF, Osadchuk AV, Page GP, Paigen B, Paigen K, Palmer AA, Pan HJ, Peltonen-Palotie L, Peirce J, Pomp D, Pravenec M, Prows DR, Qi Z, Reeves RH, Roder J, Rosen GD, Schadt EE, Schalkwyk LC, Seltzer Z, Shimomura K, Shou S, Sillanpää MJ, Siracusa LD, Snoeck HW, Spearow JL, Svenson K, Tarantino LM, Threadgill D, Toth LA, Valdar W, de Villena FP, Warden C, Whatley S, Williams RW, Wiltshire T, Yi N, Zhang D, Zhang M, Zou F. 2004. The collaborative cross, a community resource for the genetic analysis of complex traits. Nat Genet 36:1133–1137.

Clarimón J, Bertranpetit J, Calafell F, Boada M, Tɔrraga L, Comas D. 2003. Association study between Alzheimer's disease and genes involved in Aβ biosynthesis, aggregation and degradation: suggestive results with BACE1. J Neurol 250:956–961.

Clegg MS, Keen CL, Lonnerdal B, Hurley LS. 1981. Influence of ashing techniques on the analysis of trace elements in animal tissue. Biol Trace Elem Res 3:107–115.

Crabbe JC, Kosobud A, Young ER, Janowsky JS. 1983. Polygenic and single-gene determination of responses to ethanol in BXD/Ty recombinant inbred mouse strains. Neurobehav Toxicol Teratol 5:181–187.

de Deungria M, Rao R, Wobken JD, Luciana M, Nelson CA, Georgieff MK. 2000. Perinatal Fe deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain. Pediatr Res 48:169–176.

Dingwall C. 2007. A Cu-binding site in the cytoplasmic domain of BACE1 identifies a possible link to metal homeostasis and oxidative stress in Alzheimer's disease. Biochem Soc Trans 35:571–573.

Doroulee N, Yanovky Y, Haas H. 1997. Suppression of long-term potentiation in hippocampal slices by Cu. Hippocampus 7:666–669.

Donnelly PS, Xiao Z, Wedd AG. 2007. Cu and Alzheimer's disease Curr Opin Chem Biol 11:128–133.

Erikson KM, Pinero DJ, Connor JR, Beard JL. 1997. Regional brain Fe, ferritin, and transferrin concentrations during Fe deficiency and Fe repletion in developing rats. J Nutr 127:2030–2038.

Felt BT, Lozoff B. 1996. Brain Fe and behavior of rats are not normalized by treatment of Fe deficiency anemia during early development. J Nutr 126:693–701.

Felt BT, Beard JL, Schaller T, Shao J, Aldridge J, Connor JR, Georgieff MK, Lozoff B. 2006. Persistent neurochemical and behavioral abnormalities in adulthood despite early Fe supplementation for perinatal Fe deficiency anemia in rats. Behav Brain Res 10:261–270.

Flynn C, Brown CE, Galasso SL, McIntyre DC, Teskey GC, Dyck RH. 2007. Znergic innervation of the forebrain distinguishes epilepsy-prone from epilepsy-resistant rat strains. Neuroscience 144: 1409–1414.

Frederickson CJ, Koh J-Y, Bush AI. 2005. The neurobiology of Zn in health and disease. Nat Rev Neurol 6:449–462.

Gaggelli E, Kozlowski H, Valensin D, Valensin G. 2006. Cu homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). Chem Rev 106: 1995–2044.

Goldschmith A, Infante C, Leiva J, Motles E, Palestini M. 2005. Interference of chronically ingested Cu in long-term potentiation (LTP) of rat hippocampus. Brain Res 1056:176–182.

Golub MS, Takeuchi PT, Keen CL, Gershwin ME, Hendrickx AG, Lonnerdal B. 1994. Modulation of behavioral performance of prepubertal monkeys by moderate dietary Zn deprivation. Am J Clin Nutr 60:238–243.

Golub MS, Keen CL, Gershwin ME, Hendrickx AG. 1995. Developmental Zn deficiency and behavior. J Nutr 125:2263S–2271S

Groner JA, Holtzman NA, Charney E, Mellits ED. 1986. A randomized trial of oral Fe on tests of short term memory and attention span in young pregnant women. J Adolesc Health Care 7:44–48.

Halas ES, Eberhardt MJ, Diers MA, Sandstead HH. 1983. Learning and memory impairment in adult rats due to severe Zn deficiency during lactation. Physiol Behav 30:371–381.

Halas ES, Hunt CD, Eberhardt MJ. 1986. Learning and memory disabilities in young adult rats from mildly Zn deficient dams. Physiol Behav 37:451–458.

Hirate M, Takeda A, Tamano H, Enomoto S, Naoto O. 2002. Distribution of trace elements in the brain of EL (epilepsy) mice. Epilepsy Res 51:109–116.

Hitzemann R, Reed C, Malmanger B, Lawler M, Hitzemann B, Cunningham B, McWeeney S. 2004. On the integration of alcohol-related quantitative trait loci and gene expression analyses. Alcohol Clin Exp Res 28:1437–1448.

Holmes A, Yang R. 2007. Strain anxiety- and depression-like phenotypes. Unpublished data available on Genenetwork.org.

Hunt CD, Idso JP. 1995. Moderate Cu deprivation during gestation and lactation affects dentate gyrus and hippocampal maturation in immature male rats. J Nutr 124:2700–2710.

Irizarri RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. 2003. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 31:e15

Izumi Y, Auberson YP, Zorumski CF. 2006. Zn modulates bidirectional hippocampal plasticity by effects on NMDA receptors. 26: 7181–7188.

Jansen RC, Nap JP. 2001. Genetical genomics: The added value from segregation. Trends Genet 17:388–391.

Jones BC, Reed CL, Hitzemann R, Wiesinger JA, McCarthy KA, Buwen JP, Beard JL. 2003. Quantitative genetic analysis of ventral midbrain and liver Fe in BXD recombinant inbred mice. Nutr Neurosci 6:369–377.

Jones LC, McCarthy KA, Beard JL, Keen CL, Jones BC. 2006. Quantitative genetic analysis of brain Cu and Zn in BXD recombinant inbred mice. Nutr Neurosci 9:81–92.

Jones BC, Beard JL, Gibson JN, Unger EL, Allen RP, McCarthy KA, Earley CJ. 2007. Systems genetic analysis of peripheral Fe parameters in the mouse. Am J Physiol Regul Integr Comp Physiol 293: R116–R124.

Jorgenson LA, Sun M, O'Connor M, Georgieff MK. 2005. Fetal Fe deficiency disrupts the maturation of synaptic function and efficacy in area CA1 of the developing rat hippocampus. Hippocampus 15:1094–1102.

Kaleda SN, Shelton E, Kaufmann RB, Khoury MJ. 2001. Delta-aminolevulinic acid dehydratase genotype and lead toxicity: A HuGE review. Am J Epidemiol 154:1–13.

Kan R, Wang B, Zhang C, Jin F, Yang Z, Ji S, Lu Z, Zheng C, Wang L. 2005. Genetic association of BACE1 gene polymorphism C786G with late-onset Alzheimer's disease in Chinese. J Mol Neurosci 25:127–131.

Krezowski J, Knudson D, Ebeling C, Pitstick R, Giri R, Schenk D, Westaway D, Younkin L, Younkin SG, Ashe KH, Carlson GA. 2004. Identification of loci determining susceptibility to the lethal effects of amyloid precursor protein transgene overexpression. Human Mol Genet 13:1989–1997.

Kodama H, Murata Y, Kobayashi M. 1999. Clinical manifestations and treatment of Menkes disease and its variants. Pediatr Int 41:423–429.

Kwik-Uribe CL, Golub MS, Keen CL. 2000. Chronic marginal Fe intakes during early development in mice alter brain Fe concentrations and behavior despite postnatal Fe supplementation. Nutr Neurosci 130:2040–2048.

Leach SP, Salman MD, Hamar D. 2006. Trace elements and prion diseases: a review of the interactions of copper, manganese and zinc with the prion protein. Anim Health Res Rev 7:97–105

Leiva J, Palestini M, Gaete P. 2003. Cu interaction on the long-term potentiation. Arch Ital Biol 141:149–155.

Levenson CW. 2005. Trace metal regulation of neuronal apopotosis: From genes to behavior. Physiol Behav 85:399–406.

Linder MC, Hazegh-Azam M. 1996. Copper biochemistry and molecular biology. Am J Clin Nutr 63:797S–811S.

McCall RD, Frierson DD. 1981. Evidence that two loci predominantly determine the difference in susceptibility to the high pressure neurologic syndrome type I seizure in mice. Genetics 99:285–307.

McEchron MD, Cheng AY, Liu H, Connor JR, Cilmartin MR. 2005. Perinatal nutritional Fe deficiency permanently impairs hippocampus-dependent trace fear conditioning in rats. Nutr Neurosci 8:195–206.

McEchron MD, Paronish MD. 2005. Perinatal nutritional Fe deficiency reduces hippocampal synaptic transmission but does not impair short- or long-term synaptic plasticity. Nutr Neurosci 8: 277–285.

Mehrian-Shai R, Reichardt JK. 2004. A renaissance of "biochemical genetics"? SNPs haplotypes, function, and complex diseases. Mol Genet Metab 83:47–50.

Milhaud JM, Halley H, Lassalle JM. 2002. Two QTLs located on chromosomes 1 and 5 modulate different aspects of the performance of mice of the BXD Ty RI strain series in the morris navigation task. Behav Genet 32:69–78.

Morse AC, Beard JL, Jones BC. 1999. A genetic developmental model of iron deficiency: Biological aspects. PSEBM 220:147–152.

Murray-Kolb LE, Beard JL. 2007. Fe treatment normalizes cognitive functioning in young women. Am J Clin Nutr 85:778–787.

Patrick LR. 2007. Restless legs syndrome: Pathophysiology and the role of Fe and folate. Altern Med Rev 12:101–112.

Phinney AL, Drisaldi B, Schmidt SD, Lugowski S, Coronado V, Liang Y, Horne P, Yang J, Sekoulidis J, Coomaraswamy J, Chishti MA, Cox DW, Mathews PM, Nixon RA, Carlson GA, St George-Hyslop P, Westaway D. 2003. In vivo reduction of amyloid-β by a mutant Cu transporter. Proc Natl Acad Sci USA 100:14193–14198.

Pietrangelo A. 2006. Hereditary hemochromatosis. Biochim Biophys Acta 1763:700–710.

Plomin R, McClearn GE, Gora-Maslak G, Neiderhiser JM. 1991. Use of recombinant inbred strains to detect quantitative trait loci associated with behavior. Behav Genet 21:99–116.

Portala K, Levander S, Westermark K, Ekselius L, von Knorring L. 2001. Pattern of neuropsychological deficits in patients with treated Wilson's disease. Eur Arch Psych Clin Neurosci 251:262–268.

Rao R, Tkac I, Townsend EL, Gruetter R, Georgieff MK. 2003. Perinatal Fe deficiency alters the neurochemical profile of the developing rat hippocampus. J Nutr 133:3215–3221.

Sandstead HH, Fosmire GJ, Halas ES, Jacob RA, Strobel DA, Marks EO. 1977. Zn deficiency: Effects on brain and behavior of rats and rhesus monkeys. Teratology 16:229–234.

Schenck JF, Zimmerman EA, Li Z, Adak S, Saha A, Tandon R, Fish KM, Belden C, Gillen RW, Barba A, Henderson DL, Neil W, O'Keefe T. 2006. High-field magnetic resonance imaging of brain Fe in Alzheimer disease. Top Magn Reson Imaging 17:41–50.

Shcherbatykh I, Carpenter DO. 2007. The role of metals in the etiology of Alzheimer's disease. J Alzheimers Dis 11:191–205.

Schmidt AT, Waldow KJ, Grove WM, Salinas JA, Georgieff MK. 2007. Dissociating the long-term effects of fetal/neonatal Fe deficiency on three types of learning in the rat. Behav Neurosci 121: 475–482.

Schlief ML, West T, Craig AM, Holtzman DM, Gitlin JD. 2006. Role of the Menkes Cu-transporting ATPase in NMDA-receptor-mediated neuronal toxicity. Proc Natl Acad Sci USA 103:14919–14924.

Sen A, Kanani SJ. 2006. Deleterious functional impact of anemia on young adolescent girls. Indian Pediatr 43:219–226.

Shirley RL, Walter NA, Reilly MT, Fehr C, Buck KJ. 2004. Mpdz is a quantitative trait gene for drug withdrawal seizures. Nat Neurosci 7:699–700.

Siddappa AJ, Rao RB, Wobken JD, Casperson K, Leibold EA, Connor JR, Georgieff MK. 2003. Fe deficiency alters Fe regulatory protein and Fe transport protein expression in the perinatal rat brain. Pediatr Res 53:800–807.

Siddappa AM, Georgieff MK, Wewerka S, Worwa C, Nelson CA, Deregnier RA. 2004. Fe deficiency alters auditory recognition memory in newborn infants of diabetic mothers. Pediatr Res 55: 1034–1041.

Skrzydelski D, Guyon A, Daugé V, Rovère C, Apartis E, Kitabgi P, Nahon JL, Rostène W, Parsadaniantz SM. 2007. The chemokine stromal cell-derived factor-1/CXCL12 activates the nigrostriatal dopamine system. J Neurochem 102:1175–1183.

Smith AP, Lee NM. 2007. Role of Zn in ALS. Amyotroph Lateral Scler 8:131–143.

Takeda A, Takefuta S, Okada S, Oku N. 2000. Relationship between brain Zn and transient learning impairment of adult rats fed Zn-deficient diet. Brain Res 859:352–357.

Takeda A, Minami A, Takefuta S, Tochigi M, Oku N. 2001. Zn homeostasis in the brain of adult rats fed Zn-deficient diet. J Neurosci Res 63:447–52.

Takeda A, Hirate M, Tamano H, Oku N. 2003. Release of glutamate and GABA in the hippocampus under Zn deficiency. J Neurosci Res 72:537–542.

Takeda A, Tamano H, Tochigi M, Oku N. 2005. Zinc homeostasis in the hippocampus of zinc-deficient young-adult rats. Neurochem Int 46:221–225.

Takeda A, Tamano H, Kan F, Itoh H, Oku N. 2007. Anxiety-like behavior of young rats after 2-week Zn deprivation. Behav Brain Res 177:1–6.

Takeda A, Yamada K, Tamano H, Fuke S, Kawamura M, Oku N. 2008. Hippocampal calcium dyshomeostasis and long-term potentiation in 2-week zinc deficiency. Neurochem Int 52:241–246.

Tarohda T, Yamamoto M, Amano R. 2004. Regional distribution of manganese, Fe, Cu, and Zn in the rat brain during development. Anal Bioanal Chem 380:240–246.

Thompson J, Jones DD, Beasley WH. 1977. The effect of metal ions on the activity of delta-aminolevulinic acid dehydratase. Br J Ind Med 34:32–36.

Unger EL, Paul T, Murray-Kolb LE, Felt B, Jones BC, Beard JL. 2007. Early iron deficiency alters sensorimotor development and brain monoamines in rats. J Nutrit 137:118–124.

Wang J, Williams RW, Manly KF. 2003. WebQTL: Web-based complex trait analysis. Neuroinformatics 1:299–308.

White AR, Reyes R, Mercer JF, Camakaris J, Zheng H, Bush AI, Multhaup G, Beyreuther K, Masters CL, Cappai R. 1999. Cu levels are increased in the cerebral cortex and liver of APP, APLP2 knockout mice. Brain Res 842:439–444.

Williams RW, Gu J, Qi S, Lu L. 2001. The genetic structure of recombinant inbred mice: High-resolution consensus maps for complex trait analysis. Genet Biol 2:46.1–46.18

Yoo KY, Hwang IK, Kang IJ, Kang TC, Lee HJ, Kang HY, Lee HY, Oh YS, Won MH. 2007. Age-dependent changes in Fe deposition in the gerbil hippocampus. Exp Anim 56:21–28.

Zucconi GG, Cipriani S, Scattoni R, Balgkouranidou I, Hawkins DP, Ragnarsdottir KV. 2007. Copper deficiency elicits glial and neuronal response typical of neurodegenerative disorders. Neuropathol Appl Neurobiol 33:212–225.