Published in final edited form as:

Biochimie. 2009 October ; 91(10): 1255–1259. doi:10.1016/j.biochi.2009.04.009.

Systems genetics analysis of iron regulation in the brain

Leslie C. Jellen¹, John L. Beard², and Byron C. Jones^{1,*}

¹ Neuroscience Graduate Program, Pennsylvania State University, University Park, PA

² Department of Nutrition, Pennsylvania State University, University Park, PA

Abstract

Iron imbalances in the brain, including excess accumulation and deficiency, are associated with neurological disease and dysfunction; yet, their origins are poorly understood. Using systems genetics analysis, we have learned that large individual differences exist in brain iron concentrations, even in the absence of neurological disease. Much of the individual differences can be tied to the genetic makeup of the individual. This genetic-based differential regulation can be modeled in genetic reference populations of rodents. The work in our laboratory centers on iron regulation in the brain and our animal model consists of 25 BXD/Ty recombinant inbred mouse strains. By studying naturally occurring variation in iron phenotypes, such as tissue iron concentration, we can tie that variability to one or more genes by way of quantitative trait loci (QTL) analysis. Moreover, we can conduct genetic correlation analyses between our phenotypes and others previously measured in the BXD/Ty strains. We have observed several suggestive QTL related to ventral midbrain iron content, including one on chromosome 17 that contains *btd9*, a gene that in humans has been associated with restless legs syndrome and serum ferritin. We have also observed gene expression correlations with ventral midbrain iron, including *btd9* expression and dopamine receptor expression. In addition, we have observed significant correlations between ventral midbrain iron content and dopamine-related phenotypes. The following is a discussion of iron regulation in the brain and the contributions a systems genetics approach can make toward understanding the genetic underpinnings and relation to neurological disease.

Keywords

iron; genetics; mouse; brain; RLS

Introduction

Iron imbalances in the brain, including excess accumulation and deficiency, are associated with neurological disease and dysfunction; yet, their origins are poorly understood. In particular, the genetic influences on these imbalances have not been identified. Over the past five years, using the systems genetics approach and a genetic reference animal model, we have learned a great deal about individual differences in iron homeostasis that show the importance of genetic background in determining an individual's iron profile in the brain and in the periphery. The following review aims to illustrate the utility of a systems genetics approach to complex trait

*Corresponding author: Byron C. Jones, Department of Biobehavioral Health, 315 E. HHD Building, University Park, PA 16802, Telephone: 814-863-0167, E-mail: bcj1@psu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

analysis using iron homeostasis as an example. The review will first broadly discuss the role of iron in neurological disease, and then discuss evidence for genetic-based individual differences in brain iron homeostasis that could contribute to iron-related neuropathology.

Iron Regulation in the Brain

Iron homeostasis is essential to the functioning of the central nervous system (CNS). In the brain, sufficient iron supply is critical to a wide variety of biochemical pathways, ranging from basic cellular metabolism to catecholamine synthesis and myelination [1,2]. Iron is also involved in the production of reactive oxygen species (ROS) and can be toxic in excess [3]. Thus, both iron overload and deficiency must be avoided, yet imbalances do occur in some individuals for reasons that are yet unknown. Iron overload in relevant brain regions is linked to Parkinson's disease [4,5] and Alzheimer's disease [6], while iron deficiency is associated with restless legs syndrome (RLS) [7] and attention deficit hyperactivity disorder [8]. Iron deficiency also leads to specific cognitive impairment and emotional effects, which vary across developmental stages [9,10].

A complex network of iron regulatory genes and proteins manages iron's distribution and homeostasis. This network has been well studied but is not yet completely understood, particularly as concerns the brain. A detailed presentation of iron regulation is beyond the scope of this review, but, in short, iron homeostasis involves absorption, distribution, storage, and export. Iron sensors that signal as iron levels fluctuate must coordinate all of these processes. Many genes are required at each level of iron homeostasis, including the genes for transferrin, the transferrin receptors and divalent metal transporter 1, the storage genes for heavy and light chain ferritin, genes in the iron export pathways including those for HFE, the bone morphogenic proteins, hepcidin, and ferroportin, and genes involved in iron signaling, i.e. those with iron responsive elements that interact with iron responsive proteins [reviewed in 5]. While this list is not all-inclusive, it demonstrates that iron homeostasis is a polygenic trait with several levels of regulation, each of which presents a possible avenue for malfunctioning of the system. For instance, in one genetic disorder of iron overload, hemochromatosis, the classic mutation associated with the disease is a mutation of HFE that decreases hepcidin expression. However, penetrance is highly variable, suggesting involvement of other factors [11]. It was recently shown in one severe case that in addition to being homozygous for the HFE mutation, one patient had another polymorphism in the promoter region of hepcidin, in a BMP responsive element [12]. This polymorphism led to further decreased expression of hepcidin and impaired the interaction of hepcidin with the BMP pathway. Thus, three components of the iron regulatory system were malfunctioning at once, all reducing hepcidin functioning and contributing to severe iron overload. This is the nature of a complex phenotype, and as such, iron homeostasis must be studied from a systems perspective.

Iron regulation in the brain involves the same iron regulatory genes as the periphery, but is unique in two very important ways. First, while the iron regulatory network must be able to supply iron to the circulating plasma and deliver iron to bodily tissues, it must also be able to transport iron across the blood brain barrier (BBB) to enter the brain. In 2003, using a cell culture model of the BBB, Burdo and colleagues showed that iron transport in endothelial cells depends on their iron status; thus, the BBB may play the role of gatekeeper of iron transport into the brain, reducing transport when iron is high and increasing transport when iron is low [13]. Secondly, iron distribution throughout the brain is highly heterogeneous [14]. Also, the distribution of iron follows a regionally specific developmental timeline, wherein iron accumulates with age [14–17]. These together suggest local influences on iron regulation, not only from the classic iron regulatory pathways but also likely from cross talk with unrelated cellular pathways that require iron for their functioning. Thus, in investigating the molecular

mechanisms behind individual differences in brain iron homeostasis, it is important to consider brain-specific sources from both the BBB and local systems.

In sum, systemic iron regulation is well described and involves many genes. Local iron homeostatic mechanisms in the brain, on the other hand, are poorly described and could very well involve genes heretofore unnamed or not implicated in iron metabolism. For these reasons, systems genetics is an appropriate approach to identifying genetic influences on iron regulation in the brain.

Systems Genetics and the BXD/TyJ recombinant inbred strains

Systems genetics is a population-based approach to understanding complex heritable phenotypes such as drug addiction or disease [18]. This approach involves quantifying a phenotype across a population, then associating the variance in that phenotype with genetic markers in a quantitative trait loci (QTL) analysis and/or genomic variation from gene expression analyses. Instead of focusing on the effect of one gene, QTL analysis allows multiple genetic loci to be detected, including epistatic interactions. Populations of recombinant inbred strains are well suited for this purpose [19]. Recombinant inbred (RI) strains are generated when two inbred strains are crossed and the F₂ progeny are inbred for twenty consecutive generations, after which over 99% of alleles are fixed. In this way, multiple strains are established from the same two parental strains in order to create a genetic reference population. For example, the BXD/TyJ strains were derived from a pairing of C57/BL6 and DBA/2J strains. Recombinant inbred populations have several advantages for quantitative genetic research [19]. First, the progeny strains are each a unique recombination of the parental alleles. Secondly, due to the fixing of alleles, each strain provides a renewable source of biological replicates to be phenotyped for any quantifiable trait. Finally, the RI strains are genotyped and thus QTL analyses with these mice, as opposed to QTL analysis of F₂ mice, do not require genotyping. This is the case with the BXD/TyJ strains that we use to study iron homeostasis. We can use a colony of BXD/TyJ mice to measure a multitude of quantifiable phenotypic features, and then use publicly available data to correlate the genotype with the phenotype in a QTL analysis. This is all currently available on Gene Network (<http://www.genenetwork.org>), a site created for systems genetics analysis using the BXD/TyJ strains as well as other inbred and recombinant inbred strains [20]. This site features an up-to-date database of published phenotypic data and a constantly updated set of microarray data showing gene expression levels for over 40,000 genes in the 70+ BXD strains in whole brain and several brain regions. Because the BXD panel has been profiled for gene expression, QTL analysis of the gene expression as a phenotype can also expose expression QTL, or QTL that influence the expression of a given gene. The QTL, phenotypic data, and genomic data can all be integrated for QTL and correlation analysis to gain more knowledge about a phenotype in minutes than one could gather in years in an isolated laboratory.

Systems Genetics Approach: an example

The ultimate goal of QTL analysis is to identify the genes underlying the QTL that contribute to the phenotype of interest. One obstacle to QTL research is the cumbersome process of candidate gene identification when QTL confidence intervals span hundreds of genes. In the past, this obstacle has been overcome by using breeding strategies to narrow down wide confidence intervals; however, these strategies are not known for their efficiency in time and effort. An alternative candidate gene identification process is to use gene expression experiments in conjunction with the publicly available BXD gene expression datasets to identify differentially expressed genes that underlie behavioral QTL. Palmer and colleagues performed a study of methamphetamine (MA) sensitivity that demonstrated the utility of pairing gene expression experiments with the available gene expression datasets [21–23]. Having identified QTL associated with MA sensitivity, Palmer and colleagues selectively bred

two mouse lines differing in sensitivity to methamphetamine for four generations [21]. Gene expression was measured in the nucleus accumbens of fourth generation mice in the high- and low-sensitivity lines, aimed at identifying differentially expressed genes that would account for the differences in MA sensitivity. A number of genes were differentially expressed between lines, and indeed, one of these genes, Casein Kinase 1 Epsilon (Csnk1e), is known to play a functional role in a drug-related biological pathway involving dopamine in the nucleus accumbens. Using the Gene Network gene expression and phenotype databases, the researchers were then able to investigate the source of variation in Csnk1e expression, which turned out to be a cis-regulating expression QTL that co-maps with a behavioral QTL for MA responsivity on chromosome 15. Thus, a strong, testable hypothesis presented itself that an allele at this QTL influences differential MA sensitivity via differential expression of Csnk1e. Recently a role for Csnk1e in the locomotor response to MA was confirmed by administering a selective inhibitor of Csnk1e, which attenuated this response in mice [22]. In addition, a SNP has been identified in CSNK1E that is associated with increased sensitivity to d-amphetamine in humans [23]. This general approach of linking phenotype-associated QTL to differentially expressed, *cis*-regulated genes within those QTL is the model we will apply to investigating the genetic influences on brain iron homeostasis. The following is a discussion of our findings thus far.

Systems Genetics of Iron in the BXD/TyJ strains

In 2003, we published the first study to specifically address individual differences in iron regulation [24]. We used fifteen strains of the BXD/TyJ strains to measure iron concentrations in four brain regions: the ventral midbrain, caudate-putamen, nucleus accumbens, and prefrontal cortex. With the advantages of using these strains we were able to have 8–10 biological replicates in almost all of the strains, thus reducing environmental influences. We measured iron concentrations at 120 days of age in mice that had been on a standard laboratory diet containing 240 ppm iron. In QTL analysis there is no control group; rather, the strains are compared *interse*. In terms of baseline iron concentrations in the various brain regions, we found significant differences in iron concentrations in all of the regions with moderate correlations between regions. Ventral midbrain iron concentrations in this study were correlated with several dopamine-based phenotypes, including cocaine-induced hyperlocomotion ($r=-0.60$, $p<0.0156$) [25]. Microarray data on Gene Network also revealed a correlation between ventral midbrain iron and striatal dopamine D1a receptor expression ($r=0.59$, $p<0.0196$; Illumina striatum dataset; Nov, 06). QTL analysis revealed non-significant but suggestive QTL on chromosomes 3, 7, 11, 13, and 17 that were associated with ventral midbrain iron concentrations. The QTL on chromosome 17 was also associated with iron in the caudate-putamen and nucleus accumbens and has later been also associated with copper and zinc in various brain regions [26]. In several studies to follow, we confirmed that copper and zinc concentrations are positively correlated with iron concentrations in the brain. We also followed up with a study of iron, copper, and zinc in the hippocampus of over 20 strains and found significant differences and multiple QTL, but no correlation between the hippocampal iron concentrations and those in other brain regions [27]. Finally, we measured systemic iron parameters in 31 of the BXD/TyJ strains, including hemoglobin, hematocrit, plasma Fe, total iron binding capacity, liver iron, and spleen iron [28]. For each of these variables, significant differences among strains were observed in a continuous distribution across the population. Principle components analysis enabled the grouping of QTL into functional categories, such as iron transport, for example. Interestingly, the systemic variables were not correlated with brain iron concentrations. This was compatible with other evidence from clinical studies that brain iron concentrations can be independent of systemic iron status [29,30]. Overall, our work shows that even in a small population of mice generated from the same parental strains, substantial genetic-based variation in iron homeostasis exists, not only in the brain but in the periphery as well. The variation in the brain is linked to several QTL and dopamine-based phenotypes. The challenge now is to identify the genes underlying the QTL identified.

Systems genetics of iron and Restless legs syndrome

Our work shows links between iron and dopamine and may have applications for iron-related neuropathology. With the possible exception of Alzheimer's disease, iron-related neurological conditions and effects of iron deficiency can all be primarily tied to dopamine malfunction, which is interesting as dopamine and iron have been shown to be related in a number of studies [31–38]. Iron is distributed heterogeneously throughout the brain and is most highly concentrated within the substantia nigra, a region where dopaminergic cell bodies reside [32]. It is now clear that iron deficiency has a negative impact on the nigrostriatal development and maintenance of dopaminergic systems in the basal ganglia [33–38] as well as sensorimotor functioning in the rat [36]. Specifically, the D1 and D2 receptors and DA transporter (DAT) densities are reduced in the caudate-putamen of rats with neonatal iron deficiency, and D2 receptor density is also reduced in the nucleus accumbens [33,34]. Dopamine neurons in the substantia nigra are also the target of iron overload and thus provide the link between iron and Parkinson's disease [4,5]

RLS is a dopamine-based disorder related to iron deficiency in the midbrain [39]. It is a sensory-motor syndrome involving odd sensations and urges to move the lower limbs, particularly during the transition to bedtime in the evening. RLS can (but does not necessarily) also involve periodic limb movements (PLMs) during sleep. RLS is currently treated with dopamine agonists, which reduce symptoms [40].

Challenges to peripheral iron homeostasis, such as nutritional iron deficiency, pregnancy and hemodialysis-related iron deficiency anemia can produce RLS [41,42]. Alternatively, many RLS patients present with normal systemic iron status; however magnetic resonance imaging and postmortem analysis reveal low iron concentrations in the cerebrospinal fluid and substantia nigra [29,30]. Very little is known as to what exact role iron plays in the etiology of the disorder. One hypothesis is that iron deficiency in the brain impairs dopamine functioning and thus leads to the symptoms of RLS. Oral iron treatments are not always effective in treating RLS, but recent clinical studies have shown that intravenous iron dextran treatments can alleviate symptoms of RLS in some patients for up to six months [43,44].

RLS can be idiopathic or can run in families. Genetic association studies in humans have recently revealed polymorphisms within several genes that are predictive of RLS. These include *Ptprd*, *Map2k5*, *Meis1*, and *Btbd9* [45–48]. *Btbd9* was found in two independent studies to have a SNP associated with RLS [46,47]. This finding was recently replicated in a third study of three European populations [49]. In the study by Stefansson and colleagues, this association was shown to be particular to PLMs with or without RLS. Interestingly enough, Stefansson and colleagues also found an association between *btbd9* and serum ferritin, a measure of bodily iron stores [46]. The function of *BTBD9* is not well characterized, and none of the genes associated with RLS are known iron regulatory genes [46]. Understanding how these polymorphisms may play into altered iron homeostasis and the PLMs of RLS is the current challenge.

In an example of how our work can be applied to neurological disease etiology, the mouse homolog, *btbd9*, is positioned within one of the QTL we associated with iron concentrations in the ventral midbrain [24]. This QTL envelops a region of mouse chromosome 17 that is homologous to human chromosome 6p, which contains *BTBD9*. In addition, our data in the BXD strains show that striatal expression of the mouse homolog, *btbd9*, is positively correlated with iron concentrations in the ventral midbrain. It is also correlated with iron in the caudate-putamen and nucleus accumbens, and with copper and zinc in the brain. Shown in figure 1 are the strain values for iron concentrations in the ventral midbrain plotted against Illumina-based striatal expression of probe ILM101980400 in the promoter region of *Btbd9* (<http://genenetwork.org>). The correlation is $r=0.73$, $p<0.001$. While the microarray data must

be validated, the correlation is quite interesting in consideration of the association of a variant allele of *BTBD9* with RLS and serum ferritin and the QTL in the *btbd9* region that is also related to ventral midbrain iron. We are currently following up on validating the correlations observed with this gene and will continue to search for links between our work and genetic analyses in humans that may be helpful in generating animal models of disease.

Future directions: candidate gene selection with gene expression data

The studies leading up to identification of CSNK1E as a QTgene for methamphetamine sensitivity provide the framework with which we plan to follow up on the QTL analyses and correlations we have observed. Instead of selective breeding, we will take advantage of the existing variation in the BXD/TyJ strains in ventral midbrain iron concentrations and use microarray analysis and real-time pcr to test for underlying gene expression differences that map back to our QTL. We also plan to induce iron deficiency in our panel and observe gene by environment interactions that will be subject to QTL and gene expression analyses. The systems genetics approach, including gene expression analysis, shows promise for identifying genes involved in brain iron regulation and any other phenotype that can be quantified in a genetic reference population.

Conclusions

Iron is an important metal for brain function and its homeostasis is critical. Iron imbalances occur in individuals for reasons that are sometimes not apparent. Genetic analysis may help to uncover genetic differences between individuals that lead to differences in iron homeostasis. Systems genetic analysis is an approach that takes into account the complexity of iron regulatory systems, including the large number of genes known to be involved and those that are yet unidentified. We have applied the principles of systems genetics to describe variation in iron homeostasis among RI strains, identify related QTL, and perform correlation analyses. Future work will follow up on these results by integrating QTL analyses with gene expression correlates and testing candidate genes. The identification of genes involved in altered iron homeostasis may shed light on the role of iron in iron-related neurological disease.

Acknowledgments

Supported in part by USPHS Grant PO1 AG21190 and F31 NS060393, NRSA Fellowship to LCJ

References

1. Connor JR, Menzies SL. Relationship of iron to oligodendrocytes and myelination. *Glia* 1996;17:83–93. [PubMed: 8776576]
2. Wu LL, Zhang L, Shao J, Qin YF, Yang RW, Zhao ZY. Effect of perinatal iron deficiency on myelination and associated behaviors in rat pups. *Behav Brain Res* 2008;188:263–270. [PubMed: 18093669]
3. Berg D, Youdim M, Riederer P. Redox imbalance. *Cell Tissue Res* 2004;318:201–213. [PubMed: 15365815]
4. Chinta SJ, Andersen JK. Redox imbalance in Parkinson's disease. *Biochimica et Biophysica Acta* 2008;1780:1362–1367. [PubMed: 18358848]
5. Rhodes SL, Ritz B. Genetics of iron regulation and the possible role of iron in Parkinson's disease. *Neurobiol Dis* 2008;32:183–195. [PubMed: 18675357]
6. Shcherbatykh I, Carpenter DO. The role of metals in the etiology of Alzheimer's disease. *J Alzheimers Dis* 2007;11:191–205. [PubMed: 17522444]
7. Connor JR. Pathophysiology of restless legs syndrome: evidence for iron involvement. *Curr Neurol Neurosci Rep* 2008;8:162–166. [PubMed: 18460286]

8. Ghanizad A. Regarding the effects of iron supplementation on attention deficit hyperactivity disorder in children. *Pediatr Neurol* 2008;38:20–26. [PubMed: 18054688]
9. Beard JL, Hendricks MK, Perez EM. Maternal iron deficiency anemia affects postpartum emotion and cognition. *J Nutr* 2005;135:267–272. [PubMed: 15671224]
10. Lozoff B. Iron deficiency and child development. *Food Nutr Bull* 2007;38:S560–571. [PubMed: 18297894]
11. Fix OK, Kowdley KV. Hereditary hemochromatosis. *Minerva Med* 2008;99:605–617. [PubMed: 19034258]
12. Island ML, Jouanolle AM, Mosser A, et al. A new mutation in the hepcidin promoter impairs its BMP response and contributes to a severe phenotype in HFE related hemochromatosis. *Haematologica*. 2009In press
13. Burdo JR, Antonetti DA, Wolpert EB, Connor JR. Mechanisms and regulation of transferrin and iron transport in a model blood-brain barrier system. *Neuroscience* 2003;121:883–890. [PubMed: 14580938]
14. Hallgren B, Sourander P. The effect of age on the non-haemin iron in the human brain. *J Neurochem* 1958;3:41–51. [PubMed: 13611557]
15. Hock A, Demmel U, Schicha H, Kasperek K, Feinendegen LE. Trace element concentration in human brain. Activation analysis of cobalt, iron, rubidium, selenium, zinc, chromium, silver, cesium, antimony, and scandium. *Brain* 1975;98:49–64. [PubMed: 1122375]
16. Aoki S, Okada Y, Nishimura K, Barkovich AJ, Kjos BO, Brasch RC, Norman D. Normal deposition of brain iron in childhood and adolescence: MR imaging at 1.5 T. *Radiology* 1989;172:381–385. [PubMed: 2748819]
17. Roskams AJ, Connor JR. Iron transferrin, and ferritin in the rat brain during development and aging. *J Neurochem* 1994;63:709–716. [PubMed: 8035195]
18. Churchill GA. Recombinant inbred strain panels: a tool for systems genetics. *Physiol Genomics* 2007;31:174–175. [PubMed: 17698925]
19. Williams RW, Gu J, Qi S, Lu L. The genetic structure of recombinant inbred mice: high- resolution consensus maps for complex trait analysis. *Genome Biol* 2001;2:46.1–46.18.
20. Wang J, Williams RW, Manly KF. WebQTL: Web-based complex trait analysis. *Neuroinformatics* 2003;1:299–308. [PubMed: 15043217]
21. Palmer AA, Verbitsky M, Suresh R, et al. Gene expression differences in mice divergently selected for methamphetamine sensitivity. *Mamm Genome* 2005;16:291–305. [PubMed: 16104378]
22. Bryant CD, Graham ME, Distler MG, et al. A role for casein kinase 1 epsilon in the locomotor stimulant response to methamphetamine. *Psychopharmacology* 2009;203:703–711. [PubMed: 19050854]
23. Veenstra-VanerWeele J, Qaadir A, Palmer A, Cook EH Jr, de Wit H. Association between the casein kinase 1 epsilon gene region and subjective response to D-amphetamine. *Neuropsychopharmacology* 2006;31:1056–1063. [PubMed: 16237383]
24. Jones BC, Reed CL, Hitzemann R, et al. Quantitative genetic analysis of ventral midbrain and liver iron in BXD recombinant inbred mice. *Nutr Neuroscience* 2003;6:369–377.
25. Phillips TJ, Huson MG, McKinnon CS. Localization of genes mediating acute and sensitized locomotor responses to cocaine in BXD/Ty recombinant inbred mice. *J Neurosci* 1998;18:3023–3034.
26. Jones LC, McCarthy KA, Beard JL, Keen CL, Jones BC. Quantitative genetic analysis of brain copper and zinc in BXD recombinant inbred mice. *Nutr Neuroscience* 2006;9:81–92.
27. Jones LC, Beard JL, Jones BC. Genetic analysis reveals polygenic influences on iron, copper, and zinc in mouse hippocampus with neurobiological implications. *Hippocampus* 2008;18:398–410. [PubMed: 18189309]
28. Jones BC, Beard JL, Gibson JN, et al. Systems genetic analysis of peripheral iron parameters in the mouse. *Amer J Physiol Regul Integr Comp Physiol* 2007;293:R116–124. [PubMed: 17475678]
29. Earley CJ, Barker PB, Horska A, Allen RP. MRI-determined regional brain iron concentrations in early- and late-onset restless legs syndrome. *Sleep Medicine* 2006;7:458–461. [PubMed: 16740411]
30. Connor JR, Boyer PJ, Menzies SL, et al. Neuropathological examination suggests impaired brain iron acquisition in restless legs syndrome. *Neurology* 2003;61:304–309. [PubMed: 12913188]

31. Ortega R, Cloetens P, Deves G, Carmona A, Bohic S. Iron storage within dopamine neurovesicles revealed by chemical nano-imaging. *PLoS ONE* 2007;2:e925. [PubMed: 17895967]
32. Snyder AM, Connor JR. Iron, the substantia nigra and related neurological disorders. *Biochim Biophys Acta*. 2008In press
33. Erikson KM, Jones BC, Beard JL. Iron deficiency alters dopamine transporter functioning in rat striatum. *J Nutr* 2000;130:2831–2837. [PubMed: 11053528]
34. Erikson KM, Jones BC, Hess EJ, Zhang Q, Beard JL. Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. *Pharm Biochem Beh* 2001;69:409–418.
35. Bianco LE, Wiesinger J, Earley CJ, Jones BC, Beard JL. Iron deficiency alters dopamine uptake and response to L-DOPA injection in Sprague-Dawley rats. *J Neurochem* 2008;106:205–215. [PubMed: 18363828]
36. Beard JL, Erikson KM, Jones BC. Neurobehavioral analysis of developmental iron deficiency in rats. *Beh Brain Res* 2002;134:517–524.
37. Beard JL. Iron deficiency alters brain development and functioning. *J Nutr* 2003;133:1468S–1472S. [PubMed: 12730445]
38. Felt BT, Beard JL, Schallert T, et al. Persistent neurochemical and behavioral abnormalities in adulthood despite iron supplementation for perinatal iron deficiency anemia in rats. *Beh Brain Res* 2006;171:261–270.
39. Barriere G, Cazalets JR, Bioulac B, Tison F, Ghorayeb I. The restless legs syndrome. *Prog Neurobiol* 2005;77:139–165. [PubMed: 16300874]
40. Winkelman JW, Allen RP, Tenzer P, Henning W. Restless legs syndrome: nonpharmacologic and pharmacologic treatments. *Geriatrics* 2007;62:13–16. [PubMed: 17922563]
41. Kavangh D, Siddiqui S, Geddes CC. Restless legs syndrome in patients on dialysis. *Am J Kidney Dis* 2004;43:763–771. [PubMed: 15112166]
42. Manconi M, Govoni V, De Vito A, et al. Pregnancy as a risk factor for restless legs syndrome. *Sleep Med* 2004;5:305–308. [PubMed: 15165540]
43. Earley CJ, Heckler D, Allen RP. The treatment of restless legs syndrome with intravenous iron dextran. *Sleep Med* 2004;5:231–235. [PubMed: 15165528]
44. Earley CJ, Heckler D, Allen RP. Repeated IV doses of iron provides effective supplemental treatment of restless legs syndrome. *Sleep Med* 2005;6:301–305. [PubMed: 15978514]
45. Schormair B, Kemlink D, Roeske D, et al. PTPRD (protein tyrosine phosphatase receptor type delta) is associated with restless legs syndrome. *Nat Genet* 2008;40:946–948. [PubMed: 18660810]
46. Stefansson H, Rye DB, Hicks A, et al. A genetic risk factor for periodic limb movements in sleep. *N Engl J Med* 2007;357:639–647. [PubMed: 17634447]
47. Winkelmann J, Schormair B, Lichtner P, et al. Genome-wide association study of restless legs syndrome identifies common variants in three genomic regions. *Nat Genet* 2007;39:1000–1006. [PubMed: 17637780]
48. Xiong L, Catoire H, Dion P, et al. MEIS1 intronic risk haplotype associated with restless legs syndrome affects its mRNA and protein expression. *Hum Mol Genet* 2009;18:1065. [PubMed: 19126776]
49. Kemlink D, Polo O, Frauscher B. Replication of restless legs syndrome loci in three European populations. *J Med Genet*. 2009In press

VMB Iron Concentration vs. Expression of Riken gene in Btbd9 Promoter Region in 15 BXD/TyJ Strains of Mice

Figure 1.

Ventral midbrain (VMB) iron in female BXD/TyJ mice is correlated with the expression of Riken gene 0710001D07, which lies in the Btbd9 promoter region on murine chromosome 17 ($r=0.731$; $p<0.001$). A one-unit difference on the x-axis corresponds to a twofold difference in expression. Graph adapted from <http://genenetwork.org>.