Neuroscience 252 (2013) 13–23

IRON DEFICIENCY ALTERS EXPRESSION OF DOPAMINE-RELATED GENES IN THE VENTRAL MIDBRAIN IN MICE

L. C. JELLEN,[a] L. LU,[b,c] X. WANG,[b] E. L. UNGER,[d] C. J. EARLEY,[e] R. P. ALLEN,[e] R. W. WILLIAMS[b] AND B. C. JONES[a,f*]

[a] *Neuroscience Institute, The Pennsylvania State University, University Park, PA, USA*

[b] *Department of Anatomy and Neuroscience, The University of Tennessee Health Sciences Center, Memphis, TN, USA*

[c] *Jiangsu Key Laboratory of Neuroregeneration, Nantong University, China*

[d] *Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA*

[e] *Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA*

[f] *Department of Biobehavioral Health, The Pennsylvania State University, University Park, PA, USA*

Abstract—A clear link exists between iron deficiency (ID) and nigrostriatal dopamine malfunction. This link appears to play an important role in at least restless legs syndrome (RLS) if not several other neurological diseases. Yet, the underlying mechanisms remain unclear. The effects of ID on gene expression in the brain have not been studied extensively. Here, to better understand how exactly ID alters dopamine functioning, we investigated the effects of ID on gene expression in the brain, seeking to identify any potential transcription-based mechanisms. We used six strains of recombinant inbred mice (BXD type) known to differ in susceptibility to ID in the brain. Upon weaning, we subjected mice from each strain to either an iron-deficient or iron-adequate diet. After 100 days of dietary treatment, we measured the effects of ID on gene expression in the ventral midbrain, a region containing the substantia nigra. The substantia nigra is the base of the nigrostriatal dopamine pathway and a region particularly affected by iron loss in RLS. We screened for ID-induced changes in expression, including changes in that of both iron-regulating and dopamine-related genes. Results revealed a number of expression changes occurring in ID, with large strain-dependent differences in the genes involved and number of expression changes occurring. In terms of dopamine-related genes, results revealed ID-induced expression changes in three genes with direct ties to nigrostriatal dopamine functioning, two of which have never before been implicated in an iron–dopamine pathway. These were stromal cell-derived factor 1 (*Cxcl12*, or *SDF-1*), a ferritin regulator and potent dopamine neuromodulator, and hemoglobin, beta adult chain 1 (*Hbb-b1*), a gene recently shown to play a functional role in dopaminergic neurons. The extent of up-regulation of these genes varied by strain. This work not only demonstrates a wide genetic variation in the transcriptional response to ID in the brain, but also reveals two novel biochemical pathways by which iron may potentially alter dopamine function.

Key words: BXD, recombinant inbred strains, *Drd2*, *Cxcl12*, *Hbb-b1*.

INTRODUCTION

Iron deficiency (ID) has a number of effects in the brain, including dopamine malfunction, and is associated with restless legs syndrome (RLS; Allen et al., 2013), a neurological disorder with dopaminergic abnormalities. RLS is one of several dopamine-related neurological disorders associated with iron imbalance in the brain, particularly in the substantia nigra. Thus, iron and dopamine share an important link to neurological disease (Snyder and Connor, 2009).

Several lines of evidence implicate the iron–dopamine relationship in RLS. First, impaired iron acquisition (Connor et al., 2003) and iron deficits in RLS have been observed in the substantia nigra, a region housing dopaminergic neurons of the nigrostriatal pathway (Earley et al., 2006; Astrakas et al., 2008; Godau et al., 2008). This region is normally iron-rich. Secondly, the dopaminergic abnormalities of RLS overlap those of animal models of ID—in both RLS and ID, the nigrostriatal pathway is disrupted, with reduced striatal densities of dopamine D_2 receptors and increased levels of tyrosine hydroxylase (TH) and phosphorylated TH (Connor et al., 2009). Other nigrostriatal changes in animal models of ID include reductions in striatal dopamine D_1 receptors and the dopamine transporter, increases in striatal extracellular dopamine levels, and sensorimotor deficits (Beard et al., 1994, 2002; Erikson et al., 2000, 2001; Burhans et al., 2005; Felt et al., 2006; Unger et al., 2007; Bianco et al., 2008; Georgieff, 2011; Lozoff, 2011). Finally, RLS symptoms can be reduced by intravenous iron therapy (Earley et al., 2009).

*Correspondence to: B.C. Jones, Department of Biobehavioral Health, 315 E. Health and Human Development Building, University Park, PA 16802, USA. Tel: +1-814-863-0167; fax: +1-814-863-7525.

E-mail address: bcj1@psu.edu (B. C. Jones).

Abbreviations: *Alas2*, aminolevulinic acid synthase 2; ANCOVA, analysis of covariance; *Drd2*, dopamine receptor 2; FDR, false discovery rate; FHC, ferritin heavy-chain; GSEA, Gene Set Enrichment Analysis; ID, iron deficiency; LR ratio, $\log_2$ ratio; RLS, restless legs syndrome; *Rsad2*, radical S-adenosyl methionine domain containing 2; RT-PCR, Real-Time Polymerase Chain Reaction; TH, tyrosine hydroxylase; *Tfrc*, transferrin receptor; VMB, ventral midbrain.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.07.058

Nevertheless, many questions remain unanswered regarding the role of iron in RLS. Our research centers on two: (1) what causes ID to develop in the substantia nigra in RLS? and (2) by what mechanisms does ID alter dopamine functioning in this and neighboring regions? Here, we investigate the potential role of gene expression in either.

Several studies have demonstrated a number of expression changes in ID in early development, in both the whole brain (Clardy et al., 2006) and hippocampus (Carlson et al., 2007, 2008). It is thus plausible that ID-related expression changes also occur in adulthood and may influence nigral iron levels in ID and/or mediate the effects of ID on dopamine pathways. To our knowledge, however, no studies have yet profiled expression changes in response to ID in the substantia nigra, particularly in adulthood when RLS typically develops.

In previous work, we investigated the potential role of genetics in susceptibility to brain iron deficits in RLS (Jellen et al. 2009). We showed that recombinant inbred strains of mice (BXD type) differ widely in their response to ID in the brain, in terms of brain iron loss. In one study, we profiled 22 strains for susceptibility to ID in the ventral midbrain (VMB), which contains the substantia nigra. We found that in response to 100 days of being fed a low-iron diet, VMB iron loss ranged from 0% to 40%, depending on strain (Jellen et al., 2012). From this work, we identified a QTL linked to VMB iron levels and used microarray analysis of a subset of six strains with high or low susceptibility to VMB iron loss to narrow down identification of a candidate gene, *Glt-1*, which may regulate VMB iron. In the current study, we used the same set of microarray data, but expanded our focus from the effects of ID on genes within the QTL interval (i.e. genes that regulate iron concentration) to the effects of ID on expression throughout the genome, whether related to iron regulation or other downstream effects of ID, especially dopamine function.

The purpose of this study was thus to profile the effects of ID on gene expression in a dopamine- and RLS-relevant region of the brain and identify (a) genes that respond to ID that may underlie susceptibility to iron loss in this region and/or (b) genes that respond to ID and may in turn regulate dopamine neurobiology.

To perform this work, we chose a subset of six of the original 22 strains, which exhibited relatively high- or low-susceptibility to VMB iron loss. We then fed mice from these strains a low- or adequate-iron diet for 100 days and profiled each strain's response to ID in terms of changes in VMB gene expression.

Among our findings, we identified a large number of ID-responsive genes in the VMB, and found a wide variation among the six strains in this response. We did not find evidence linking gene expression profiles to differences in susceptibility to VMB iron loss in ID among the strains. We did, however, reveal expression changes in three genes in ID that do indeed have direct ties to nigrostriatal dopamine functioning. Two of these have never before been implicated in an iron–dopamine pathway.

This study demonstrates wide genetic variation in the transcriptional response to ID in the VMB, even among strains with nearly identical VMB iron loss profiles, and reveals two genes potentially involved in novel biochemical pathways linking iron to dopamine.

EXPERIMENTAL PROCEDURES

Animals

All experimental protocols were conducted in accordance with the National Institutes of Health Animal Care guidelines and were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Rearing and housing was controlled, with a constant light–dark cycle (06:00–18:00, on–off), ambient temperature (23 ± 2 °C) and relative humidity (40%). All mice were weighed weekly. Mice were from the BXD panel of recombinant inbred strains, derived from a C57BL/6J × DBA/2J pairing. The BXDs exhibit differential VMB iron regulation; varying widely in both baseline VMB iron content (Jones et al., 2003) and susceptibility to VMB iron loss in response to a low-iron diet (Jellen et al., 2012). The BXDs also vary in systemic response to ID (Yin et al., 2012).

Strain selection

Six strains were selected for gene expression profiling; these were a subset of twenty-two BXD strains

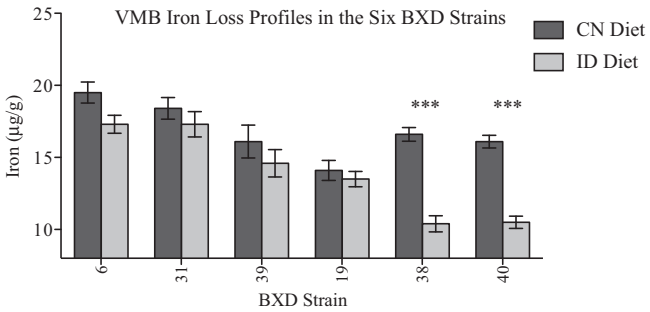

Fig. 1. VMB iron concentration in the six BXD strains (adapted from Jellen et al., 2012). In previous work, mean (±SEM) iron concentration (µg/g) was measured in mice from the BXD strains used in the current analysis. While these iron values were generated from a separate cohort, mice from BXD strains are genetically identical, thus the data provide predictive iron values for mice in the current study.