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Adipocytokine signaling is altered in flinders sensitive line rats, and adiponectin correlates in humans with some symptoms of depression

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Abstract

Major depression is a complex multi-factorial disorder with a lifetime diagnosis of nearly 1 out of 6. We used the Flinders Sensitive Line (FSL) of rats, a model of depression, and the parent Sprague–Dawley (SD) rats to identify genes, gene ontology categories and pathways associated with depression. Depression-like behavior was verified in the FSL line by forced swim testing, with FSL animals exhibiting greater immobility compared to SD rats. RNA samples from the hippocampus were isolated from a group of experimentally naïve FSL and SD rats for microarray analysis. Microarray analysis yielded a total of 361 genes that were differentially regulated between FSL and SD rats, with catechol-O-methyltransferase (COMT) being the most up-regulated. The genes that were differentially regulated between FSL and SD rats were subjected to bioinformatic analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID), which yielded several gene ontology categories that were overrepresented. Subsequent pathway analysis indicated dysregulation of the adipocytokine signaling pathway. To test the translational impact of this pathway, metabolic factors and psychiatric symptoms were evaluated in a sample of human research participants. Results from our human subjects indicated that anxiety and a subset of depressive symptoms were correlated with adiponectin levels (but not leptin levels). Our results and those of others suggest that disruption of the adipocytokine signaling pathway may be a critical component of the depressive-like behaviors observed in the FSL rats and may also be an important indicator of depressive and anxiety symptoms in humans.

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Conflict of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.pbb.2012.11.001>.

Keywords

Depression; Forced swim test; Hippocampus; Behavioral despair; Adipocytokine

1. Introduction

Major depressive disorder (MDD) is a serious illness characterized by deep sadness, feelings of hopelessness and despair, and increased somatic symptoms (e.g., disturbed sleep, appetite changes, and pain). By the year 2020, depression will be the 2nd most disabling health problem in the world. Up to 15% of individuals with severe MDD die by suicide (American Psychiatric Association, 2000). Current medications to treat depression exhibit limited efficacy (e.g. Fountoulakis and Möller, 2011). Residual depressive symptoms are common, increase the risk for relapse, and may result in a more chronic course of illness (reviewed in Kurian et al., 2009). Thus, new treatments for major depression are needed to address the high rates of resistance to current interventions and the chronic residual symptoms in many patients treated for depression. A better understanding of the pathophysiological mechanisms associated with depression will contribute to this need for improved therapeutic strategies.

The heterogeneity of MDD suggests that multiple neurocircuits and neurochemicals are involved in its pathogenesis. The most widely accepted neurochemical theory of depression is the monoamine hypothesis which postulates that depression is a pathology caused by alterations in brain serotonergic and noradrenergic systems. However, this theory is incomplete (Lee et al., 2010), and the role of the immune system in depression is increasingly appreciated and supported (reviewed in Loftis et al., 2010; Raedler, 2011; Leonard and Maes, 2012).

To identify genes that contribute to depression and the biological mechanisms through which they act, we used the Sprague Dawley (SD) derived Flinders Sensitive Line (FSL) rats to perform genome-wide expression profiling. The FSL rats, a well-validated genetic model for depression, were previously generated by selective breeding of out-bred SD rats for differences in the effects of the anticholin-esterase agent diisopropylfluorophosphate (DFP) (Overstreet and Russell, 1982; Overstreet, 1986). FSL rats are more sensitive to DFP and cholinergic agonists, a feature shared by depressed humans (Janowsky et al., 1994). Subsequent evaluations of the FSL rats revealed that, in addition to their cholinergic hypersensitivity, they express behavioral (e.g., reduced locomotor activity, increased immobility, and cognitive deficits (Overstreet, 1993)) and physiological (e.g., psychomotor retardation, lower body weight, and reduced appetite (Overstreet et al., 2005)) features similar to those found in MDD. Recent proteomic analyses found that a number of analytes previously associated with MDD were similarly altered in hippocampus and prefrontal cortex of FSL rats (Carboni et al., 2010; Piubelli et al., 2011), including alterations in proteins associated with energy metabolism, cellular localization and transport, cytoskeleton organization, and apoptosis.

The hippocampus is increasingly thought to be involved in the patho-physiological mechanisms of depression (Duman, 2002; Santarelli et al., 2003; Campbell et al., 2004;

Videbach and Ravnkilde, 2004), and therefore was selected for gene expression profiling. Repeated intra-hippocampal administration of prednisolone (a commonly prescribed glucocorticoid) increased anxiety and depression-like behavior in mice, and altered expression of genes associated with cell death and inflammation (Kajiyama et al., 2010). Imaging studies show that hippocampal volumes are reduced in some patients with MDD (Bremner et al., 2000; MacQueen et al., 2003; Sheline et al., 1996), possibly due to reduced neuropil sizes (Rosoklija et al., 2000). Early life stress may underlie the reduced hippocampal volumes observed in some patients with MDD (see Frodl and O'Keane, in press for review). Efficacious antidepressant treatments function in part, by normalizing disturbed neuroplasticity (Michael-Titus et al., 2008) and facilitating axonal and dendritic sprouting (Vaidya et al., 1999) — processes that can help restore synaptic connections within the neuropil. Although the role of hippocampal neurogenesis in the development and persistence of depression is not completely understood, its requirement for antidepressant efficacy is well accepted (Lewitus et al., 2009; Santarelli et al., 2003; Malberg et al., 2000).

In the present study we identified associations with several genes and found dysregulation of the adipocytokine signaling pathway in the FSL rat model of depression. We chose to conduct the microarray investigation under resting conditions (i.e., using experimentally naïve rats) to conform to studies involving depressed patients (e.g., Shelton et al., 2011) and to determine whether differences in gene expression would be evident in the absence of stress or pharmacological manipulation. We found significant gene expression differences at all levels of analysis, including at the single gene level, at the biological process level (gene ontology) and at the pathway level. We followed up these preclinical findings with a study in humans to determine whether disrupted adipocytokine signaling was similarly associated with symptoms of depression or anxiety. Our translational findings highlight important new directions for depression research and diagnosis and further support the utility and relevance of the FSL rat model.

2. Materials and methods

2.1. Animals

Male FSL rats (302.9±23.4 g; Dr. Amir H. Rezvani, Duke University) and male SD rats (294.9±17.6 g; Harlan Laboratories) were housed in the same room for 3 months prior to the start of the experiments. Rats were pair-housed under conditions of constant temperature (20–22 °C) and humidity (30–45%) with free access to food and water. The room was maintained on a 12:12 h light:dark cycle with lights off at 1800 h. All animal studies were approved by the Institutional Animal Care and Use Committee at the Portland VA Medical Center and were performed in accordance with the guidelines of the National Institutes of Health.

2.2. Forced swim test

To evaluate depressive-like behavior in FSL and SD rats, forced swim testing (FST) was performed to assess behavioral despair (immobility). Rats participating in the behavioral testing were not used for the microarray experiment because the stress associated with the swim test could alter gene expression (e.g. Drossopoulou et al., 2004). The FST was

performed as previously described (Loftis et al., 2006; Wilhelm et al., 2011). Briefly, rats were placed in a clear acrylic cylinder (40 cm height; 18 cm diameter) filled to 30 cm high with 25 °C (± 2 °C) water. The water was sufficiently deep that the rats would swim or float in the water without limbs or tail touching the floor of the container. Rats were exposed to a 15-min practice swim (training session), followed 24 h later by a 5-min test swim. Both the training and test sessions were video recorded, and the test sessions were scored by an independent observer. Three behaviors were measured: swimming, climbing and immobility. These behaviors were defined by Cryan et al. (2002):

- Immobility — floating in the water without struggling and using only small movements to keep the head above water
- Swimming — moving limbs in an active manner (more than required to keep head above water) causing movement around the cylinder
- Climbing — making active movements with the forepaws in and out of the water, usually directed against the wall.

The predominant behavior during 5-s intervals of the 5-min test swim was assessed. The main dependent variables for this task were time spent immobile and latency to start floating. The total time spent immobile was calculated and subjected to a *t*-test (two-tailed, unpaired).

2.3. Flow cytometry

Animals ($n=7$ from each group) were euthanized via CO₂ asphyxiation and whole brains were rapidly removed. A single-cell suspension of mononuclear cells was prepared using a 100 μ m cell strainer (BD Biosciences, San Jose, CA, USA). Recovered cells were washed in RPMI 1640, resuspended in 8 ml of 40% Percoll (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) underlaid with 3 ml of 80% Percoll to form a discontinuous gradient in a 15-ml centrifuge tube. The gradient was centrifuged at 500 $\times g$ for 30 min, and the cells at the 40% to 80% interface harvested. Cells were washed, stained for flow cytometric analysis (antibodies for CD11b/c-APC, CD45-PE, CD3-FITC, and CD161a-PE), and acquired on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

2.4. RNA isolation, amplification and microarray analysis

Animals were euthanized by rapid decapitation and hippocampal sections were removed on dry ice, as previously described (Loftis and Janowsky, 2002). Hippocampi were placed in RNAlater (Qiagen, Inc., Valencia, CA, USA) and stored at -80 °C until processed for microarray analysis. RNA was isolated from tissue samples using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) as described in the product manual.

Microarray experiments were conducted in the Affymetrix Microarray Core of the Oregon Health & Science University Gene Microarray Shared Resource. Messenger RNA was amplified and labeled from 200 ng of total RNA in two steps using the MessageAMP Premier RNA Amplification kit (Ambion, Inc., Austin, TX, USA) as described in the product manual. Target yield was measured by UV₂₆₀ absorbance and quality was assessed by examining yield and size distribution of the *in vitro* synthesis reaction products using a

2100 Bioanalyzer and RNA 6000 LabChip (Agilent Technologies, Santa Clara, CA, USA). Labeled target was fragmented at 95 °C in the presence of high magnesium concentration to produce a uniform distribution of short cRNAs. Ten micrograms of the fragmented material was combined with biotinylated hybridization control oligomer and biotinylated control cRNAs for BioB, BioC, BioD and CreX (Affymetrix, Santa Clara, CA, USA) in hybridization buffer and hybridized on the Affymetrix GeneChip Rat Genome 230 2.0 array for 18 h as described in the Affymetrix expression analysis technical manual. The array image scan was processed with Affymetrix GeneChip Command Console (AGCC).

2.5. Statistical analysis of microarray data

The “affy” and “gcrma” packages of Bioconductor (<http://www.bioconductor.org>) were employed to normalize the intensities following import of CEL files into the R statistical program. Then, the GeneChip Robust Multiarray Analysis (GCRMA) was used to adjust perfect match (PM) probe data for background noise and further normalized by quantile normalization. Gene expression values were determined using a linear model estimated by the median polish algorithm. The normalized intensities between the SD and FSL lines were compared by using the Significant Analysis of Microarrays with q-value less than 5% and up- or down-regulated by 1.5 fold changes or more (corresponding to fold changes of >1.5 for genes up-regulated or <0.67 (1/1.5) for genes down-regulated). These cutoffs make the gene selection process symmetric on a log₂ scale. The q value is a Bayesian equivalent to the false discovery rate adjusted p-value. This allowed us to identify a large set of genes that were differentially regulated between FSL animals and SD animals which could then be subjected to pathway enrichment analyses. Functional analysis of the data was performed using the DAVID (the Database for Annotation, Visualization and Integrated Discovery v6.7) Bioinformatics Resources (<http://david.abcc.ncifcrf.gov/home.jsp>) (Huang da et al., 2009a, 2009b). Genes identified as differentially expressed between the FSL and SD rats were assessed for significant enrichment of biological processes using the terms of the fifth level of Gene Ontology (GO). Pathway analysis was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) module within DAVID. Statistical analysis for GO and pathway analyses were carried out using a modified Fisher Exact Test (Expression Analysis Systematic Explorer (EASE); Reviewed in Hosack et al., 2003).

2.6. Human research participants

A total of 40 adults were recruited from the Portland Veterans Affairs Medical Center (PVAMC) and community hospitals via study advertisements posted throughout the hospital, or word of mouth. Participants were excluded if they met any of the following criteria: 1) History of antiviral therapy or chemotherapy for any purpose, 2) History of a major medical condition, or currently unstable medical condition, that is likely to be associated with severe neurological or immune dysfunction (e.g., stroke, seizures, brain tumors, Parkinson's disease, neurodegenerative dementia, mental retardation, hepatic encephalopathy, and HIV). Participants with common well-controlled or stable conditions were included as long as severe cognitive or immunological effects (conditions that were excluded: stroke, seizure, brain tumors, Parkinson's disease, neurodegenerative dementia, mental retardation, hepatic encephalopathy, and human immunodeficiency virus (HIV)) were not currently suspected (conditions that were allowed: well-controlled diabetes,

hypertension, or asthma). 3) History of traumatic brain injury with known loss of consciousness 30 min. 4) Use of alcohol, illicit substances, or medications with acute cognitive effects such as sedation or intoxication (e.g., benzodiazepines, opiates, and muscle relaxants) on the day of testing, or chronic use of medications with long-term cognitive effects (e.g. topiramate, remicade, anticholinergics and steroids). 5) Advanced liver disease as indicated by any of the following: a) classified as having stage 4 liver disease or grade 4 inflammation upon biopsy, OR, b) classified by a hepatologist as having probable decompensated cirrhosis based on clinical indicators and standard liver labs, OR, and c) aspartate aminotransferase (AST) to platelet (PLT) ratio index (APRI) ≥ 1.5 . 6) Current pregnancy. 7) History of schizophrenia or schizoaffective disorder, OR, current psychotic or manic episode, OR currently unstable and severe psychiatric disorder. Patients with mild but stable depression, anxiety, or post-traumatic stress disorder were included. 8) Alcohol or drug abuse or dependence within the past 90 days (except nicotine or caffeine), based on Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria (American Psychiatric Association, 2000), confirmed with the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998).

2.7. Procedures for human participants

All research was conducted with permission from the PVAMC's Institutional Review Board and in accordance with the Helsinki Declaration. All participants gave written informed consent and were paid \$75 to complete the following study procedures: clinical interview, comprehensive medical record review, questionnaires to evaluate symptoms of anxiety and depression [i.e., Generalized Anxiety Disorder Inventory (GADI) (Argyropoulos et al., 2007); Beck Depression Inventory (BDI-II) (Beck et al., 1996)], and blood sample collection. Questionnaires and interviews were administered by one of four advanced doctoral candidates in clinical psychology; all graduate students were trained and supervised by a clinical neuropsychologist (M.H.). Study data was entered into a database initially and then double-checked by separate study personnel prior to analyses.

Clinical interviews were conducted using a structured case report form (developed specifically for this study) including prompts to screen patients based on each inclusion criteria, gather relevant demographic data, assess for a full range of current and past Axis I psychiatric and substance use disorders using DSM-IV (American Psychiatric Association, 2000) criteria (confirmed with the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998)), evaluate for history of head injuries, and record a comprehensive list of current and previous medical conditions. Study personnel additionally reviewed each participant's complete electronic medical record to collect recent medical laboratory results and to cross-validate the psychiatric, substance use, and medical history gathered in the clinical interview.

Symptoms of depression were assessed using the Beck Depression Inventory, Second Edition (BDI-II) (Beck et al., 1996). This is a well-validated and widely used measure of depression. Symptoms of anxiety were assessed using the Generalized Anxiety Disorder Inventory (GADI) (Argyropoulos et al., 2007). This brief inventory measures severity of current anxiety symptoms.

2.8. Multiplex metabolic factor assessments

Following collection of the neuropsychiatric data, blood was drawn in the afternoon (mean time was 12:57 PM, SD=1:45 h) by one-time venipuncture into cell preparation tubes (BD Vacutainer Systems, Franklin Lakes, NJ) containing 1 ml of 0.1 M sodium citrate solution. The blood was then centrifuged at 1500 RCF for 20 min at room temperature (22–25 °C). Plasma was separated, collected and immediately aliquoted in polypropylene tubes (Phoenix Research Products, Hayward, CA) and frozen at –80 °C until assayed. Metabolic factors (adiponectin and leptin) were measured by Myriad Rules-Based Medicine, Inc., (Austin, TX) using multi-analyte testing technology. Myriad Rules-Based Medicine, Inc. operates using Good Laboratory Practices (GLP) and has maintained CLIA (Clinical Laboratory Improvement Amendments) accreditation from the Commission on Office Laboratory Accreditation continuously since 2005. The lower limits of detection were as follows: adiponectin 0.02 µg/ml; leptin=0.074 ng/ml.

2.9. Statistical analyses of human data

Analyses were conducted with SPSS or Microsoft Excel, and p-values<0.05 were considered significant unless otherwise noted. Correlations between questionnaire measures (i.e., BDI-II and GADI) and metabolic factors were carried out using Pearson's correlation coefficient unless data were non-normal, in which case Spearman's Rho was used. For the BDI-II, in addition to BDI-II total scores, the Cognitive–Affective and Somatic factor scores derived from our recent BDI-II factor analysis were also used to evaluate depressive symptoms in our participants (Patterson et al., 2011). The Cognitive–Affective factor consists of 11 BDI-II items and the Somatic factor consists of 7 items (Supplementary Table S4).

3. Results

3.1. Behavioral assessment

As expected FSL animals spent more time immobile in the forced swim test (FST; a measure of behavioral despair often used as an antidepressant screen) relative to SD rats ($t=4.45$, $p<0.01$; Fig. 1). This test confirms increased depression-like behavior in FSL animals relative to SD animals and is consistent with previous studies examining this behavior in FSL rats (e.g. Overstreet, 1986; Wegener et al., 2011).

3.2. Brain immune cell expression

To determine whether phenotypic immune cell differences play a role in differential gene expression, we conducted flow cytometry of brain monocytes from FSL ($n=7$) and SD ($n=7$) rats. We found no significant differences between FSL and SD animals in proportions of CD3 (T cells), CD161a (natural killer cells), or CD45/CD11b/c (macro-phages) (Supplementary Table S1) indicating that differences in immune cell expression in brain is not likely to be a source of differential gene expression.

3.3. Gene expression and validation

Gene expression studies were carried out on five FSL rats and five SD rats that were experimentally naïve. We identified 130 genes that were up-regulated and 231 genes that

were down-regulated in FSL rats relative to SD rats (Supplementary Table S2). The ten most up- or down-regulated named transcripts are listed in Table 1.

3.4. Bioinformatic functional analysis

We used the database for annotation, visualization and integrated discovery (DAVID) to perform systematic and integrative gene expression analysis as outlined in Huang et al. (2009a, 2009b). Using this technique, we subjected genes that were up- or down-regulated to GO-based enrichment and pathway analyses. The GO enrichment analysis identified several overrepresented categories for both up- and down-regulated genes (Table 2). We then used functional annotation to determine whether distinct physiological pathways were differentially regulated between FSL and SD rats. No pathways were identified for genes that were down-regulated. The adipocytokine signaling pathway was identified for genes that were up-regulated ($p=0.06$). Within this pathway, three genes were differentially regulated between FSL and SD rats: tumor necrosis factor receptor superfamily, member 1A (TNFRSF1A)-associated via death domain (TRADD; ratio 2.02), acyl-CoA synthetase long-chain family member 1 (Acs11; ratio 1.73), and mitogen activated protein kinase 10 (MAPK10, JNK; ratio 1.59). The ratio value is the average intensity of the gene derived from the microarray for FSL animals relative to the average gene intensity measured for the SD animals. No other pathways were identified from the up-regulated genes. Quantitative real-time PCR validated the microarray findings on four (C3, TRADD, Acs11, and Rnase4) of the genes of interest (Supplementary Table S3; Supplementary methods).

3.5. Translational impact of metabolic factors

To assess the potential role of components of the adipocytokine signaling pathway (leptin and adiponectin) on symptoms of depression and anxiety, we collected blood samples and questionnaire data from 40 adults. Table 3 shows the demographic, psychiatric and medical characteristics of the research participants. Leptin and adiponectin levels were not significantly correlated ($p=0.75$). There was also no significant correlation between leptin levels and symptoms of anxiety or depression. Adiponectin, however, was significantly correlated with GADI ($r^2=0.13$; $p<0.05$) (Fig. 2). To specifically examine individuals with some symptoms of depression, we removed individuals with BDI-II Cognitive–Affective factor scores of zero ($n=16$), which resulted in a marginal correlation ($r^2=0.15$; $p=0.07$). Subsequent removal of a single outlier individual with a history of alcohol dependence led to a very strong correlation ($r^2=0.44$, $p<0.001$) (Fig. 2). It should be noted that the individuals with BDI-II Cognitive–Affective factor score of zero had plasma adiponectin levels that ranged from 0.5 to 2.8 $\mu\text{g/ml}$. Nevertheless, the presence of a strong correlation among those with non-zero BDI-II Cognitive–Affective factor scores is strongly suggestive of a role for adiponectin in depressive symptoms.

4. Discussion

We identified 361 transcripts that were differentially regulated between experimentally naïve FSL and SD control rats (Supplementary Table S2). The benefit of using naïve animals is that they have not been exposed to the stress induced by depression-related tests such as the FST (Drossopoulou et al., 2004). The limitation of this design is that measures of

depressive-like behavior were not directly correlated with microarray expression findings. The gene most up-regulated in the FSL rats compared to SD rats was catechol-O-methyltransferase (COMT) (Table 1). COMT is an enzyme involved in the degradation of dopamine, epinephrine and norepinephrine. Increased COMT activity in FSL rats would imply reductions in levels of catecholamine neuro-transmitters. In agreement with this finding are the results from Roth-Deri et al. (2009) who found sub-sensitivity to cocaine, a dopa-mine transporter inhibitor, in FSL rats. Characterization of neurotransmitter signaling in FSL rats revealed low extracellular levels of dopamine within the nucleus accumbens following cocaine administration, which was a consequence of attenuated dopamine release (Roth-Deri et al., 2009). Similarly, reduced dopamine signaling was observed in the ventral tegmental area of FSL rats (Friedman et al., 2008). Clinical studies have examined the val158met polymorphism of COMT to determine whether it is associated with MDD. These results have largely been equivocal, however (see Opmeer et al., 2010 for review), suggesting that the val158met polymorphism does not have a large effect on the development of MDD. Nevertheless, treatment with the COMT inhibitor tolcapone reduced symptoms of depression on several depression scales among subjects with MDD (Fava et al., 1999). Clearly, the role of COMT in MDD and in FSL rats has yet to be fully elucidated and should be examined in future studies.

In addition to COMT and consistent with Blaveri et al. (2010), we also found differential expression of the genes, TMEM176A and RNase 4 (Supplementary Table S2), with reduced expression in FSL rats as compared to SD rats (or Flinders Resistant Line (FRL) rats in Blaveri et al., 2010). We found the transcript for FAM111A to be one of the most up-regulated transcripts in FSL rats compared with SD rats (Table 1). This compares with Blaveri et al., who found FAM111A to be one of the most down-regulated transcripts in the hippocampus and prefrontal/frontal cortex between FSL and FRL rats. It is unclear what underlies the apparent differences in gene expression between our study and that of Blaveri et al. (2010). One possibility is that the rats used by Blaveri et al. had previously been stressed in the FST one week prior to collection of samples leading to the possibility that swim stress may have impacted the gene expression data. An additional difference is that Blaveri et al. used the FRL rats for comparison as opposed to SD (parent line) rats. Thus, the experimental design of the two studies varied considerably and therefore it is not surprising that some profiling differences exist.

To better understand how differential expression of neurotransmitter and immune system genes may affect biological mechanisms and contribute to depression, we used DAVID to identify biological processes that were overrepresented in our sample of regulated transcripts. We found several GO categories that exhibited enrichment in FSL rats compared with SD rats (Table 2). The list of biological processes associated with genes exhibiting reduced expression was dominated by immune-related processes (e.g., regulation of myeloid leukocyte mediated immunity; immunoglobulin mediated immune response; B cell mediated immunity; and positive regulation of immune effector process). Interestingly, complement component 3 (C3) was found to be down-regulated in three different animal models of treatment- or surgery-induced depression (Uriquen et al., 2008). C3 levels were significantly elevated in plasma from schizophrenic or depressed subjects relative to healthy controls, and were normalized in medicated patients with the same disorders (Maes et al., 1997). A

sibling-pair linkage study also found a significant association with the C3 locus and depression spectrum disease (Tanna et al., 1976). The role of C3 and other immune related molecules in MDD remains unclear; however increasing evidence indicates that the immune system is centrally involved in depression.

To examine overrepresentation and dysregulation of gene networks, we used the functional annotation chart feature in DAVID. The adipocytokine signaling pathway was identified as overrepresented, albeit with a marginal p value of 0.06 (Fig. 3). Adipocytes (a component of white adipose tissue) are primarily localized to the subcutaneous region and the viscera and combined with infiltrating immune cells (mostly macrophages and T cells) are the primary cells involved in adipocytokine signaling (Guzik et al., 2006). Impaired adipocytokine signaling, as in the case of obesity, is associated with generalized inflammation and altered cytokine production (Ouchi et al., 2003).

Leptin and adiponectin are the two best-studied adipocytokines, with leptin playing a key role in satiety and generally promoting inflammation and adiponectin being anti-inflammatory (reviewed in Guzik et al., 2006). FSL rats have reduced leptin levels when compared with FRL rats (Husum et al., 2003). Interestingly, leptin levels are reduced following chronic unpredictable stress, or chronic social defeat (models of depression) in rats and injection of leptin reduces immobility time in the FST in both rats (Lu et al., 2006) and mice (Liu et al., 2009; Yamada et al., 2011). Recent studies have found reduced leptin receptor expression in men with MDD who committed suicide (Lalovic et al., 2010) and several studies report reduced serum or plasma leptin in subjects with depression (Pasco et al., 2008; Yang et al., 2007; Eikelis et al., 2006; Jow et al., 2006; Kraus et al., 2002). Furthermore a ghrelin polymorphism (ghrelin enhances appetite thereby acting in opposition to leptin) has been associated with depression (Nakashima et al., 2008). Also, though not a component of the adipocytokine signaling pathway, RNase4 (a.k.a. angiogenin) was found to be significantly down-regulated (Table 1) in FSL animals, and along with adiponectin, has been associated with coronary disease and may be an additional component of inflammatory signaling within adipose tissue (Krecki et al., 2010). Thus, adipocytokine signaling may play a critical role in depression. Future studies are needed to determine the role of leptin and adiponectin signaling: 1) in depression, and 2) in relation to putative neurotransmitter and immune system alterations associated with depression.

As a preliminary step toward confirming the findings from our animal experiments, we evaluated adipocytokine levels (e.g., leptin and adiponectin) and psychiatric symptoms (e.g., anxiety and depressive symptoms) in a sample of human subjects. The relationship between central gene expression, peripheral factors and disease states is unclear. A microarray analysis of tissue from the brain and blood of schizophrenics revealed 177 and 123 putative schizophrenia bio-markers respectively. Of these, six were present in both the blood and the brain (Glatt et al., 2005). A recent study by Savitz et al. (in press) using whole genome expression analysis of mRNA derived from peripheral blood mononuclear cells found significant correlations between genes associated with depression and brain activity assessed by fMRI within the amygdala, ventromedial prefrontal cortex and hippocampus, supporting the influence of peripheral factors on neurological function. Future studies will be needed to

identify disease-associated genes that generalize across tissues as well as across species, specifically within a context of mood disorders.

Human studies of associations between leptin and depression have been inconsistent, with studies finding elevations (Antonijevic et al., 1998; Rubin et al., 2002), reductions (Jow et al., 2006; Kraus et al., 2002), or no differences (Deuschle et al., 1996) in leptin levels between control and depressed patients. We did not find significant associations between leptin levels and measures of depression or related factors. The inconsistency between leptin studies of depression could be due to circadian cycle variations in leptin levels or failure to control for body mass index differences. We cannot rule out the possibility that similar circadian variations obscured an association between leptin and our behavioral measures. In contrast, we identified significant correlations between levels of adiponectin and a measure of anxiety (GADI) and the Cognitive–Affective BDI-II factor. It should be noted that the correlation between adiponectin and GADI has a relatively low $r^2=0.13$, suggesting that this relationship is fairly weak. Furthermore, the correlation between adiponectin and the Cognitive–Affective BDI-II factor was only observed following removal of participants with scores of 0 on this measure as well as the removal of an outlier with a history of alcohol dependence.

Despite our encouraging initial results, caution should be taken in interpreting these findings, as generalizing gene expression data obtained from the rat hippocampus to the human condition of major depression will require extensive validation which is outside the realm of the current study. In addition, depression is a stress-related disorder, and behavioral differences between FSL and SD rats are seen exclusively in the stressed state. Thus, our study may identify factors underlying risk for depression (or depressive-like behaviors), such as poor stress management.

Depression and anxiety are common co-morbid conditions with significant symptom overlap (e.g. Starr and Davila, 2012). Recent studies suggest that more than half of individuals with major depression exhibit a co-morbid anxiety disorder (Kessler et al., 2007). Previous studies have identified similar increases in adiponectin associated with symptoms of depression (Jeong et al., 2012), however, like leptin, the relationship between adiponectin and depression remains complex. Others have found reductions in adiponectin levels in subjects with major depression (Diniz et al., 2012; Cizza et al., 2010). The adipocytokine signaling pathway brings together immune system and metabolic factors that provide an intriguing integration of factors known to be associated with depression. Future studies identifying the specific factors that underlie depression will provide critical new targets for the development of more effective therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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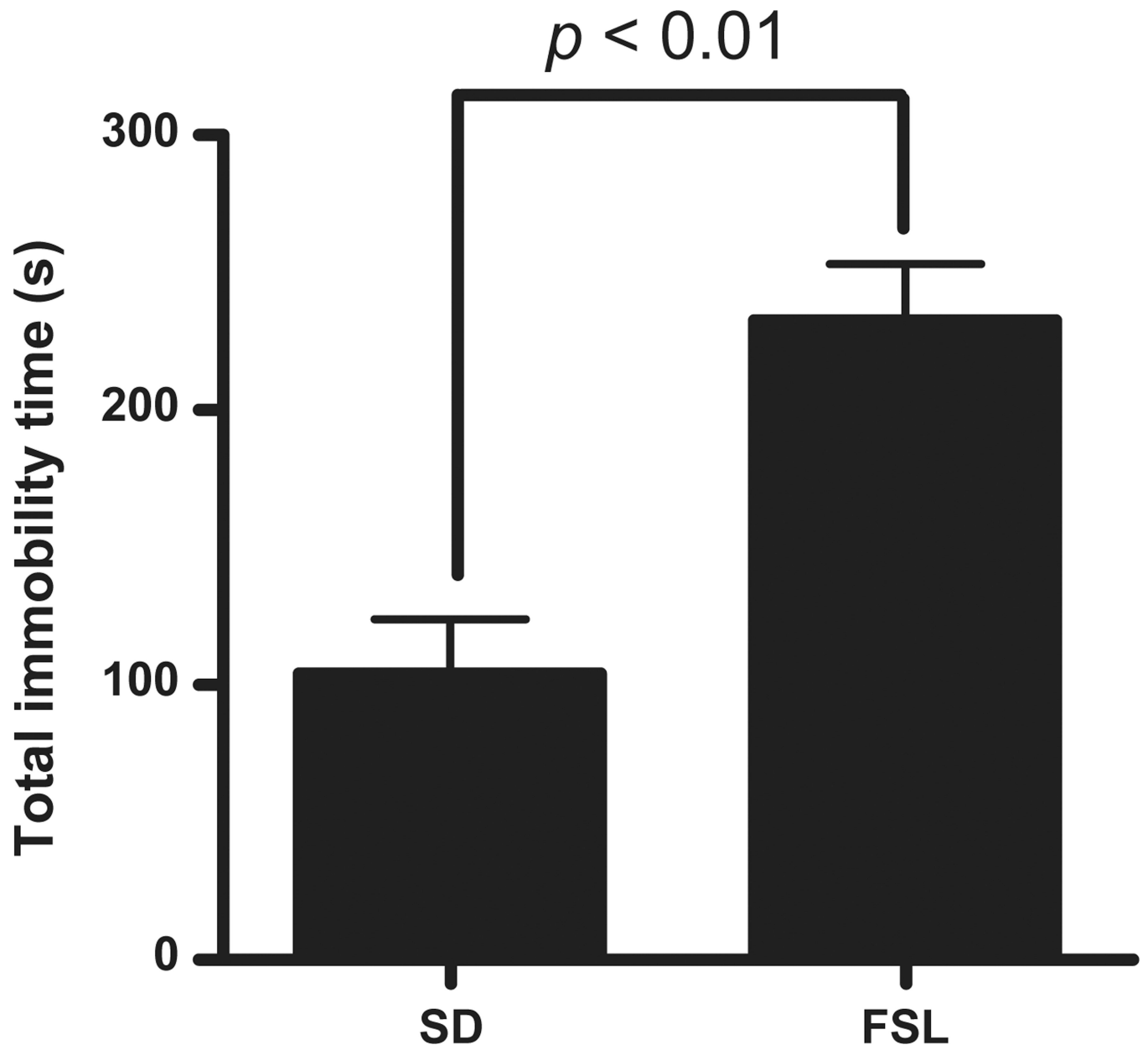


Fig. 1. Forced swim test results for FSL (n=4) and SD rats (n=5). FSL rats exhibit greater immobility compared with SD rats.

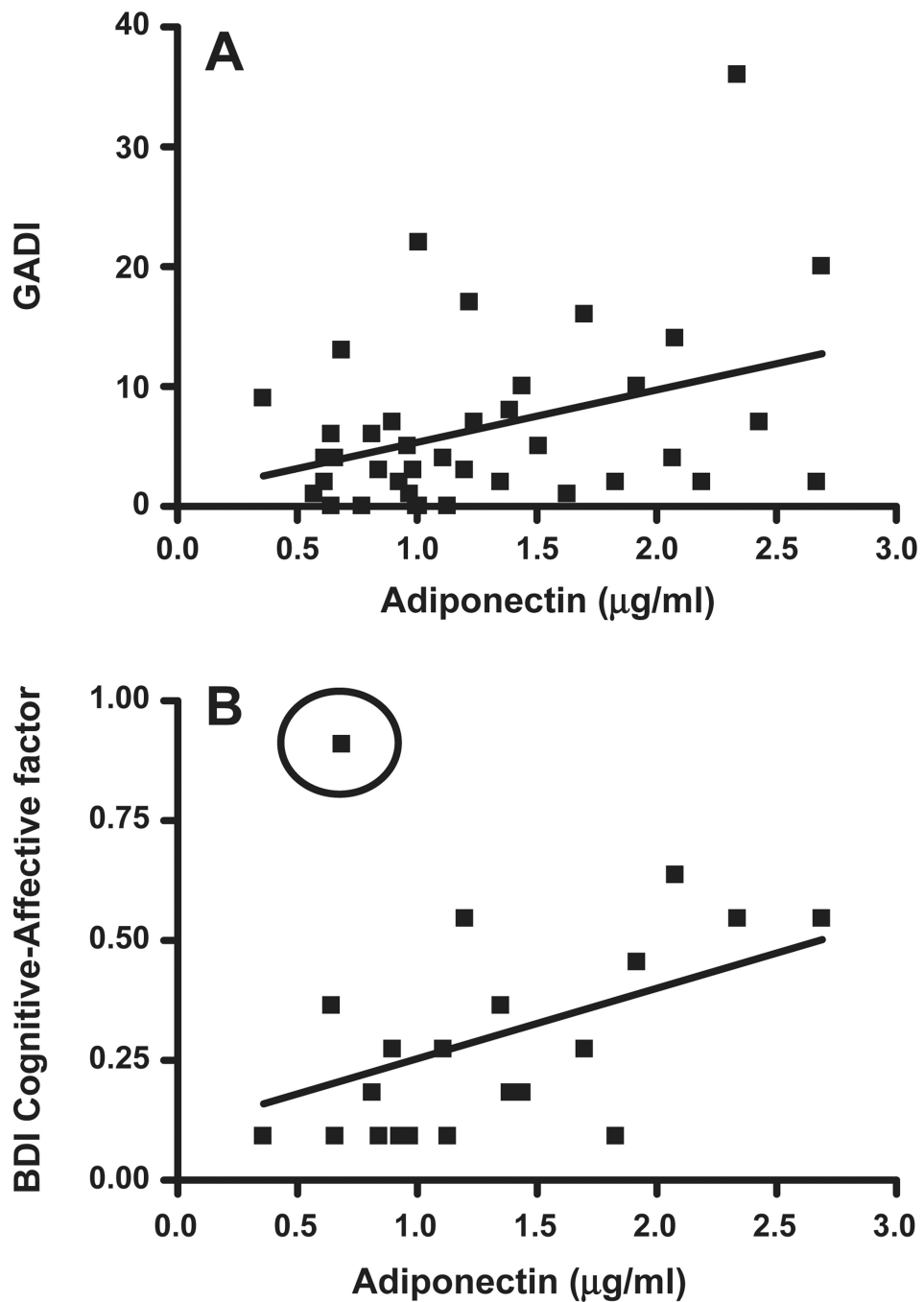


Fig. 2. (A) Significant positive correlation between plasma levels of adiponectin and GADI (Generalized Anxiety Disorder Inventory) anxiety measure (Pearson's correlation $p < 0.05$). (B) Significant positive correlation between plasma levels of adiponectin and BDI-II Cognitive-Affective factor measure. The point circled was excluded. All subjects with BDI-II Cognitive-Affective factor scores of 0 were removed. Pearson's correlation ($p = 0.07$ with outlier) ($p < 0.001$ with outlier removed).

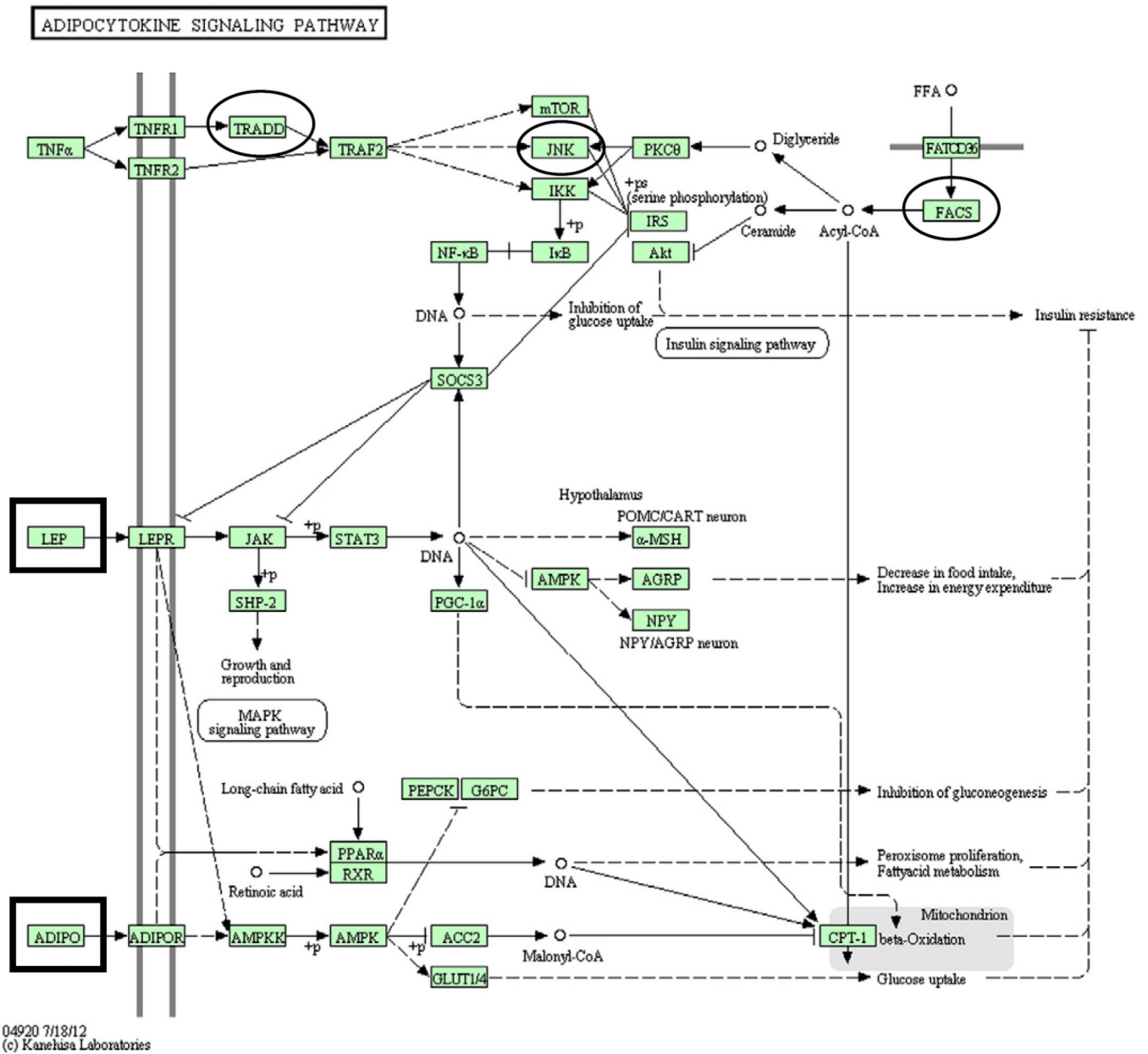


Fig. 3. The adipocytokine signaling pathway as represented in the Kyoto Encyclopedia of Genes and Genomes (KEGG; <http://www.genome.jp/kegg/kegg1.html>) (Kanehisa and Goto, 2000; Kanehisa et al., 2006, 2010). Connections represent relations between adjacent molecules, with arrows representing activation and blunted lines representing inhibition. Dashed lines indicate an indirect effect. Microarray results found the following genes from this pathway to be up-regulated in FSL rats relative to SD rats: TRADD, acyl-CoA synthetase long-chain family member 1 (FACS; *Acs11*), and mitogen activated protein kinase 10 (MAPK10, JNK). A conservative statistical analysis (EASE) indicated overrepresentation of this pathway ($p=0.06$). Results from our human studies indicated significant correlations between adiponectin (Adipo) levels and questionnaire measures of anxiety and depression.

Significant correlations were not found between these measures and leptin (Lep) levels. Factors examined in the human component of this study are outlined in rectangles, while those factors found to be differentially regulated in FSL rats are circled.

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Table 1

The most highly up- (A) or down- (B) regulated named sequences were identified from the list of 361 regulated transcripts. Transcripts are listed from most to least regulated. \log_2 (Fold change (FC)) represents the \log_2 of the difference in \log_2 probe intensity between FSL animals relative to SD animals. Positive \log_2 (FC) values indicate increased gene expression within FSL animals relative to SD animals, while negative \log_2 (FC) values indicate decreased gene expression within FSL animals relative to SD animals. Probe set ID represents the proprietary Affymetrix identification method associated with the specific microarray chip.

Probe set ID	Gene name	Gene symbol	\log_2 (FC)
<i>A. Up-regulated sequences</i>			
1368826_at	Catechol-O-methyltransferase	COMT	6.14
1371970_at	Family with sequence similarity 111, member A	Fam111a	5.51
1383211_at	Tuftelin 1	Tuft1	5.26
1395020_at	Pleckstrin homology domain containing, family H (with MyTH4 domain) member 1	Plekhh1	5.25
1387839_at	RT1 class Ib gene, H2-TL-like, grc region (N1)//RT1 class Ib gene, H2-TL-like, grc region(N2)//RT1 class Ib gene, H2-TL-like, grc region (N3)	RT1-N1//RT1-N2//RT1-N3	4.62
1369144_a_at	Potassium voltage gated channel, Shal-related family, member 3	Kcnd3	3.61
1390777_at	Sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, Saccharomyces cerevisiae)-like	Sc5dl	2.69
1393842_at	Coiled-coil domain containing 77	Ccdc77	2.54
1380577_at	ATP-binding cassette, sub-family G (WHITE), member 2	Abcg2	2.53
1378346_at	Lipin 2	Lpin2	2.27
<i>B. Down-regulated sequences</i>			
1391262_at	Similar to SUMO/sentrin specific protease 5//SUMO/sentrin specific protease 5	RGD1564247//Senp5	-6.21
1393623_at	Tripartite motif-containing 25	TRIM25	-5.72
1397439_at	Similar to diacylglycerol kinase epsilon	LOC497978	-4.81
1377133_at	Hypothetical protein LOC680687	LOC680687	-4.81
1372120_at	Ubiquitin-like modifier activating enzyme 5	Uba5	-3.98
1377442_at	HtrA serine peptidase 4	HTRA4	-3.81
1368943_at	Ribonuclease, RNase A family 4	Rnase4	-3.72
1395126_at	Fc receptor-like S, scavenger receptor	Fcrls	-3.66
1388071_x_at	RT1 class Ib, locus Aw2	RT1-Aw2	-3.43
1388060_at	Synaptotagmin XII	Syt12	-2.74

Table 2

Overrepresented GO categories identified by DAVID analysis for the group of significantly regulated transcripts between FSL and SD rats. Counts represent the number of genes from the up- (A) or down- (B) regulated gene lists submitted that were identified as components of the noted GO term. Statistical testing for significant enrichment was carried out using EASE, a modified, more conservative version of the Fisher Exact Test.

Term	Count	p-Value	Gene symbols
<i>A. FSL up-regulated</i>			
Protein complex assembly	7	0.014	CNTF, P2RX4, pfdn6, TRADD, MIS12, polr2i, ATL1
Protein complex biogenesis	7	0.014	CNTF, P2RX4, pfdn6, TRADD, MIS12, polr2i, ATL1
Response to drug	6	0.020	mgst1, sst, COMT, SLC1A3, Abcg2, ACSL1
Negative regulation of cell proliferation	5	0.038	COMT, THY1, GTPBP4, Fntb, CDH13
Regulation of axonogenesis	3	0.042	CNTF, THY1, MBP
Regulation of cell migration	4	0.045	sst, THY1, GTPBP4, CDH13
Macromolecular complex assembly	7	0.045	CNTF, P2RX4, pfdn6, TRADD, MIS12, polr2i, ATL1
Cellular amino acid derivative metabolic process	4	0.047	mgst1, ddc, COMT, SLC1A3
<i>B. FSL down-regulated</i>			
Oxidation reduction	16	0.001	DPYD, cat, Cyp4f1, retsat, NQO2, RTN4IP1, PXDN, ME3, RGD1304982, PYROXD2, etfB, FMO5, AOX1, Cyp4f4, DEGS1, Aldh3b1
Regulation of myeloid leukocyte mediated immunity	3	0.009	C3, PLD2, Fcgr2b
Immunoglobulin mediated immune response	4	0.011	C3, MLH1, Fcgr2b, C1qa
B cell mediated immunity	4	0.013	C3, MLH1, Fcgr2b, C1qa
Positive regulation of immune effector process	4	0.013	C3, PLD2, Fcgr2b, NP
Antigen processing and presentation of peptide antigen via MHC class I	3	0.021	Fcgr2b, RT1-S3, RT1-EC2
Phosphoinositide-mediated signaling	4	0.022	F2R, PIK3C3, Pthr1, HCRT
Lymphocyte mediated immunity	4	0.022	C3, MLH1, Fcgr2b, C1qa
Regulation of endocytosis	4	0.024	C3, PLD2, Fcgr2b, STON2
Positive regulation of transport	7	0.026	F2R, C3, PLD2, Fcgr2b, HCRT, AKAP5, ACSL5
Adaptive immune response	4	0.027	C3, MLH1, Fcgr2b, C1qa
Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	4	0.027	C3, MLH1, Fcgr2b, C1qa
Activation of protein kinase C activity by G-protein coupled receptor protein signaling pathway	3	0.028	F2R, HCRT, LOC497978
dGTP metabolic process	2	0.029	Dguok, NP
Regulation of leukocyte mediated immunity	4	0.036	C3, PLD2, Fcgr2b, NP
Leukocyte mediated immunity	4	0.037	C3, MLH1, Fcgr2b, C1qa
Deoxyribonucleoside catabolic process	2	0.038	DPYD, NP
Positive regulation of calcium ion transport	3	0.041	F2R, HCRT, AKAP5
Positive regulation of type IIa hypersensitivity	2	0.048	C3, Fcgr2b
Regulation of type IIa hypersensitivity	2	0.048	C3, Fcgr2b
Positive regulation of myeloid leukocyte mediated immunity	2	0.048	C3, Fcgr2b

Term	Count	p-Value	Gene symbols
Regulation of type II hypersensitivity	2	0.048	C3, Fcgr2b
Positive regulation of type II hypersensitivity	2	0.048	C3, Fcgr2b
Positive regulation of endocytosis	3	0.048	C3, PLD2, Fcgr2b

Abbreviations: Ciliary neurotropic factor, CNTF; purinergic receptor P2X, ligand-gated ion channel 4, P2RX4; prefoldin 6, pfdn6; TNFRSF1A-associated via death domain, TRADD; MIS12, MIND kinetochore complex component, homolog (yeast), MIS12; polymerase (RNA) II (DNA directed) polypeptide 1, 14.5 kDa, polr2i; spastic paraplegia 3A homolog (human), ATL1; microsomal glutathione S-transferase 1, mgst1; somatostatin, sst; catechol-O-methyltransferase, COMT; solute carrier family 1 (glial high affinity glutamate transporter), member 3, SLC1A3; ATP-binding cassette, sub-family G (WHITE), member 2, Abcg2; acyl-CoA synthetase long-chain family member 1, ACSL1; Thy-1 cell surface antigen, THY1; similar to Nucleolar GTP-binding protein 1 (chronic renal failure gene protein) (GTP-binding protein NGB); similar to G protein-binding protein CRFG; GTP binding protein 4; similar to isopentenyl diphosphate delta-isomerase type 2, GTPBP4; farnesyltransferase, CAAX box, beta, Fntb; cadherin 13, CDH13; myelin basic protein, MBP; dopa decarboxylase (aromatic L-amino acid decarboxylase), ddc; dihydropyrimidine dehydrogenase, DPYD; catalase, cat; cytochrome P450, family 4, subfamily f, polypeptide 1, Cyp4f1; retinol saturase (all trans retinol 13,14 reductase), retsat; NAD(P)H dehydrogenase, quinone 2, NQO2; reticulon 4 interacting protein 1, RTN4IP1; peroxidase homolog (Drosophila), PXDN; malic enzyme 3, NADP(+)-dependent, mitochondrial, ME3; similar to RIKEN cDNA 2810025M15, RGD1304982; pyridine nucleotide-disulphide oxidoreductase domain 2, PYROXD2; electron-transfer-flavoprotein, beta polypeptide, etfB; flavin containing monooxygenase 5, FMO5; aldehyde oxidase 1, AOX1; cytochrome P450, family 4, subfamily f, polypeptide 4, Cyp4f4; degenerative spermatocyte homolog 1, lipid desaturase (Drosophila), DEGS1; aldehyde dehydrogenase 3 family, member B1, Aldh3b1; complement component 3, C3; phospholipase D2, PLD2; Fc fragment of IgG, low affinity IIb, receptor (CD32); Fc fragment of IgG, low affinity IIa, receptor (CD32), Fcgr2b; mutL homolog 1 (E. coli), MLH1; complement component 1, q subcomponent, alpha polypeptide, C1qa; nucleoside phosphorylase, NP; histocompatibility 2, T region locus 23; histocompatibility 2, T region locus 24, RT1-S3; RT1 class Ib, locus Aw2, RT1-EC2; coagulation factor II (thrombin) receptor, F2R; phosphoinositide-3-kinase, class 3, PIK3C3; parathyroid hormone receptor 1, Pthr1; hypocretin, HCRT; stonin 2, STON2; A kinase (PRKA) anchor protein 5, AKAP5; acyl-CoA synthetase long-chain family member 5, ACSL5; similar to diacylglycerol kinase epsilon, LOC497978; deoxyguanosine kinase, Dguok.

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Table 3Demographic, psychiatric and medical characteristics^a

	N=40
Demographics	
Age (mean years \pm SD) ^b	47.9 \pm 13.4
Body mass index	28.1 \pm 5.1
Male gender	29 (72.5%)
Caucasian	28 (70.0%)
Veteran status	20 (50.0%)
Years of education (mean \pm SD) ^b	13.8 \pm 2.3
Current psychiatric status	
Current psychiatric diagnosis (any)	4 (10.0%)
Major depressive disorder	3 (7.5%)
PTSD ^c	1 (2.5%)
Other anxiety disorder	2 (5.0%)
Neuropsychiatric symptom severity	
Depression (BDI-II)	4.5 \pm 5.1
Anxiety (GADI)	6.6 \pm 7.5
Medical history	
Past medical diagnoses (any)	17 (42.5%)
Diabetes	5 (12.5%)
Hyperlipidemia	4 (10.0%)
Hypertension	9 (22.5%)
Other cardiovascular	3 (7.5%)
Asthma/pulmonary	5 (12.5%)

^aData are expressed as n, with (%) in terms of n over total N unless otherwise stated.

^bPsychiatric diagnoses were based on DSM-IV criteria verified using the MINI (Mini-International Neuropsychiatric Interview), medical records, and clinical interviewing. Abbreviations: PTSD, posttraumatic stress disorder; BDI-II, Beck Depression Inventory, II; GADI, Generalized Anxiety Disorder Inventory. Data shown are mean \pm standard deviation.