Biomarkers of mood disorders in blood

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Introduction

Chronic stress is known to exert long lasting effects in the brain including genomic modification, physiological alterations and dysregulation of the HPA axis[1]. This type of stress has been implicated as a trigger for depression as well as other mood disorders. Rodent models have provided some insight into the genomic changes that occur following chronic subordination stress. Nestler has shown that chronic subordination in mice produced long lasting down regulation of brain derived neurotrophic factor (BDNF) in the brain[2], yet little is known about gene expression changes detected in the blood following chronic stress. Blood biomarkers may offer an unexpected informative window into brain functioning and disease state. To determine the effect of chronic subordination stress on blood gene expression, whole blood samples were collected from aggressive and subordinate animals prior to and following a ten day chronic subordination paradigm.

Materials and Methods

Animals and Treatment
CF-1 male mice were housed in groups of 3 in 12:12 hour light:dark cycle with food and water available *ad libitum*. Aggressive animals were established by isolation for 6 weeks prior to use in the test paradigms. On Day 1 of the chronic social subordination procedure, group housed animals were exposed to an aggressive animal for a five minute attack. A partition was inserted between the animals to prevent physical but not sensory isolation from the aggressor. Each subordinated animal was exposed to a new aggressor for 10 days.

Repeat Sampling
Before subordination, blood was collected from all animals via the saphenous vein. Rapid sampling from the saphenous vein was used because it causes minimal discomfort or stress to the animal. The blood was stored in RNAlater at -70 °C until use. After the second round of behavior testing, animals were sacrificed and blood was collected via truncation into RNAlater and frozen at -70°C until use.

Animal Testing and Selection
Animals (n=4) were selected for use based on behavioral data from light-dark testing. Previous experiments showed no learning with repeat testing in the light – dark paradigm[11]. Both aggressive and subordinated animals were chosen for this study based on the change in dark entries from pre testing to post testing. Aggressive animals selected showed an increase in dark entries while subordinate animals showed a decrease in dark entries. Figure 1 shows these changes.

RNA Isolation and Analysis
RNA was isolated using Mouse RiboPure™- Blood RNA Isolation Kit (Ambion®). RNA isolation was completed according to the manufacturer directions. RNA isolates were analyzed by spectrophotometry (Nanodrop, Beckman). All samples contained high amounts of RNA with the most concentrated sample containing 125 ng/ul . The 260/280 ratio was used to determine the quality of RNA present, samples with a value under 1.7 were not used. Total RNA (400ng) was reverse transcribed (First Strand Kit, SABiosciences) into cDNA and quantified by qRT-PCR.[7] qRT-PCR was performed using a custom PCR array plate designed to detect genes of interest researched based on findings from other studies on genetics and depression, anxiety and aggression[8,9,10] (SABiosciences). (Figure 2) SAB software available to users on their website (www.SABiosciences.com/pcr) was used to analyze data to determine Ct, fold change and regulation.

Figures

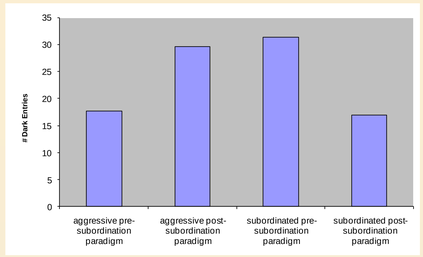

Figure 1. Average change in dark entries of aggressive and subordinated mice

Gene	Fold Change
Bdnf	4.50
Avpr1a	-2.91
Avpr1b	-3.66
Drd1a	-3.41
Gabra1	-3.63
Gabrg2	-2.53
Nmur2	-3.46
Sstr2	-3.41
Sstr4	-3.07

Figure 3. Genes showing greater than a 2.5-fold change in aggressive CF-1 male mouse following the chronic subordination paradigm. Data values shown are fold change vs pre- subordination. Hsp90ab1 served as a housekeeper gene.

Gene	Fold Regulation
Ar	-2.92
Gabra1	-2.50
Gabra3	-2.54
Itpr1	-3.04

Figure 4. Genes showing greater than a 2.5-fold change in subordinated CF-1 male mouse following the chronic subordination paradigm. Data values shown are fold change vs pre- subordination. Hsp90ab1 served as a housekeeper gene.

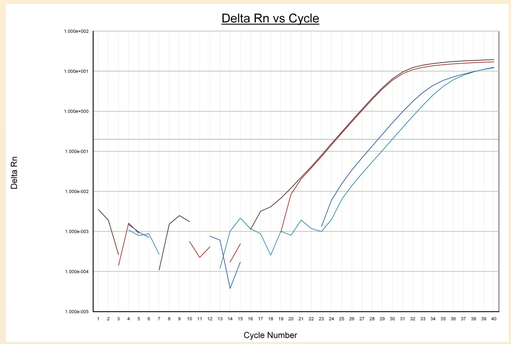

Figure 2. Representative amplification plots of average fold change for aggressive animal BDNF (above) and subordinated animals AR (below). qRT-PCR performed using SABioscinces PCR array plate.

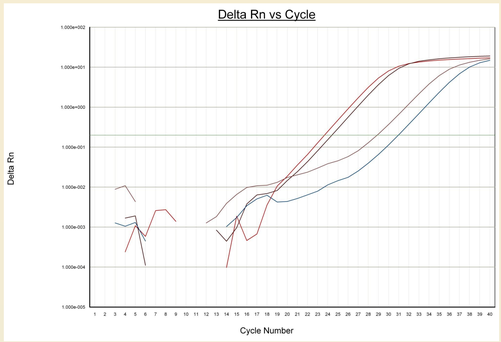

Figure 5. Genes that changed more than 2.5-fold in the study that have been implicated in mood disorders

Gene Symbol	Gene	Relation to Mood Disorders
Ar	Androgen Receptor	Androgen Receptor isotype is a marker of vulnerability to developing depression[12]
Avpr1a	Vasopressin Receptor 1A	An increase of vasopressin receptors in the brain is found in aggressive animals[13]
Avpr1b	Vasopressin Receptor 1B	
Bdnf	Brain derived neurotrophic factor	Stress decreases Bdnf in animals[14]
Drd1a	Dopamine receptor D1A	Increased dopamine associated with aggression[15]
Gabra1	GABA A Receptor Alpha 1	$GABA_a$ receptors regulate rapid changes in anxiety and the acute stress response[16]
Gabra3	GABA A Receptor Alpha 3	
Gabrg2	GABA A Receptor Gamma 2	
Itpr1	Inositol 1,4,5-trisphosphate receptor type 1	Implicated in long term depression g-protein[17]
Nmur2	Neuromedin U receptor 2	Expression of *Nmur2* in the PVN suggests a role in mediating stress response[18]
Sstr2	Somatostatin Receptor 2	Reduced hypothalamic somatostatin receptor expression is seen in aggressive animals[19]
Sstr4	Somatostatin Receptor 4	

Results and Conclusions

- PCR array analysis of subordinated mice resulted in four down regulated genes (>2.5 fold change): Androgen Receptor, two GABA receptor subunits and inositol triphosphate 1a receptor.
- PCR array analysis of 48 genes in aggressive CF-1 male mice produced pronounced changes in BDNF (upregulation) and Avpr1b (down-regulation), vasopressin receptor 1b.
- Aggressive and subordinated mice showed different patterns in gene expression after ten days of chronic subordination stress.
- The stress paradigm produced changes in gene expression in blood that are consistent with findings in brain.

Future Research

- Determine the pathways of regulated genes to find relationships linked to stress, depression, and mood disorders
- Analyze brain regions of interest to compare and contrast gene regulation with blood sample data

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Many thanks to Dr. Neal Simon, Carrie Garripa, and Dr. Shifang Lu.