

Aberrant response to dexamethasone suppression test associated with inflammatory response in MDD patients

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SUMMARY

Major depressive disorder (MDD) is a prevalent mood disorder. The direct causes and biological mechanisms of depression still elude understanding, though genetic factors have been implicated. This study looked to identify the mechanism behind the aberrant response to the dexamethasone suppression test (DST) displayed by MDD patients, in which they display a lack of cortisol suppression. Understanding the reason behind the response may offer insight into the mechanisms behind MDD and even potentially offer a pathway to new diagnostic or treatment solutions. We analyzed previously generated microarray gene expression profiling data from whole blood samples to obtain a view of the genes affected by the DST and the aberrant response. We used GEO2R to run analyses. We also used STRINGdb and Genecards to further explore these genes. Analysis revealed several pro-inflammatory genes that were significant and differentially expressed between affected and non-affected groups in response to the DST. The presence of a steroid should cause a decrease in the expression of inflammatory genes, but in this study, we saw a pro-inflammatory response after steroid administration in diagnosed MDD subjects, while decreased expression of inflammatory genes was seen in subjects unaffected by depression. Inflammation can cause cellular injury and can activate other inflammatory pathways, and may be a driving factor behind MDD as a result. Thus, looking at ways to decrease the inflammatory response could have implications for treatment and may explain why some people treated for depression still display symptoms or may lead researchers to different classes of drugs for treatment.

INTRODUCTION

Major depressive disorder (MDD) is a highly prevalent mood disorder characterized by persistent feelings of sadness, worthlessness, or guilt; fatigue; loss of interest in previously enjoyed activities or daily activities; and other physical or emotional symptoms (1). Due to the COVID-19 pandemic, the incidence of MDD is estimated to have risen 27.6% globally in 2020 (2). The National Institute of Mental Health also estimates that, as of 2020, 17% of adolescents aged 12–17

had experienced at least one major depressive episode, though only 41.6% of these adolescents received treatment (3). Lack of treatment can lead to complications, such as the development of anxiety or substance misuse disorders and a higher risk of developing self-destructive behaviors (4). The direct causes and biological mechanisms of MDD still elude understanding, although hardship or negative experiences an individual may undergo or a family history of depression may be risk factors (5). MDD is often characterized by frequent relapses and remissions, the condition has a high mortality and morbidity, and many affected individuals have a poor quality of life (6). Treatment options do exist to minimize the impairment that symptoms may cause in an individual's key areas of functioning in daily life, but there currently is no cure (6).

The dexamethasone suppression test (DST) assesses whether the administration of dexamethasone, an exogenous glucocorticoid, suppresses the hypothalamic-pituitary-adrenal (HPA) axis production of endogenous cortisol (7). In the DST, a healthy patient would demonstrate suppression of cortisol production after administration of dexamethasone via the HPA feedback loop (7). Despite the current gap in knowledge regarding MDD, patients with depression have a well-documented abnormal response to the DST, likely because of hyperactivity in the neuroendocrine system (8). Patients with depression display either a complete lack of cortisol suppression or cortisol suppression with early escape (loss of sustained suppression) in response to the DST (9).

In our study, we sought to understand how MDD affects responses to the DST in relation to gene expression. To address this, we analyzed a dataset that contained only male patients with and without depression and their response to the DST, based on the knowledge that administration of the exogenous steroid dexamethasone (a glucocorticoid) should result in decreased cortisol production and that MDD patients often show dysregulation of HPA system in response to the DST (10, 11). Glucocorticoids, which are expressed by the HPA axis as a response to stress, are well known for their anti-inflammatory properties (12). Dexamethasone is a potent, short-acting steroid, and in addition to the well-known anti-inflammatory effects of steroids, it is the standard chemical used in the DST to examine the neuroendocrine feedback loop (13,7). This research used publicly available gene expression data derived from peripheral blood samples

to identify unique gene responses that would explain why responses to the DST differ between those with MDD and those without. Prior research has demonstrated that stress can cause an inflammatory response in the brain as well as peripherally, which allows the use of peripheral blood samples to be used as a mirror of the central nervous system response (8). Transcriptional profiling in whole blood has previously been used to search for biomarkers for neurological and psychiatric disorders (14).

In this study, we sought to determine whether gene expression could explain the aberrant response to the DST in patients with depression. In these analyses, we compared control (individuals unaffected by MDD) baseline (before treatment with dexamethasone) versus control stimulated (after treatment with dexamethasone) and case (individuals diagnosed with MDD) baseline versus case stimulated groups in the hopes of uncovering the biological mechanisms that result in the aberrant DST response in patients diagnosed with MDD. Through our gene expression analyses of control and case patients before and after treatment with a DST, we hypothesized that there will be differential gene expression between patients diagnosed with MDD and patients who have not been diagnosed with MDD patients who have been exposed to dexamethasone.

Since current research notes that MDD patients treated with a DST display a well-documented aberrant response, further understanding of the biological mechanisms and

implications of this response may provide opportunities for future research or even the development of future treatment options for MDD, given that normalization of the DST response has been observed to have an association with clinical improvement in MDD patients (8).

RESULTS

Using publicly available gene expression data by microarray from peripheral blood showing gene expression differences in the immediate transcriptome response to stress, we performed an analysis between male patients with depression and male patients who have never had depression in response to the DST (15). We used GEO2R, a function of Gene Expression Omnibus (GEO), to run an analysis between three comparisons: control baseline versus case baseline; case stimulated versus case baseline, referred to as the case analysis; and control stimulated versus control baseline, referred to as the control analysis (16). The GEO2R analyses produced results with p -values, adjusted p -values, Log Fold Change (LogFC) values, gene symbols, and gene IDs, among other data. The upregulation of genes is denoted by positive LogFC values, while a negative LogFC value denotes downregulation as compared to baseline. We also obtained UMAP (Uniform Manifold Approximation and Projection) plots from GEO2r for the case analysis and control analyses, respectively (Figures 1 and 2). Since both UMAPs in this study show this clear separation between

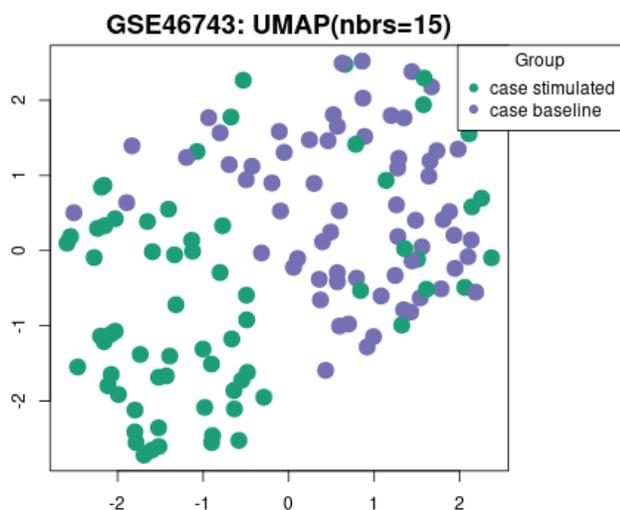


Figure 1. Case stimulated vs case baseline UMAP. The predictive clustering and separation of samples from baseline to stimulated for the case comparison. “Nbrs = 15” indicates that the number of nearest neighbors used during the calculation of data is fifteen, a number chosen by GEO2r during analysis in reclassifying data points during dimension reduction. Fifteen neighbors is a relatively local view of the manifold, but still allows for a clear picture of the overall structure of the data, which is helpful in determining the separation of the case stimulated and case baseline groups. The data in this figure were obtained through GEO2R analysis of case (individuals affected by MDD) baseline (before treatment with dexamethasone) versus case stimulated (after treatment with dexamethasone) groups of the publicly available dataset GSE46743.

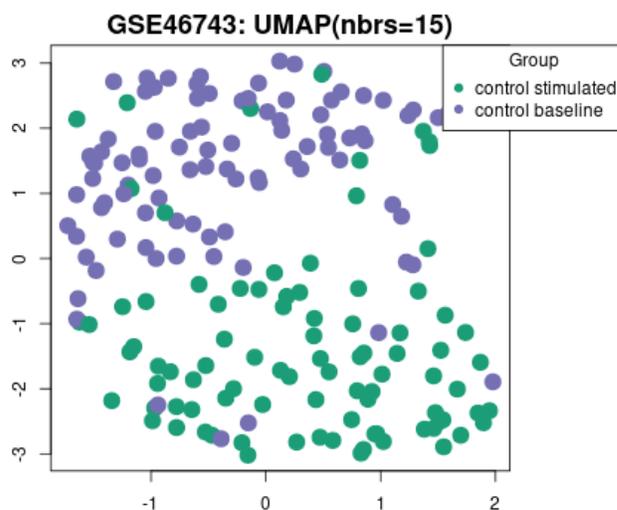


Figure 2. Control stimulated versus control baseline UMAP. The predictive clustering and separation of samples from baseline to stimulated for the control comparison. “Nbrs = 15” indicates that the number of nearest neighbors used during the calculation of data is fifteen, a number chosen by GEO2r during analysis in reclassifying data points during dimension reduction. Fifteen neighbors is a relatively local view of the manifold, but still allows for a clear picture of the overall structure of the data, which is helpful in determining separation of the control stimulated and control baseline groups. The data in this figure were obtained through GEO2R analysis of control (individuals unaffected by MDD) baseline (before treatment with dexamethasone) versus control stimulated (after treatment with dexamethasone) groups of the publicly available dataset GSE46743.

two distinct, color-coded groups, these figures suggest that there is an underlying difference between both groups in each comparison beyond a difference in disease state; in this case, this separation indicates differences in terms of gene expression. UMAP plots for each comparison revealed not only differential gene populations but also opposite movements of the sample populations between the groups.

These differences in expression were further confirmed by investigation with volcano plots, which showed significant populations of upregulated and downregulated genes (Figures 3 and 4). Volcano plots of the case analysis and the control analysis showed the difference in gene expression between the baseline and stimulated groups, with red representing upregulated genes and blue indicating downregulated genes that are statistically significant (adj. $p < 0.05$). Here, upregulation indicates a higher level of expression in the stimulated group compared to the baseline group, while downregulation means a lower level of expression compared to the baseline group. Each dot in the volcano plot corresponds to a unique gene which can be identified by hovering a cursor over the dot on the original volcano plot. Since both the UMAP and volcano plot indicate unique populations of genes with differential expression, we conducted further evaluation involving the data table and STRING-db.

An initial evaluation of baseline control versus baseline case comparison revealed no statistically significant gene

expression difference between the groups at baseline. Since the adjusted p -values in this comparison were also not statistically significant (adj. $p < 0.05$), a STRING diagram was not produced. These results establish that the baseline case and baseline control groups were comparable and not significantly different at baseline prior to the DST, thereby permitting the following analysis.

The more appropriate investigations of the case stimulated versus case baseline (case analysis) and control stimulated versus control baseline (control analysis) revealed several differentially expressed genes common to both maps with many STRING connections in each map (Figures 5 and 6). These genes were *SOCS1*, *TLR4*, *TLR2*, *SLC11A1*, *CXCR4*, *IRAK3*, *LTA*, and *ARG1*. These genes were present in both STRING diagrams and had similar linkage patterns in both as well. The fact that they were in both STRING diagrams means that they displayed similar degrees of statistical significance in the data from both analyses and were in the 250 most significant genes in both data tables (Table 1). However, the control analysis also had several significant genes (as determined by STRING connections) that were not present in the case STRING diagram. These were *CD86*, *FOS*, *MMP9*, *PRKCZ*, and *SOD1*. All genes discussed in this paper reached statistical significance by adjusted p -value (adj. $p < 0.05$) in both case and control data regardless of presence in STRING diagrams.

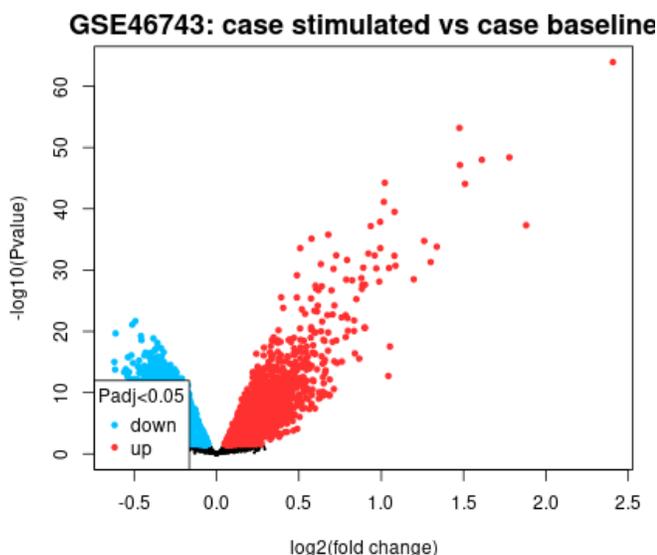


Figure 3. Volcano plot of GSE46743 case stimulated versus case baseline. Volcano plot comparing the gene expression changes between the case stimulated and case baseline groups. The extreme points represent the most highly up- or downregulated genes based on the largest or smallest LogFC values in the comparison. Dots that are colored either red or blue are statistically significant (adj. $p < 0.05$) within this comparison. Dots represented in black are not statistically significant. The data in this figure were obtained through GEO2R analysis of case (individuals affected by MDD) baseline (before treatment with dexamethasone) versus case stimulated (after treatment with dexamethasone) groups of the publicly available dataset GSE46743.

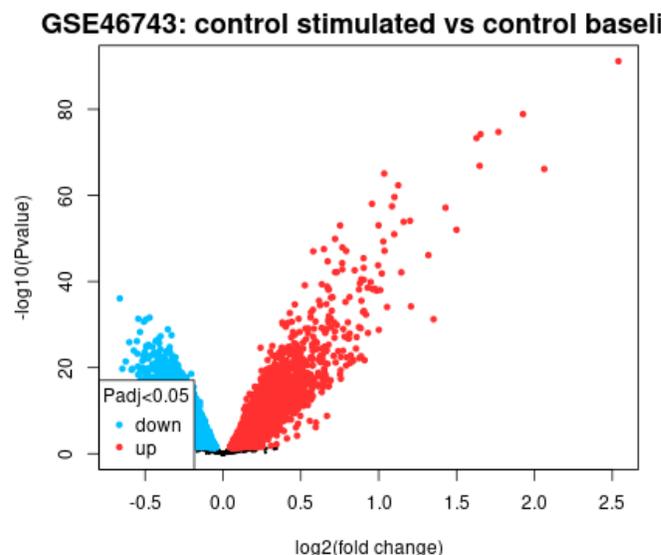


Figure 4. Volcano Plot of GSE46743 control stimulated versus control baseline. Volcano plot comparing the gene expression changes between the control stimulated and control baseline groups. The extreme points represent the most highly up- or downregulated genes based on the largest or smallest LogFC values in the comparison. Dots that are colored either red or blue are statistically significant (adj. $p < 0.05$) within this comparison. Dots represented in black are not statistically significant. The data in this figure were obtained through GEO2R analysis of control (individuals unaffected by MDD) baseline (before treatment with dexamethasone) versus control stimulated (after treatment with dexamethasone) groups of the publicly available dataset GSE46743.

Table 1. Genes common to both case and control comparisons in STRING.

Gene Name	Control LogFC	Case LogFC	Control adj. <i>p</i> -value	Case adj. <i>p</i> -value	Gene ID
TLR2	-1.1250331	1.0806999	7.07e-60	1.86e-37	ILMN_1772387
TLR4	-0.5975919	0.5055261	4.75e-23	2.35e-14	ILMN_1706217
CXCR4	-0.7870243	0.6726273	1.31e-33	3.17e-21	ILMN_2320888
IRAK3	-0.9989241	0.8846404	9.52e-31	3.47e-27	ILMN_1661695
LTA	0.4015930	-0.0369019	4.88e-23	1.67e-15	ILMN_1795464
ARG1	-0.9619422	0.8426668	1.87e-36	1.65e-20	ILMN_1812281
SLC11A1	-0.7942520	0.3123882	2.87e-21	1.40e-09	ILMN_1741165
SOCS1	-0.5795212	0.3510283	5.36e-45	7.67e-32	ILMN_1774733

NOTE: The genes of focus that were present in the STRING diagrams for both the case and control analyses, along with the LogFC values and adjusted *p*-values each gene displayed in both the case and control comparisons. These values indicate the significant changes in expression that occurred in the test group (the stimulated samples) as compared to the control group (the baseline samples), though all genes listed were statistically significant in both case and control comparisons (adj. *p*<0.05). The Gene IDs reference specific genetic isotypes. The data in this table were obtained through GEO2R analysis of control (individuals unaffected by MDD) baseline (before treatment with dexamethasone) versus control stimulated (after treatment with dexamethasone) groups and case (individuals affected by MDD) baseline (before treatment with dexamethasone) versus case stimulated (after treatment with dexamethasone) groups of the publicly available dataset GSE46743.

Several genes were noted in the STRING-db diagram for the control analysis that were not seen in the STRING-db diagram for the case analysis, which prompted further inquiry into those genes. Those genes were significant by adjusted *p*-value (adj. *p*<0.05) in the case analysis as well as the control analysis but were not in the top 250 most significant genes used for STRING evaluation for the case analysis. The genes identified also had differential expression between the control analysis and case analysis similar to the genes noted in common; the expression of the genes in the case analysis was in the opposite direction as in the control analysis, in terms of upregulation (positive LogFC) versus downregulation (negative LogFC).

The first three genes show downregulation in the control analysis and upregulation in the case analysis: *PRKCZ* (control LogFC = -0.381, case LogFC = 0.334), *FOS* (control LogFC = -0.724, case LogFC value = 0.533), and *MMP9* (control LogFC = -1.352, case LogFC = 1.037) (Table 2). The next two genes, in juxtaposition, show upregulation in the control analysis and downregulation in the case analysis: *CD86* (control LogFC = 0.494, case LogFC = -0.256), and *SOD1* (control LogFC = 0.480, case LogFC = -0.398) (Table 2).

The STRING-db diagrams of case analysis and control analysis, respectively, revealed several significantly linked genes in common. The genes were in the top 250 statistically significant genes by *p*-value, and again showed differential expression (Figures 5 and 6). These genes were: *TLR2*, *TLR4*, *CXR4*, *IRAK3*, *LTA*, *ARG1*, *SOCS1*, and *SLC11A1*. In addition to being present in both diagrams, they also had similar linkage patterns and a similar number of

connections between each diagram. These genes can be divided by function into being either pro-inflammatory or anti-inflammatory when upregulated.

Most of these genes were pro-inflammatory in function when upregulated. These pro-inflammatory genes were *TLR4*, *TLR2*, *CXCR4*, *ARG1*, and *SLC11A1* (16, 17, 18, 19, 20, 21) (Table 1). As before most of these genes were down-regulated in the control analysis and upregulated in the case analysis: *TLR4* (control LogFC = -0.598, case LogFC = 0.506) Toll-Like Receptor 2 (*TLR2*) (control LogFC = -1.125, case LogFC = 1.081). *CXCR4* (control LogFC = -0.787, case LogFC = 0.673), *ARG1* (control LogFC = -0.962, case LogFC = 0.843), and *SLC11A1* (control LogFC = -0.794, case LogFC = 0.312) (Table 1). The final gene encodes a protein that is a known counter-regulator of the anti-inflammatory and immunosuppressive properties of glucocorticoids and shows upregulation in the control analysis and downregulation in the case analysis: *LTA* (control LogFC = 0.402, case LogFC = -0.037) (Table 1).

In this study, there was only one gene with anti-inflammatory functions when upregulated: *SOCS1* (control LogFC = -0.580, case LogFC = 0.351) (Table 1).

DISCUSSION

Aberrant responses to the DST have been used to diagnose endogenous depression or major depressive disorder with high sensitivity and specificity (17). The effect of steroids, such as dexamethasone, on inflammation and the HPA feedback loop are well known and described in the literature (7). The expected response to dexamethasone, a potent glucocorticoid, should be anti-inflammatory and

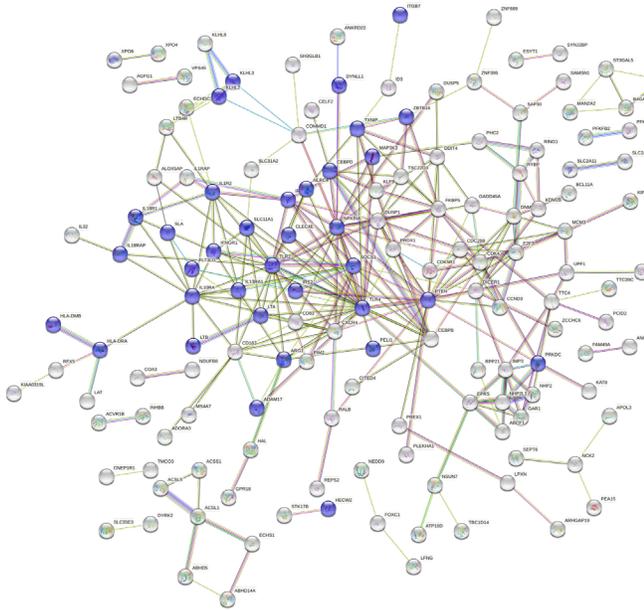


Figure 6. Case stimulated versus case baseline STRING-db diagram. The STRING-db analysis for the case (individuals affected by MDD) baseline (before treatment with dexamethasone) versus case stimulated (after treatment with dexamethasone) comparison demonstrates connections between the top 250 statistically significant (adj. $p < 0.05$) genes in the publicly-available dataset GSE36743. Noticeably, TLR2, TLR4, IRAK3, SOCS1, SLC11A1, LTA, and ARG1 all show a substantial number of connections. All of these genes are also highlighted in blue, which signifies that they are involved in the immune system Reactome pathway.

regulatory of the cortisol production pathways (7). Our research question was whether gene expression can explain the aberrant response in depressed patients.

This study consisted of two comparisons after establishing that the baseline groups were equivalent: the case and control analyses. The analysis of the STRING-db diagrams and GEO2R data table revealed significant differences between the case and control analyses in genes that are associated with the immune Reactome pathway, meaning that the genes associated with this pathway had an impact on immune system regulation or function. Regarding the genes noted in this paper, in all instances, the responses of case and control to the DST were opposite in terms of gene up or downregulation as denoted by the LogFC values in the tables.

When looking at the function of the genes discussed in this paper, we identified three groups of gene function. The first group, when upregulated, was pro-inflammatory or regulatory of pro-inflammatory function. The second group, when upregulated, had cellular protective functions. The final group functioned in glucocorticoid regulation. The genes noted in the control analysis largely show the expected anti-inflammatory response as demonstrated by the downregulation of *PRKCZ*, *FOS*, *MMP9*, *TLR2*, *TLR4*, *CXCR4*, *SLC11A1*, and *ARG1*. While the upregulation of *CD86* was surprising, it may be due to the shortened time

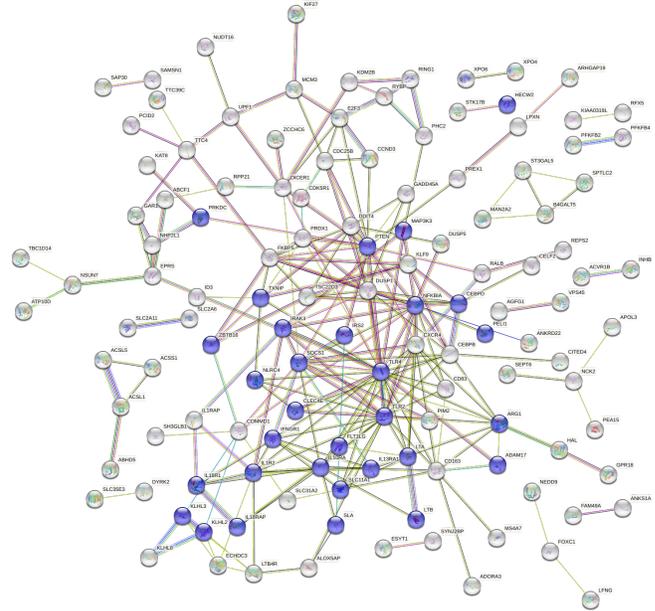


Figure 5. Case stimulated versus case baseline STRING-db diagram. The STRING-db analysis for the case (individuals affected by MDD) baseline (before treatment with dexamethasone) versus case stimulated (after treatment with dexamethasone) comparison demonstrates connections between the top 250 statistically significant (adj. $p < 0.05$) genes in the publicly-available dataset GSE36743. Noticeably, TLR2, TLR4, IRAK3, SOCS1, SLC11A1, LTA, and ARG1 all show a substantial number of connections. All of these genes are also highlighted in blue, which signifies that they are involved in the immune system Reactome pathway.

of exposure in the DST. Upregulation of *SOD1* would be consistent with its function in cellular protective responses, where it encodes a protein that functions to protect cells from interior cell stressors such as free oxygen radicals, thus preventing cell injury and death (18). The upregulation of *LTA* may reflect its function in glucocorticoid regulation where it is attempting to decrease cortisol production rather than an inflammatory response. The downregulation of *IRAK3* and *SOCS1* may reflect their function in the regulation of *TLR2* and *TLR4* mirroring their decrease in response to the DST.

The genes noted in the case analysis largely show an unexpected inflammatory response as demonstrated by upregulation of *PRKCZ*, *FOS*, *MMP9*, *TLR2*, *TLR4*, *CXCR4*, *SLC11A1*, and *ARG1*. Upregulation of *IRAK3* and *SOCS1* may reflect their regulatory function of *TLR2* and *TLR4* in that increased production of the toll-like receptors results in an attempt at inhibitory feedback (19, 20). The downregulation of *LTA* may reflect a decreased ability of case patients to regulate glucocorticoid production (21).

Most of the genes discussed in the case and control analysis are relatively simple to understand in relation to the Reactome Pathway discussed in this paper, a few, as discussed below, require more discussion.

TLR4 is involved in the activation of the immune response and *TLR2* is involved in activation of innate immunity (22, 23).

Table 2. Genes unique to control stimulated versus control baseline in STRING.

Gene name	Control LogFC	Case LogFC	Control adj. p-value	Case adj. p-value	Gene ID
CD86	0.49403141	-0.25559092	1.22e-29	4.95e-11	ILMN_1782560
FOS	-0.72390695	0.53259936	3.51e-20	1.70e-09	ILMN_1669523
MMP9	-1.35228405	1.03650556	1.03e-29	8.98e-12	ILMN_1796316
PRKCZ	-0.38060498	0.33382996	3.51e-20	2.81e-10	ILMN_1697267
SOD1	0.48028996	-0.39845522	1.64e-21	2.65e-11	ILMN_1662438

NOTE: The genes of focus that were present in the STRING diagrams for only the control comparison and appeared to drop out in the case analysis, along with the LogFC values and adjusted p-values each gene displayed in both the case and control comparisons. Though the genes listed here were present and statistically significant (adj. $p < 0.05$) in both the case and control comparisons, they demonstrated a lesser degree of statistical significance in the case analysis, and thus were not above the 250 gene cutoff used to generate the case STRING diagram, but did have p -values small enough to be above the 250 gene cutoff for the control analysis and were able to appear in the STRING diagram as a result. The values in this table indicate the significant changes in expression that occurred in the test group (the stimulated samples) as compared to the control group (the baseline samples). The Gene IDs reference specific genetic isotypes. The data in this table were obtained through GEO2R analysis of control (individuals unaffected by MDD) baseline (before treatment with dexamethasone) versus control stimulated (after treatment with dexamethasone) groups and case (individuals affected by MDD) baseline (before treatment with dexamethasone) versus case stimulated (after treatment with dexamethasone) groups of the publicly available dataset GSE46743.

In one study of the relationship between toll-like receptors and MDD, toll-like receptor mRNA levels were differentially expressed in MDD compared to healthy controls (24). *TLR4* was also found to be an independent risk factor relating to the severity of MDD (24). Additional studies indicate that TLR expression correlates to the severity of depression (24). The current body of research regarding MDD and TLRs indicates several additional findings. The first is that TLR signaling stands as a theoretical suspect in inflammation-mediated depression (24). The second is that numerous studies have implicated TLRs in depression (24).

The *LTA* gene mediates inflammatory, immunostimulatory, and antiviral responses (21). Additionally, it functions in the macrophage migration inhibitory factor (MIF) mediated glucocorticoid regulation superpathway which is a counter-regulator of immunosuppressive and anti-inflammatory activities of glucocorticoids (25). *LTA*'s encoded protein is highly inducible and pro-inflammatory in the immune system (21). Based on this knowledge, the upregulation in the control analysis is either an inflammatory response or more likely a glucocorticoid-induced response to limit the immune suppressive effects of glucocorticoids.

SOCS1 is a suppressor of cytokine signaling and is thus also a negative regulator of inflammation (19). It is involved in superpathways for the regulation of *TLR2* and *TLR4* (19). The downregulation seen in the control analysis may represent a response to the induced downregulation of *TLR2* and *TLR4*.

IRAK3 is a member of IRAK signaling proteins and acts to suppress TLR signaling resulting in decreased pro-inflammatory cytokine production (20). Moreover, *IRAK3* expression is upregulated in response to dexamethasone and thus forms part of the glucocorticoid-mediated immunosuppressive pathways (20). Downregulation in the

control group may represent a response to the induced downregulation of *TLR2* and *TLR4*, with a similar but opposite response in the case analysis for the same genes.

As we have found in this study, activation of inflammatory pathways may be related to the aberrant response of depressed patients to the DST. Persistent elevated levels of stress may be associated with exogenous MDD (5). Physiologic stress can release mediators that cause inflammation (12). In depressed people, there is an increased inflammatory response (26). Specifically, inflammation is displayed in some depressed patients through increased levels of pro-inflammatory mediators in the blood and cerebrospinal fluid (24). Another study showed inflammatory mediators in peripheral blood correlate to inflammatory mediators in the brain (27). Based on that, we may assume that our results may correlate to an inflammatory response in the brain for individuals who are diagnosed with MDD and it certainly correlates with the documented aberrant responses to dexamethasone suppression tests in depressed patients as demonstrated in the case analysis (27).

Gene activity in peripheral blood often overlaps with or mirrors gene activity in the brain, and transcriptional profiling in whole blood has been used to search for biomarkers for neurological and psychiatric disorders (14). In our study, the inflammatory mediators are often active in the brain. Furthermore, the blood-brain barrier (BBB) is a selectively permeable barrier that serves to isolate the central nervous system from the rest of the body (28). In addition to this, one gene studied in this analysis increases the permeability of the BBB and the inflammatory response (29). This gene, *MMP9*, is notably upregulated in the case comparison. This altered brain chemistry could be in part behind the manifestation of depression.

Ultimately, looking to see if inflammation is a driving force behind depression may prompt further research to find non-steroid agents to control the inflammatory response or normalize this response and help treat depression. This aberrant response to the DST could be a biomarker to see the efficacy of the treatment of depression on a cellular level. Inflammation certainly changes the brain's function. If a patient were to feel better emotionally but still display an aberrant response, then a patient may still be suffering from depression. Some treatments may simply mask symptoms.

If the DST could be considered a physiologic model for a stress response, it could be possible to see that in subjects not diagnosed with MDD, the immune system attempts to downregulate the steroid production and activates anti-inflammatory pathways, all of which serve to protect the subject by normalizing the system. In the same situation, patients who are diagnosed with MDD respond by not regulating steroid production and by activating inflammatory pathways. This aberrant response would nearly produce a feedback loop potentiating inflammation and a stress response. The cellular injury from increased inflammatory activity could also result in further cascading activation of an inflammatory response.

However, our study also has several limitations. First, this study only assessed male patients with depression. Future studies could be performed to assess if female patients display similar responses, in terms of the expression levels of the genes considered in this study. Sex differences between males and females may lead to different responses in an all-female study as compared to an all-male one. In addition, this study analyzed samples after only three hours after dexamethasone administration. In both low- and high-dose DST studies, dexamethasone is administered either overnight (8 hours) or over a two-day period (48 hours) (7). In the study we analyzed, dexamethasone exposure was noticeably short compared to a normal DST. Future studies may similarly evaluate whole blood samples taken from depression patients at baseline and case points at 8 hours instead of 3 hours to determine if the patterns realized in this analysis persist. The upregulation of the gene *CD86* in the control analysis was surprising considering that other studies indicate that *CD86* should be downregulated with longer-term exposure to dexamethasone (30). This difference in procedure could potentially lead to the differences described above in the expression of *CD86* since the study cited above took data after 48 hours of dexamethasone incubation (30). While the *CD86* result is unexpected, most studies indicate that long-term exposure to dexamethasone would lead to a decrease in the expression of *CD86*, but the reason for these changes is yet to be determined.

Our study also leaves several questions unanswered. For instance, do drugs used to treat depression impact inflammation levels or alter the inflammatory response to the DST? Would drugs that are primarily anti-inflammatory normalize the inflammatory response to the DST help treat depression? This study does imply a relationship between

inflammation, stress, and depression, as determined through the administration of the DST. Future studies could work towards answering the above questions. These answers could potentially have future implications on the medical community, providing data useful for formulating future treatment or diagnostic options for MDD, or perhaps for a better understanding of the biological mechanisms behind MDD.

In conclusion, the aberrant response to the DST in patients diagnosed with depression appears to be caused by a pro-inflammatory response to exogenous steroids. The genes discussed in this study are unified by their relation to the immune system Reactome pathway, which regulates the innate immune system, adaptive immune system, and cytokine signaling in the immune system (31). As expected, in the control analysis there is a dampening of the immune system and inflammatory pathways; yet the case analysis shows the opposite. Depression may be caused by stress or the response to stress, and the result would be the endogenous secretion of cortisol. This may then result in an inflammatory cascade, causing immune system activation, inflammation, or cellular injury.

MATERIALS AND METHODS

The dataset GSE46743, originally generated for use in a 2015 paper, was selected for use in the study from the NCBI Gene Expression Omnibus (GEO) database, which gave access to microarray expression profiling data derived from whole blood samples from 160 male subjects analyzed using Illumina Human HT-12 v3 arrays (15, 16). This dataset was made up of 320 samples, one taken from each subject before treatment with a DST and one taken after treatment. The data used in this study were derived from multiple comparisons of two groups, which were performed using GEO2r (16). These comparisons were baseline control versus baseline case, case stimulated versus case baseline (3 hours after 1.5 mg dexamethasone administered orally), and control stimulated versus control baseline. String maps were not produced for insignificant comparisons.

Next, GEO2r was used to analyze the other two two-group comparisons to identify trends in gene expression between the groups (16). In preparing to compare the groups of samples, GEO2r's 'define groups' tool was used to sort samples into baseline and stimulated groups for both the control and case samples. Since the order in which the groups are defined does impact downstream results, the test group (the stimulated samples) was defined first and then the control group (the baseline samples) so that the resultant LogFC values would be positive for genes that were upregulated in stimulated samples compared to the baseline ones, and negative for the genes that were downregulated (32). GEO2r uses the Linear Models for Microarray Analysis (Limma) R package to identify differentially expressed genes, which uses a series of linear regression models based on the data to identify patterns (33). Of particular interest to this

analysis were the LogFC values, p -values, and adj. p -values of each gene, so these values were included, along with GENE_SYMBOL, in the spreadsheets of data downloaded from the GEO database for each comparison. After opening the spreadsheets, the data were organized by statistical significance, with the smallest p -values at the top of the table. Included were the adj. p -values in spreadsheet tables, which were calculated using the Benjamini-Hochberg procedure, which serves to decrease the false discovery rate and thus ensures the statistical significance of the differences in gene expression (34).

The top 250 statistically significant named genes were selected with the lowest adjusted p -values from the GENE_SYMBOL category of both the case stimulated versus case baseline and control stimulated versus control baseline comparison data sheets and input each into separate tabs of STRING-db under the 'multiple proteins' tab (35). The top 250 genes by adjusted p -value were selected (as is standard in this type of study) because it provides information on the most statistically significant genes while not generating too dense of a STRING diagram, and because the number is reasonable for enrichment testing. The LogFC values of the genes input into STRING for each comparison were noted. For both comparisons, the organism selected was 'Homo sapiens.' Once the data were input and the organism selected, the STRING diagrams were able to generate and demonstrate the known and predicted protein-protein interactions of the genes that were input. From there, we took note of the genes with the most pathways and connections to other genes, though not all these genes are mentioned in the analysis. The function of these genes was further explored on Genecards.

Once a list of the genes with significant connections from each STRING diagram existed, the lists were compared to see which genes were similar and which ones were not. The genes that were similar in the diagrams for control stimulated vs control baseline and case stimulated vs case baseline were marked for further exploration, as well as those that were unique to the STRING diagram of the control analysis. Appearing under each STRING diagram, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, Reactome pathways, as well as Gene Ontology (GO) pathways, also offered further information on the genes of interest (36, 31, 37). The 'count in network' number of each pathway in the STRING diagram was examined, as well as the false discovery rate. A relatively high 'count in network' and a low false discovery rate would be ideal in finding a pathway to study. Finally, having used these tools to identify genes of interest, further exploration of the functions of these genes was performed using the Genecards database (38).

ACKNOWLEDGMENTS

We would like to thank Dr. Lee and the miRcore volunteer program for guiding us through the research process and exposing us to the various computational genetic resources that were used in this study. We would also like to thank

Bianca Done for helping us find a dataset.

Received: June 8, 2022

Accepted: September 1, 2023

Published: November 6, 2023

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