

Identification of Co-Expressed Genes to BDNF and trk-B as Major Depressive Disorder Related

Biomarkers Using Microarray Data

Jennifer Hu

Nikola Tesla STEM High School

Abstract

Depression is a leading cause of death and disability with more than 264 million people of all ages suffering from the disorder worldwide. Understanding how brain function is altered in depressed patients is crucial for determining novel biological targets that can be used to prevent suicidal behavior. This project aims to identify potential biomarkers for major depressive disorder by finding co-expressed genes with brain-derived neurotrophic factor (BDNF) and receptor tyrosine kinase B (trk-B), two genes that contribute to the pathophysiology of depression through neuron growth and survival (neuroplasticity). 3055 probes for genes involved in nervous system development were screened from public microarray data in the Allen Human Brain Atlas. Average gene expression level and Pearsons correlation coefficient were calculated for each probe using Java programming and Excel to determine correlations between either BDNF or trk-B and a potential gene biomarker. A total of 93 and 27 co-expressed genes were identified for trk-B and BDNF respectively with a significant Pearsons correlation of above +0.5 (trk-B: $0.82 > x > 0.50$, BDNF: $0.68 > x > 0.50$). In addition, 72 biomarkers were further identified by gene function and trends in data as especially important to depression research. These biomarkers may give new insight into genetic factors for vulnerability to depression involving brain neuroplasticity and can be used to create new therapies as drug targets or to improve the remission rates of existing therapies.

Keywords: BDNF, trk-B, neuroplasticity

Identification of Co-Expressed Genes to BDNF and trk-B as Major Depressive Disorder Related Biomarkers Using Microarray Data

Worldwide, depression is a leading cause of death and disability. More than 264 million people of all ages suffer from depression globally and close to 800,000 people die due to suicide every year (WHO, 2020). Antidepressants have been developed that largely focus on the serotonin hypothesis for depression, which explains depression symptoms through a lack of neurotransmitter such as serotonin or norepinephrine in the brain. To relieve these symptoms, drugs such as selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) have been created to block the reuptake in order to increase the amount present in the synapse and increase binding. However, these medications are largely ineffective for major depressive disorder patients. In the STAR*D study conducted by the National Institute of Mental Health, patient remission rates for SSRIs and SNRIs in the first two levels of treatment were only 37% and 31% respectively (Rush, 2006). This illustrates the need for new research that moves away from the serotonin hypothesis to other factors that may contribute to the pathophysiology of depression, such as neuroplasticity.

Neuroplasticity is the brain's ability to adapt and change overtime in response to environmental stressors and it underlies our ability to adjust, learn, and remember. It is thought that reduced neuroplasticity results in the loss of neurons and glia that lead to histological changes in the brain that make a patient more vulnerable to depression. This is supported by studies that show changes in the hippocampus, amygdala, and prefrontal cortex in depressed brains, such as reduced hippocampal volume in depressed patients in comparison to healthy controls (Liu W., 2017).

Two genes important to the neuroplasticity hypothesis are brain-derived neurotrophic factor (BDNF) and receptor tyrosine kinase B (trk-B) which is the receptor that BDNF binds to and activates. The BDNF/trk-B pathway contributes to neuronal proliferation, growth, and survival. Research has repeatedly found that depressed patients tend to have reduced expression of BDNF and trk-B, as shown in a study performed on areas of postmortem brains of suicide subjects where messenger RNA levels of BDNF and trk-B were significantly reduced when compared to control subjects (Dwivedi, 2003). Stress rat models have also shown correspondence between hippocampal BDNF concentrations and vulnerability to depression (Blugeot, 2011). However, inconsistencies in existing scientific literature indicate that BDNF and trk-B may be more complex than directly causing depression pathophysiology (Groves, 2007), demonstrating the need to gain insight into linked genes that could be a new focus for research or used to better understand the current pathway.

This project aims to identify novel biomarkers related to major depressive disorder by finding co-expressed genes to BDNF and trk-B in all grey matter of the brain. Co-expression of a potential biomarker to BDNF/trk-B will be determined by Pearsons correlation co-efficient, with an above +0.5 coefficient indicating a significant correlation. These co-expressed genes may prove to be significant for brain neuroplasticity which can have large implications for new therapies to improve patient remission from depression and improve depression diagnosis.

Methodology

Existing microarray data was obtained from the publicly available open access Human Brain Atlas through the Allen Institute. Gene expression maps contain microarray data across 6

adult donor patient brains of healthy individuals. All data is anonymous and the only donor information provided are age, sex, and ethnicity.

Data Collection

The Allen Human Brain Atlas contains over 58,000 probes of each gene. Probes specifically involved in nervous system development were chosen for the focus of this project because the BDNF/trk-B pathway is involved in nervous system development, therefore increasing the likelihood that correlated genes found would be relevant to neuroplasticity and depression research. From this screening of data, 3055 probes were chosen for download.

Raw data download. The heatmap displaying gene expression on the database was set to log2 intensity values in order to download raw probe data instead of calculated z-scores. The resolution was set to samples instead of default brain structures to obtain all samples that the probe took from each brain structure instead of an average value for each structure. All 3055 nervous system development probes were then downloaded from the database, each with 3702 log2 intensity values, and copied into Excel spreadsheets.

Data Analysis

Average gene expression level. The Allen Human Brain Atlas contains multiple probes for each gene. To single out one probe to represent each nervous system development gene, average gene expression level was calculated. The probe with the highest average expression level best represents the underlying gene expression and would be chosen for further analysis. A Java program was written to automatically find average expression level for all 3055 probes. Probe data was held by a Java file titled “numbers”. This program used Scanner to read the file and assign each log2 intensity into a 2D array with one row containing the values for each probe. The average of each row is then calculated and printed automatically in the console. After

calculations were completed, the data was manually screened to identify the probe with the highest average per gene. 1228 gene probes were screened for next analysis.

Pearsons correlation co-efficient. To identify co-expressed genes, Pearsons correlation co-efficient, or r-score, was calculated between each potential biomarker and both BDNF and trk-B. An r-score of above +0.5 indicated a statistically significant correlation in gene expression. A Correlation Class was written in Java to automatically calculate the r-score when given a BDNF or trk-B array of data values as the x-variable and a row of the 2D array of all genes from the previous program for finding average expression level as the y-variable. The program incorporates code from stackoverflow.com that uses the following formula to calculate r-score:

$$Correl(X, Y) = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2 \sum (y - \bar{y})^2}}$$

The r-scores are printed in the console for each gene between BDNF and trk-B. Genes with an r-score above +0.5 are identified as a potential neuroplasticity biomarker as a BDNF correlate or trk-B correlate.

Researching results. After statistically identifying the co-expressed genes, the correlates are further researched for involvement in neuroplasticity and depression. Research into gene function and existing scientific literature is completed to identify important trends.

Results

The results of the statistical data analysis identified 93 significantly co-expressed genes with trk-B (r-score: $0.82 < x < 0.50$) and 27 significantly co-expressed genes with BDNF (r-score: $0.68 < 0.50$). Further research determined 72 of these genes to be especially important to depression research through trends of neurogenesis, cell adhesion, and involvement in MAPK pathways.

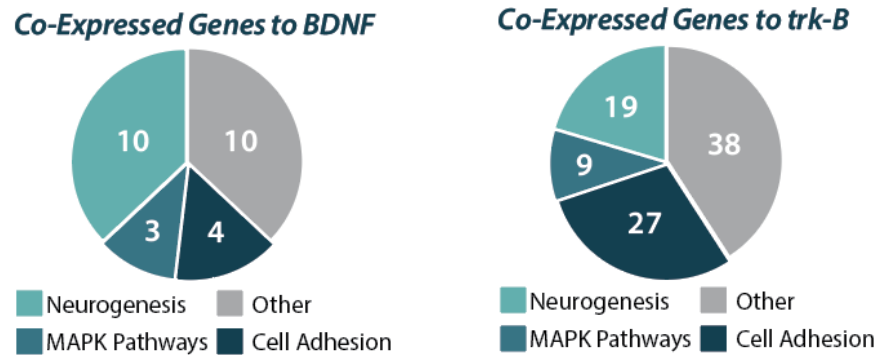


Figure 1. These pie charts indicate the number of genes that correspond to the identified trends of neurogenesis, cell adhesion, and involvement in MAPK pathways out of a total of 27 for BDNF (left) and total of 93 for trk-B (right).

Table 1

Table of 72 Co-Expressed Genes Involved in Identified Trends

trk-B Correlates									BDNF Correlates					
r-score	Gene Symbol	Trend	r-score	Gene Symbl	Trend	r-score	Gene Symbol	Trend	r-score	Gene Symbol	Trend			
0.804251	NOTCH2		0.62803	SPTBN1		0.544695	PCDHGC3		0.678776	NEUROD2				
0.794085	FGFR3		0.618904	CDH2		0.54405	MACF1		0.612815	CDH10				
0.782925	MERTK		0.607976	PCDH11X		0.540986	NPNT		0.595405	OLFM1				
0.741973	EFEMP1		0.607974	CDH11		0.539193	EFNB1		0.587809	L1CAM				
0.73503	BCAN		0.605709	MSI2		0.536575	HES5		0.571706	LRFN2				
0.722813	PDGFRA		0.603669	PCDH10		0.534996	ERBB2		0.570584	NEUROD1				
0.718167	ANGPTL4		0.597871	PDGFRB		0.533861	GHR		0.556112	GPM6A				
0.71051	FGF2		0.594199	FYN		0.532842	PTPRA		0.549768	STXBP5L				
0.702757	PCDH17		0.579421	NCAN		0.529563	PTPRJ		0.546803	NELL2				
0.685308	EGFR		0.571557	PTPN14		0.522763	CHL1		0.54265	NRXN2				
0.67933	CDH20		0.566048	FAT3		0.514131	NCAM1		0.541575	PTPRR				
0.676406	FBLN1		0.56595	DCX		0.512286	PCDH11Y		0.53678	CNTN1				
0.657216	PTPN11		0.562813	MAPK1		0.51029	TNFAIP6		0.533739	SEMA4F				
0.65661	ALK		0.562563	NOTCH2NL		0.508807	NCAM2		0.533729	LRFN4				
0.651435	PTPRF		0.560786	CDH4		0.507124	CREB3L2		0.523172	RTN1				
0.650287	PDGFC		0.554443	TCF3		0.505631	CDH6		0.521389	PLXNA1				
0.641926	MAPKAPK3		0.554086	DTNA		0.50356	FER		0.510956	PPFIA4				
0.629647	ALCAM		0.550485	TNC		0.503415	COBLL1		Neurogenesis Other					
						0.502087	FGF13		MAPK Pathways Cell Adhesion					

Note: This table summarizes the gene symbols and r-scores of the 72 identified neuroplasticity biomarkers and which trend they correspond to.

Discussion and Future Applications

This project succeeded in determining 72 genes to be potential depression biomarkers through significant gene to gene correlations. Additionally, identified trends give insight into the BDNF/trk-B pathway and how it affects brain neuroplasticity. Neurogenesis includes the functions of neuron differentiation, regeneration, synapse formation, and survival, which would be necessary functions for increasing brain volume. The regulation of genes relating to neurogenesis may play a role in reversing the observed reduction of hippocampal volume in depressed brains to reduce depressive symptoms. Another trend, involvement in mitogen-activated protein kinase (MAPK) pathways, was also found among co-expressed genes and may prove important for depression research because of its influence in our inflammatory responses to stress that would cause apoptosis, cell differentiation, and other neurogenesis functions. Previous research provides support for the inclusion of this trend in the results of this project. Extracellular signal-regulated kinase (ERK) pathways, which is a subclass of MAPKs, have been specifically investigated and found to be downregulated in the prefrontal cortex and hippocampus, two areas that contribute to the pathophysiology of depression, in human and animal depression models (Wang, J. Q., 2019). Lastly, the gene function of cell adhesion contributes to synaptic plasticity, axonal growth, and regeneration, which demonstrate connection to neuroplasticity. Cadherin (CDH) genes correspond to the trend of cell adhesion and appear frequently in Table 1, as shown by the presence of CDH20, CDH2, CDH11, CDH4, CDH6, and CDH10 as significantly correlated genes to the BDNF/trk-B pathway. This may indicate novel importance of the cadherin family to neuroplasticity. Another gene of note is neural cell adhesion molecule with NCAM1 and NCAM2 co-expressed with trk-B. This gene finding has been reflected in existing scientific literature; however, co-expression found with the

BDNF/trk-B pathway is novel research. NCAM and its polysialylated form (PSA-NCAM) has been previously examined for antidepressant potential and increased gene expression may contribute to brain neuroplasticity, which supports the identification of NCAM as a potential depression biomarker in this project (Wędzony, 2014).

It is important to note that these results are limited to only 6 donor brains from the Allen Human Brain Atlas. More trials should be completed to confirm the linkage of these genes with the BDNF/trk-B pathway. Furthermore, while human error and experimenter bias were reduced through the usage of computational programs for analysis, sources of error may occur during the transfer of data from one site to another. Future research should be performed by comparing the control human brain data used in this project to new microarray data from depressed brains to determine if an increased or reduced expression in one of the 72 genes causes vulnerability to depression. Observable effects of depressive behavior or decreased neuroplasticity from the manipulated expression of a certain gene can validate its status as a depression biomarker found from this project and is necessary for determining how it may be used in antidepressant therapies.

After experimenting with the effect of identified biomarkers on vulnerability to depression in future experiments, the results from this research can be widely applied to improve the treatment of major depressive disorder patients. First, these genes can be used as drug targets for new drugs that reverse the histological changes prevalent in depressed brains. An example of a potential drug target would be upregulating NCAM in order to increase neuroplasticity in depressed patients. Increasing NCAM would increase binding with trk-B, particularly in the presence of its ligand BDNF, that would promote neurite outgrowth from hippocampal neurons (Cassens, 2010).

The development of neuroplasticity drugs can also be used to improve existing medication. When prescribed in conjunction with existing therapies such as SSRIs, the effects of the SSRI may be amplified by an increase in neuroplasticity that protects the brain from environmental stressors. This demonstrates the potential of these results for building upon current therapies instead of replacing them altogether. Additionally, ketamine is used to induce unconsciousness during electroconvulsive therapy, which is given to patients with very severe depression. Ketamine is greatly associated with BDNF levels, where ketamine's mechanism for action may be associated with an increase in plasma BDNF (Haile, 2014). Despite evidence of ketamine's rapid antidepressant effects, patients' response to the treatment vary considerably. Boosting BDNF levels through changing the expression of identified co-expressed genes with the pathway may better the usage of ketamine to improve its reliability in eliciting a consistent response in patients.

Lastly, this research can also contribute to diagnostic tools with potential for adding a genetic component to depression diagnosis. This would be able to detect depression earlier and more accurately than current questionnaires such as the HAM-D scale. An example could be to detect low serum or blood levels of identified genes such as NCAM that would indicate vulnerability to depression. Doctors would be able to prescribe treatment faster and prevent suicides before traumatic events that trigger extreme depressive behavior occur.

To summarize the implications of these results, the present study identified 72 biomarkers co-expressed with the BDNF/trk-B pathway that further the neuroplasticity hypothesis for depression. These gene findings could be influential for creating effective treatment for depression through the development of novel antidepressants as drug targets, assisting existing

therapies such as SSRIs and ketamine, and creating genetic diagnostic tools to accurately detect vulnerability to depression.

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