Transcriptomic Deconvolution of Dorsal Striata Reveals Increased Monocyte Fractions in Bipolar Disorder

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Keywords

Neuroinflammation · Bipolar disorder · Monocytes · RNA deconvolution

Abstract

Introduction: Accumulating evidence suggests a relationship between the immune system, neuroinflammation, and mood disorders such as bipolar disorder (BD). However, the immunological landscape of critical brain structures implicated with BD, such as the dorsal striatum, has yet to be characterized. This study sought to investigate the immunological composition of dorsal striata in patients with BD. **Methods:** CIBERSORTx, an established RNA deconvolution algorithm, was applied on RNA-sequencing data developed from dorsal striata of 18 BD patients and 17 controls. A validated gene signature matrix for 22 human hematopoietic cell subsets was used to infer the relative proportions of immune cells that were present in the original brain tissue. **Results:** Deconvolution of the bulk gene expression data showed that dorsal striata from BD subjects had a significantly greater relative abundance of monocytes compared to control samples. **Conclusion:** Monocytes may play a role in the pathogenesis of BD in dorsal striata. Further studies are warranted to confirm the computational results presented herein.

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Introduction

Bipolar disorder (BD) is a heterogeneous mental illness characterized by interspersed episodes of mania or hypomania, usually intermixed with bouts of depression. Studies have shown such manic episodes often present with impulsive, risk-taking behavior [1, 2]. Certain models for BD implicate reward-predicting stimuli, and abnormal processing thereof, in the mental illness [3]. From these models evolved the hypothesis that the dorsal striatum, a region of the brain implicated in processing reward and risk information, is involved in BD pathogenesis [4]. Not only is this proposition supported by the fact that dorsal striatum is involved in action-contingent learning and is one of the main input areas for the basal ganglia, but also because previous imaging studies have shown aberrations in function and volume of the striatum in BD subjects [5–7].

In addition to elucidating the dorsal striatum as one of the major brain structures in BD, evidence has accumulated for the involvement of the immune system in mood disorders [8–11]. The majority of case-control studies have discovered that BD patients have increased proinflammatory cytokines during manic episodes compared with controls [12–20]. One recently proposed theory postulates that reduced maturation of T-regulatory cells plays a role in the progression of BD [21]. These studies that have described immune alterations in the periphery

have led to the theory that neuroinflammation, defined broadly as the inflammatory state of the central nervous system, plays a role in BD. Indeed, recent transcriptomic analyses have enriched pathways and discovered differentially expressed genes involved in immune system regulation from brains of postmortem BD subjects [22].

Although previous work has shown a putative role of the immune system in BD, most studies involving the brain have focused on microglia, with no substantial evidence for microglial immune activation [23–27]. Thus, it is imperative to search for the involvement of other immune elements. There has been no investigation to date, as per our knowledge, characterizing the broad spectrum of immune cell subtypes as possible infiltrates in BD-implicated brain structures. Therefore, the present study explored the immune composition of dorsal striata from 18 BD subjects and 17 control subjects through digital cytometry. Bulk RNA-sequencing data derived from dorsal striata were computationally profiled to estimate relative proportions of immune subsets present in the original tissue. The proportions of immune cell subtypes were compared between BD patients and control subjects to examine any differences. It was found that dorsal striata from BD patients were comprised of significantly more monocytes. The present work underscores the importance of examining the striatum to explore possible immune factors involved in BD, which may aid in development of novel neuroinflammation-based therapies.

Materials and Methods

The dataset used for deconvolution consists of human postmortem dorsal striatum gene expression data for 18 BD patients and 18 control subjects (Table 1) [27]. The frozen postmortem samples were retrieved from the Harvard Brain Tissue Resource Center (Belmont, MA, USA). The groups were comparable as no significant differences were found in terms of age, sex, RNA integrity number, and postmortem interval [27]. The read count data are readily available in the Gene Expression Omnibus database (GEO) database (<http://www.ncbi.nlm.nih.gov/geo>) under accession GSE80336. Since the data are publicly available, approval to conduct the present study by an institutional review board was not required.

As per the original study, the raw reads were generated with the Illumina HiSeq2000 platform and were processed by removing low quality base calls with PHRED score <30 and contaminating adapter sequences. Reads were then aligned with BowTie2 [28] to a custom ribosomal DNA reference to remove those aligning to ribosomal RNA genes. TopHat (v2.0.12) [29] mapped the remaining reads against the reference human genome GRCh38. HTSeq-count within HTSeq (v0.6.0) was run in union mode [30]. TopHat and HTSeq parameters included a reverse setting for library strandedness with other parameters set as default. For the present study, the

Table 1. Demographics of bipolar and control subjects

Status	Age, years	Sex	PMI
Control	65.0±15.7	10 F/7 M	22.8±6.4
Bipolar	69.7±15.3	12 F/6 M	25.1±15.8

Adapted from Pacifico and Davis [27]. F, female; M, male; PMI, postmortem interval. Age and PMI reported as mean ± standard deviation.

Table 2. Signature genes used for monocyte relative enumeration

AIF1	CHST15	HNMT	NOD2
APOBEC3A	CLEC4A	HPSE	P2RY13
AQP9	CLEC7A	IGSF6	PADI4
ASGR1	CREB5	LILRA2	RNASE2
ASGR2	CSF3R	LILRA3	RNASE6
BST1	FAM198B	LILRB2	S100A12
C5AR1	FCN1	LST1	SLC15A3
CCR2	FES	MEFV	TLR2
CD1D	FOSB	MNDA	TLR7
CD33	FPR1	MS4A6A	TLR8
CD68	FZD2	NCF2	UPK3A
CDA	HCK	NFE2	VNN1
CFP	HK3	NLRP3	VNN2

read count matrix was retrieved from the GEO database and gene length normalized in transcripts per million space prior to analysis with CIBERSORTx. However, 1 control subject was removed from further analysis due to being an outlier after visualization through hierarchical clustering based on Euclidean distance [27].

CIBERSORTx, an established machine-learning RNA deconvolution algorithm, was implemented to provide an estimation of the relative abundances of immune cells from the original previously published gene expression dataset described above [31] (shown in Fig. 1). Although other deconvolution methodologies are available, such as least-square regression and microarray microdissection, these algorithms are sensitive to experimental noise, closely related cell types, and unknown mixture content, which limits their ability to enumerate cell type proportions [32–34]. CIBERSORTx differs from these methods by implementing support vector regression, a machine-learning approach that improves deconvolution ability through feature selection and allows resolution of closely related subsets. Furthermore, the algorithm accurately quantifies the relative fractions of distinct cell types within a complex gene expression mixture given a priori cell type-specific gene signatures. Specifically, the algorithm requires (1) a matrix of bulk tissue gene expression profiles to be deconvoluted and (2) a signature matrix containing signature gene expression profiles for cell subsets of interest. To estimate the proportion of a specific cell type, the algorithm assesses the relative expression changes of signature genes related to the cell type of interest compared with the expression of all other genes in the sample [31].

Fig. 1. Illustration of workflow. Previously published RNA-sequencing data from bulk dorsal striata were retrieved and deconvoluted through machine learning, allowing for estimation of cell types present in the original tissue.

To estimate the amount of immune cell subtypes, a validated signature matrix containing a total of 547 genes for distinguishing 22 human hematopoietic cell subsets, termed “LM22,” was used to deconvolute the bulk dorsal striata gene mixture matrix [31]. The CIBERSORTx algorithm was run with bulk-mode batch correction and 500 permutations in relative mode. Quantile normalization was not implemented as dataset was generated from RNA-sequencing. Deconvoluted samples were deemed significant if CIBERSORTx p value <0.05 , which represents the significance of the deconvolution results across all cell subsets for goodness-of-fit. This p value is determined for each sample using Monte Carlo sampling, effectively providing a confidence measure in the results [31]. The data output from CIBERSORTx was downloaded and analyzed with R programming language.

The relative proportions estimated by the algorithm for each cell subtype were compared between BD patients and control subjects. Normality was tested with the Shapiro-Wilk method and visually inspected through a Q-Q plot. Homogeneity of variance was tested with Levene’s test. Differences between group means were tested by the two-sided Student’s t test assuming equal variance or with Welch’s test for heteroskedastic cell subtypes. If the normality assumption was not met, the non-parametric Mann Whitney U test was used for comparisons. A false discovery rate-adjusted p value <0.05 was deemed significant (Benjamini-Hochberg).

Results

After RNA deconvolution for immune cell subset identification, all 35 samples were significant at the criteria for CIBERSORTx p value <0.05 and were deemed suitable for further analysis. When comparing relative abundances between BD subjects and controls, BD dorsal stri-

ata exhibited a significantly higher monocyte fraction (shown in Fig. 2) ($p = 0.013$). The algorithm estimated the relative fractions of this cell type through unique signature genes specific to monocytes taken from the input LM22 signature matrix (Table 2) [31]. Significant changes in the relative proportions were not observed amongst the other 21 immune cell subtypes evaluated, including related subtypes such as M2 and M1 macrophages.

Discussion/Conclusion

As presented here, the digital dissection of the immunological landscape from existing gene expression data has shown a bias for monocytes in dorsal striata of BD subjects. These findings corroborate a previous report that implicates monocytes in the pathophysiology of BD. Jakobsson et al. [32] found increased levels of monocyte chemoattractant protein-1 and chitinase-3-like protein-1 (YKL-40) in cerebrospinal fluid from patients with BD. These proteins are primarily secreted by cells of monocytic origin and have been associated with neuroinflammation in neurologic disorders [33]. Although these proteins were measured in cerebrospinal fluid as opposed to brain tissue, cerebrospinal fluid marker levels have been shown to accurately reflect immunological activity in the brain [34, 35]. Marzi et al. [36] also observed an increased frequency of circulating monocytes in the plasma of BD patients. The explanations regarding this inflammatory

Fig. 2. Bar plot displaying mean relative proportions of immune cells estimated from dorsal striata of BD subjects and CNTRL. Asterisk (*) denotes significance at $p < 0.05$. Error bars indicate 95% confidence intervals. BD, bipolar disorder; CNTRL, controls.

profile can be summarized as follows: (1) stress precipitated by mood episodes may increase monocytes; (2) increased monocytes trigger mood disorder as proposed by the macrophage theory of depression [37]; (3) there is a common factor underlying both BD and increased monocyte frequency; or (4) monocyte overabundance and BD are actually 2 separate entities caused by 2 different factors present in the same environment [38]. Nonetheless, further studies are required to elucidate the purpose and relevance of monocytes in BD.

A limitation of the current study is the *in silico* approach to deconvolute gene expression data from bulk tissue sample. The data processing does not account for the spatial relationship of tissue and cytoarchitecture. Further studies are required to confirm the present computationally derived results. It is also important to note that an increased blood-brain barrier permeability in pa-

tients with BD may be a potential confounder in this study. Disruptions in the blood-brain barrier integrity may cause an abnormal influx of proinflammatory substances, such as immune cells, from peripheral blood into brain parenchyma [39]. Nonetheless, this digital cytometric technique has previously replicated conventional flow cytometry data with significant clinical implications [40–43]. Although medication use and mood state were unknown in the current cohort, and therefore could not be controlled for, this current article is the first to characterize possible differences in immune cell subtype composition in brain tissue, specifically among dorsal striata, in BD through an unbiased computational perspective.

Based on the hypothesis-generating data presented here, further studies are warranted to elucidate whether this immune element of BD is a causal factor of the illness or is a finding precipitated by the illness itself. In conclu-

sion, the digital approach used herein resolved monocytes as potential immune infiltrates in dorsal striata of BD subjects.

Statement of Ethics

Not applicable. This article does not contain any studies with human participants performed by the author.

Conflict of Interest Statement

The author has no conflicts of interest to declare.

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Author Contributions

S.B. formulated design and analyzed data. Results were interpreted by S.B. Manuscript was written and edited by S.B. All authors read and approved the final manuscript.

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