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C4A mRNA expression in PBMCs predicts the presence and severity of delusions in schizophrenia and bipolar disorder with psychosis

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ABSTRACT

Altered immune function is an established finding in psychotic disorders such as schizophrenia and bipolar disorder with psychosis, though its role in their development and progression remains to be understood. Evidence suggests altered JAK-STAT1 pathway activity in peripheral blood cells from participants with schizophrenia compared to controls. Activation of this pathway leads to increased expression of complement component 4A (C4A), which has recently been implicated in schizophrenia. Here, we examine mRNA expression of C4A in peripheral blood cells from participants with schizophrenia, bipolar disorder and controls. STAT1 and IRF-1 mRNA expression are included as measures of JAK-STAT1 pathway activation in the same participants. Further, we examine the association of each genes mRNA expression with clinical symptom measures using the Positive and Negative Syndrome Scale (PANSS) and the Psychotic Symptom Rating Scale (PSYRATS). We demonstrate that C4A, STAT1 and IRF-1 mRNA expression levels are correlated across the entire sample, indicating shared transcriptional regulatory mechanisms. Further, we show that C4A mRNA expression alone is positively associated with psychotic symptomatology, specifically the presence and severity of delusions. These findings are noteworthy given recent findings that demonstrate a critical role for complement proteins in synaptic pruning, alterations of which are proposed to contribute to psychopathology in psychosis.

1. Background

Altered immune system activity has been repeatedly demonstrated in individuals with schizophrenia and bipolar disorder, though a clear mechanistic understanding of the cause and consequence of immune activation remains to be elucidated (Beumer et al., 2012; Miller and Goldsmith, 2016). We have recently shown that a subset of individuals with schizophrenia have elevated levels of phosphorylated STAT1 (pSTAT1-Y701) in peripheral blood mononuclear cell (PBMC) nuclear extracts, indicating activation of the JAK/STAT1 signaling pathway (Sharma et al., 2016). This pathway is involved in the activation of myeloid cells to an inflammatory phenotype and is downstream of a number of key membrane cytokine receptors, including interferon gamma (IFN-γ), which demonstrates altered expression in schizophrenia (Miller et al., 2011; Rauch et al., 2013). Complement component 4A (C4A) was among the top 20 most significantly enriched genes in a genome-wide study of STAT1 binding regions, and demonstrated a strong transcriptional response measured by increased mRNA expression following cytokine stimulation (Satoh and Tabunoki, 2013). Interestingly, C4A has recently been implicated in schizophrenia (Sekar et al., 2016). The authors demonstrated that common structural variants of the C4 gene region which lead to the greatest expression of C4A mRNA also confer risk for developing schizophrenia. Additionally, C4A mRNA expression was elevated in multiple areas of post-mortem brain from individuals with schizophrenia compared to controls.

As C4A is strongly induced by activation of the JAK-STAT1 pathway we hypothesized that elevated C4A mRNA expression in schizophrenia could also reflect altered immune activity. While we have not investigated JAK-STAT1 signaling in participants with bipolar disorder, the overlap between these disorders with regards to the presence of peripheral inflammation is well documented (Drexhage et al., 2010; Goldsmith et al., 2016). Previous reports comparing serum C4 protein levels in schizophrenia and bipolar disorder to controls have had mixed results (dos Santos Soria et al., 2012; Mayilyan et al., 2008; Wadee et al., 2002). However, these studies are somewhat difficult to interpret because they do not distinguish between C4A and C4B protein isoforms. To our knowledge, the JAK-STAT1 signaling pathway has not been directly investigated in post-mortem tissue from individuals with a diagnosis of schizophrenia or bipolar disorder. However, results of microarray studies often show differential expression of genes involved in the immune response in both blood and brain, and mega-

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analysis of these findings revealed a significant enrichment of the hallmark IFN-γ response gene set in schizophrenia post-mortem brain (Bergon et al., 2015; Hess et al., 2016).

Additionally, while a number of peripheral immune markers have been associated with specific categories of psychopathology such as positive symptomatology and cognitive deficits in both schizophrenia and bipolar disorder (Cabrera et al., 2016; Chase et al., 2016; Hope et al., 2015, 2013), the relationship of complement expression and clinical variables in these disorders has not been previously addressed in the scientific literature. Given the complexity, heterogeneity and lack of definitive diagnostic borders of schizophrenia and bipolar disorder, it is critical to consider not only diagnostic categories but to also examine more closely the association between immune activation and symptom clusters (i.e. delusions, hallucinations, thought disorder).

Our objective, therefore, was a) to measure C4A mRNA expression in a sample of participants with schizophrenia, bipolar disorder with psychosis and controls, b) to determine whether C4A mRNA expression is associated with mRNA expression of STAT1 and IRF-1, used as measures of JAK-STAT1 pathway activity (Satoh and Tabunoki, 2013; Waddell et al., 2010), and c) to examine the relationship of C4A mRNA expression with diagnosis, clinical metrics and symptomatology.

2. Methods

2.1. Participant information and clinical measures

The study was approved by the IRB of the University of Illinois, and signed consent was obtained prior to the initiation of study procedures. Inclusion criteria for the study included persons between the ages of 21 and 60 who met DSM-IV diagnostic criteria for schizophrenia or bipolar disorder with psychosis, or persons with no history of a psychiatric disorder. Exclusion criteria included treatment with VPA, carbamazepine, or clozapine in the previous 30 days, current substance dependence, seizure disorders, and neurological conditions. Consensus diagnoses were determined by both the clinical and research team using the Structured Clinical Interview for DSM-IVTR (First et al., 2002) and available collateral information. Demographic characteristics for the sample were obtained at the study evaluation and are outlined in Table 1. Antipsychotic use for participants with schizophrenia was converted to chlorpromazine equivalents (CPZE) (Danivas and Venkatasubramanian, 2013; Gardner et al., 2010). The Positive and Negative Syndrome Scale (PANSS) was administered to participants with schizophrenia and bipolar disorder with psychosis, and the Psychotic Symptom Rating Scale (PSYRATS) was administered to a subset of 54 participants with schizophrenia or bipolar with psychosis (Haddock et al., 1999; Kay et al., 1989).

Table 1
Demographic metrics displayed by participant group.

Demographic	Control		Schizophrenia		Bipolar	
Sex	*n*		*n*		*n*	
Female	25		24		11	
Male	19		39		12	
Total	44		63		23	
Age (Mean ± SD)	38.8 ± 12.8		40.8 ± 13.2		40.9 ± 13.5	
Race**	*n*		*n*		*n*	
Caucasian, non-Hispanic	12		4		4	
Black, non-Hispanic	18		49		16	
Asian or other Pacific Islander	10		2		0	
Hispanic	4		8		3	
BMI (Mean ± SD)*	29.3 ± 8.1		32.8 ± 8.6		28.8 ± 7.7	
Nicotine*	Yes	No	Yes	No	Yes	No
	7	35	27	35	10	13
Medication	Yes	No	Yes	No	Yes	No
Typical Antipsychotics	0	44	10	53	1	22
Atypical Antipsychotics	0	44	50	10	16	7
mRNA Expression	*Mean ± SD*		*Mean ± SD*		*Mean ± SD*	
C4A	3.94 ± 1.55		4.14 ± 1.52		3.66 ± 1.20	
STAT1	6.38 ± 5.02		5.31 ± 4.01		4.89 ± 3.23	
IRF-1*	10.87 ± 5.65		8.50 ± 4.48		7.69 ± 3.05	

* $p \le .05$.
** $p \le .01$.

2.2. Sample collection, processing and quantitative PCR

Collection of blood samples, PBMC isolation, and RNA extraction were carried out according to previously described protocols (Chase et al., 2015). RNA extracts were treated with DNase (Ambion) to remove any possible genomic DNA contaminants, and reverse transcribed using the Applied Biosystems High Capacity Archive Kit. Maxima SYBR Green/ROX qPCR Master Mix (#K0222) was used for detection of PCR product and mixtures were run on a Thermo Scientific™ PikoReal. Relative quantification values were calculated using the delta delta ct method relative to the geometric mean of the housekeeping genes GAPDH and ACTB (Vandesompele et al., 2002). Primers were designed using NCBI primer-BLAST. Primers sequences were as follows: C4A forward, 5′-GGCTCACAGCCTTTGTGTTG-3′; C4A reverse, 5′-CCCTGCATGCTCCTGTCTAA-3′; STAT1 forward, 5′-GCCAAAGGAA GCACCAGAGCCAAT-3′; STAT1 reverse, 5′-AGGAGACATGGGGAG CAGGTTGT-3′; IRF-1 forward, 5′-ATGAGACCCTGGCTAGAG-3′, IRF-1 reverse, 5′-AAGCATCCGGTACACTCG-3′; GAPDH forward, 5′-CGAGATCCCTCCAAAATCAA-3′; GAPDH reverse, 5′-TTCACACCCATGAC GAACAT-3′; ACTB forward, 5′s-TGAAGGTAGTTTCGTGGATGC-3′; ACTB reverse, 5′-TCCCTGGAGAAGAGCTACGA-3′. The C4A PCR product was sent to University of Illinois at Chicago DNA Services for sequencing to confirm specificity for C4A mRNA.

2.3. Cell culture

THP-1 cells (ATCC TIB-202) were maintained in culture at 37 °C and 5% CO_2 with RPMI 1640 medium supplemented with 10% FBS, l. glut, and 50u/mL each of penicillin and streptomycin. Cells were treated with either vehicle, 10 ng/μl IFN-γ, or 100 ng/μl lipopolysaccharide (LPS) for 1, 6 and 24 h prior to harvesting.

2.4. Primary clinical measures

The primary clinical measure for this study was the PANSS (Kay et al., 1989). PANSS items were scored along a continuum of severity between 1 (asymptomatic) and 7 (extreme symptom severity). The coefficient alpha for inter-rater reliability was between 0.83 and 0.87. Analysis was conducted via data reduction strategies guided by prior empirical studies of symptom domains assessed by the PANSS. Scores were calculated for five-factors assessing Positive symptoms (delusions, grandiosity, suspiciousness/persecution, unusual thought content), Negative symptoms (blunted affect, emotional withdrawal, poor rapport, passive/apathetic social withdrawal, lack of spontaneity and flow of conversation, and active social avoidance), Cognitive Disorganization (conceptual disorganization, difficulty in abstract thinking, mannerisms and posturing, disorientation, and poor attention), Excitement (excitement, hostility, tension, and poor impulse control), and Depression (somatic concern, anxiety, guilt feelings, depression, and preoccupation). Items were grouped in this way based on previous factor analytic findings (Lindenmayer et al., 1994).

To further evaluate the characteristics of the delusions, the PSYRATS was administered. The PSYRATS consists of two sub-scales that measure the dimensions or characteristics of auditory verbal hallucinations and delusions (Haddock et al., 1999). For the purposes of this study, we utilized the delusions subscale that measures 6 characteristics of delusions. The dimensions of delusions consisted of items that measured frequency, duration, conviction, emotional valence of content and disruption caused by delusional activity. Each dimension of this scale is evaluated on a 5-point Likert Scale ranging from 0 to 4. The coefficient alpha for inter-rater reliability was over 0.80 for each dimension.

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