Psychiatry Research: Neuroimaging 224 (2014) 107–111

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

journal homepage: www.elsevier.com/locate/psychresns

# Correlations of inflammatory gene pathways, corticolimbic functional activities, and aggression in pediatric bipolar disorder: A preliminary study

Drew Barzman [a,b], Jim Eliassen [b,c], Robert McNamara [b], Pablo Abonia [d], Douglas Mossman [b], Michele Durling [b], Caleb Adler [b,c], Melissa DelBello [a,b], Ping-I Lin [b,e,*]

[a] Division of Child and Adolescent Psychiatry, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[b] Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[c] Center for Image Research, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[d] Division of Allergy and Clinical Immunology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[e] Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

ARTICLE INFO

*Article history:*
Received 4 November 2013
Received in revised form
17 February 2014
Accepted 30 July 2014
Available online 15 August 2014

*Keywords:*
Tumor necrosis factor
Frustrative non-reward
Anterior cingulate gyrus
Amygdala
Prefrontal cortex
RNA-sequencing

ABSTRACT

The mechanisms underlying aggression in adolescents with bipolar disorder have been poorly understood. The present study has investigated the associations among TNF gene expressions, functional brain activations under the frustrative non-reward task, and aggression in adolescents with bipolar disorder. Baseline gene expressions and aggressive tendencies were measured with the RNA-sequencing and Brief Rating of Aggression by Children and Adolescents (BRACHA), respectively. Our results show that activity levels of left subgenual anterior cingulate gyrus (ACG), right amygdala, left Brodmann area 10 (orbitofrontal cortex), and right thalamus were inversely correlated with BRACHA scores and were activated with frustrative non-reward during the affective Posner Task. In addition, 11 TNF related gene expressions were significantly correlated with activation of amygdala or ACG during the affective Posner Task. Three TNF gene expressions were inversely correlated with BRACHA score while one TNF gene (TNFAIP3) expression was positively correlated with BRACHA score. Therefore, TNF-related inflammatory cytokine genes may play a role in neural activity associated with frustrative non-reward and aggressive behaviors in pediatric bipolar disorder.



## 1. Introduction

Pediatric-onset bipolar disorder, which is characterized by mood episodes, often initially present in adolescents (Perlis et al., 2009) and is frequently associated with irritability, impulsiveness, and aggression. A longitudinal prospective study found that episodic irritability in early adolescence ($13.8 \pm 2.6$ years) predicted the emergence of mania in mid-adolescence ($16.2 \pm 2.8$ years) (Leibenluft et al., 2006). Regardless of the severity of identified mood symptoms, pediatric aggression is a common and major public health problem that increases the risks of drug and alcohol abuse, violence in adulthood, suicide, and incarceration while also facing the reiterative path of being both the target and eventual source of abusive parenting (Tremblay et al., 2004). Developing a better understanding of the neurobiological mechanisms underlying aggression in adolescents with bipolar disorder may provide novel avenues for developing intervention strategies to reduce aggression.

Inflammatory cytokines have been associated with various psychiatric conditions. Tumor necrosis factor-alpha (TNFα), a cytokine involved in both systemic inflammation and the acute phase reaction, may influence neuronal and neurochemical processes associated with aggression in preclinical and clinical studies (Patel et al., 2010; Suarez et al., 2002). In comparison to mice without mutations, mice with combined deletions of TNF receptor type 1 (TNF-R1) and type 2 (TNF-R2) did not exhibit aggression during a task that typically elicits physical aggression in mice (Patel et al., 2010). A study of 62 healthy men (18 to 45 years old) suggests that hostility and aggression are correlated with the level of expression of TNFα (Suarez et al., 2002). In the latter study, peripheral blood monocyte TNFα expression was measured after stimulation with lipopolysaccharide (LPS), a general activator of the immune system. LPS-stimulated TNFα expression was positively correlated with physical aggression, verbal aggression, and hostility. In other studies, LPS-induced increases in TNFα correlated with stress-induced emotion arousal,(Moons et al., 2010; Suarez et al., 2006) and interferon-induced labile anger (Lotrich et al., 2010).

* Corresponding author at: Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, MLC 5041, 3333 Burnet Avenue, Cincinnati, OH 45241, USA.
*E-mail address:* pingi.lin@gmail.com (P.-I. Lin).

http://dx.doi.org/10.1016/j.pscychresns.2014.07.009
0925-4927/

Despite the association between TNFα and aggression, to our knowledge, little is known about the relationship between TNFα and aggression in adolescents with bipolar disorder. Serum TNFα levels are found to be higher overall in children than in adults and increase with age between 3 and 14 years old, peak at age 13–14 years old, and then significantly decrease in adulthood (Sack et al., 1998). Therefore, research on the link between TNFα and aggression in adolescents needs to factor in age-dependent effects of TNFα levels. Moreover, meta-analysis studies have consistently reported higher concentrations of TNFα in individuals with bipolar disorder (Modabbernia et al., 2013; Munkholm et al., 2013b). Taken together, these findings suggest that TNFα may also play a role in linking aggression and pediatric bipolar disorder.

Prior evidence has suggested that TNFα may mediate the neuroinflammatory process involved in bipolar disorder (Munkholm et al., 2013a). TNFα has been found to be involved in neuroinflammation in several brain regions associated with emotion regulation or impulse control, including amygdala (McAlpine et al., 2009), and prefrontal cortex (Dargahi et al., 2011). In addition, TNFα may enhance synaptic transmission through increased neurotransmitter release in the anterior cingulate cortex (ACC), a structure in the limbic system associated with impulse control (Jia et al., 2007). With these considerations in mind, we proposed to investigate associations among TNF family gene expressions, functional brain activity in response to frustrative non-reward (i.e., amygdala, prefrontal cortex, and ACC), and aggression in adolescents with bipolar disorder. Our primary hypothesis is that the TNF gene expression of non-stimulated peripheral blood mononuclear cells (PBMC) would correlate with both aggression and brain activations in corticolimbic circuits (with a focus on amygdala, anterior cingulate gyrus (ACG), and orbitofrontal cortex) in adolescents with bipolar disorder.

## 2. Methods

### 2.1. Subjects

Ten adolescents aged 12–17 years (15 ± 1 years) (five females/five males) with a primary diagnosis of bipolar disorder, type I, (DSM-IV-TR criteria) were recruited as part of a larger study examining genetic predisposition in children with bipolar disorder. Clinical diagnoses were confirmed with the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS). The WASH-U-KSADS has established diagnosis and symptom reliability (diagnostic kappa = 0.94) (DelBello et al., 2001). Written and informed assent and consent were obtained from the participants and their respective guardians. The study was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center (CCHMC).

### 2.2. Assessments of aggressive tendencies

The Brief Rating of Aggression by Children and Adolescents (BRACHA) is a valid and reliable 14-item instrument for predicting pediatric inpatient aggression in the psychiatric units (Barzman et al., 2012; Barzman et al., 2011). The BRACHA, which includes assessment items for previous aggression and current impulsiveness, was completed on each study participant. Each of the 14 BRACHA items has a range of scores from 0 (negative for the risk factor) to 1 (positive for the risk factor).

### 2.3. Neuroimaging

Following the screening, all subjects underwent brain scans with a 4.0 T Varian Unity INOVA Whole Body MRI/MRS system (Varian Inc., Palo Alto, CA) located at the Center for Imaging Research at the University of Cincinnati. To provide anatomical localization for activation maps, a high-resolution, $T_1$ -weighted, 3-D brain scan was obtained using a modified driven equilibrium Fourier transform (MDEFT) sequence after which a multi-echo reference scan was obtained (Lee et al., 1995). A midsagittal localizer scan was acquired to place 30 contiguous 5-mm axial slices to encompass the entire brain. Next, a multi-echo reference scan was obtained to correct for ghost and geometric distortions (Schmithorst et al., 2001). Subjects completed the fMRI session in which whole-brain images (volumes) were acquired every 2 s while performing the continuous processing affective Posner Task (described below) (Rich et al., 2011) using a $T_2$-weighted gradient-echo echoplanar imaging (EPI) pulse sequence. Visual stimuli were presented using high-resolution video goggles (Resonance Technologies, Inc., Northridge, California).

### 2.4. General image processing

The fMRI data were evaluated using Analysis of Functional NeuroImages (AFNI) (National Institutes of Health, Bethesda, Maryland); http://afni.nimh.nih.gov/afni (Cox, 1996; Cox and Hyde, 1997) Magnetic resonance images were reconstructed to convert raw scanner data into AFNI format. Structural and echo-planar (functional) images were co-registered based upon scanner coordinates. Subject motion was determined in six directions of rotation and translation, and the maximum motion of any analyzed subject was $< 5$ mm. In addition, each volume was inspected for signal artifacts using a semi-automated algorithm in AFNI and excluded from further analysis if uncorrectable head movement occurred. Anatomical and functional maps were transformed into stereotactic Talairach space using the International Consortium for Brain Mapping 452 template (Laboratory of NeuroImaging, University of California-Los Angeles, Los Angeles, California).

### 2.5. Voxel-wise analyses

Individual voxel-wise event-related activation maps were created following standard AFNI procedures using an algorithm that compares the actual hemodynamic response to a canonical hemodynamic response function. Event-related response functions were calculated. Cubes provided the baseline against which hemodynamic responses were assessed. A voxel-wise statistical analysis was performed to identify regions that exhibited significant interactions.

### 2.6. Frustrative non-reward task: affective Posner Task

During the fMRI scan, participants performed a spatial selective attention task modified to provoke negative emotions. This affective "Posner Task" was designed to induce frustration by the use of rigged negative performance feedback. The affective Posner Task has been used to study irritability, a marker of reactive aggression by adolescents with bipolar disorder (Leibenluft et al., 2003; Rich et al., 2011). Our version of the task was designed based on these prior publications. The task is divided into three parts, with 50 trials in part 1 (sincere feedback, no monetary reward), 50 trials in part 2 (sincere feedback, monetary reward for correct performance, monetary punishment for errors), and 50 trials in part 3 (rigged feedback). An individual trial is comprised of several events. First a black fixation cross appears in the center of the computer screen flanked by two empty boxes outlined in black, which alerts the subject to the beginning of a trial. Next either the left or right box is momentarily flashed with blue interior. Following this peripheral box-brightening cue, a black circle appears momentarily in either the left or right box. The subject's task is to press a button in response to the position of the circle on the left or right. Performance feedback is presented following the response on each trial. Participants respond by pressing the left or right button on a button box held in the right hand using the index or middle finger. After a variable delay performance feedback is provided. Performance feedback is systematically manipulated over the course of three runs to increase arousal and induce frustration. In part 1, feedback consists of the word "correct" after accurate responses and "incorrect" after errors or responses that were longer than a certain amount of msec. In part 2 the words "correct" and "incorrect" were accompanied by a monetary reward or punishment (+ or −$0.10) with the objective of inducing arousal about performance. At the end of part 2 subjects were told that they had responded too slowly and would have to repeat part 2. In fact, we then conducted part 3, and rigged the feedback to induce frustration in addition to arousal. In part 3 ~50% of correct trials received "correct" feedback and a reward of +$0.10. The other roughly 50% of correct trials received "too slow" as feedback and a monetary penalty of −$0.10. All response errors received "incorrect" and a penalty of $0.10. Responses that were slower than a certain amount of msec received "too slow" and a monetary penalty.

During practice and performance of the task, participants were told to work as quickly and accurately as possible and that the computer would inform them that their answers were either "correct" or "incorrect." In addition they were told that they would be paid according to their performance. In addition, participants were asked to rate their subjective emotions using a self-assessment manikin (SAM) method (Bradley and Lang, 1994). The SAM process was conducted before and after each part of the fMRI task. The SAM ratings are based on personal assessment of one's feelings relative to three different visual scales. The scales are not explicitly described to the subject, but they have graphical representations of emotional states. The subject places a cursor in a position relative to the range of emotions displayed that best represents how they are feeling. The positions of the cursor placements are recorded in order to assess arousal and frustration levels before and after each part of the fMRI task. Participants received compensation of 50 U.S. dollars regardless of their performance during the task but participants did not know this during the tasks.