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Differences in perceived life stress in bipolar I and II disorder: Implications for future epigenetic quantification

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ABSTRACT

Objective: Few instruments measuring life events over the course of bipolar disorder distinguish the valence of events or consider cumulative stress burden. In the current study, we used a valence-focused life event questionnaire to assess stress in the last 12 months in patients with bipolar I (n = 863) and bipolar II (n = 362) disorder.

Methods: Associations between recent stress and lifetime illness severity features were evaluated via linear and logistic regression, adjusting for age and gender. We additionally investigated the feasibility of quantifying recent stress burden by measuring methylation at a known bipolar susceptibility locus, *SLC1A2* in a subset of bipolar I patients (n = 150) with or without comorbid substance use.

Results: Bipolar II patients endorsed higher total, negative, and positive stress burden than their bipolar I counterparts, but the latter displayed more significant stress-illness severity associations, notably to all forms of substance abuse (e.g., alcohol, nicotine, food, other drugs). Irrespective of bipolar subtype, negative stress burden was significantly associated with illness severity features. High versus low total stress predicted hypomethylation of the *SLC1A2* promoter ($p < 0.05$).

Conclusion: Together, these findings reveal substantial differences in how bipolar subtypes experience and perceive stress. The observed degree of association between recent stress and substance abuse in bipolar I lend further support to the multidirectional effects of stress, affective episodes, and substance abuse on illness severity. Quantification of recent total stress using the methylation status of the *SLC1A2* promoter is feasible, although a whole-methylome approach will likely prove more effective in disaggregating other environmental influences.

1. Introduction

Quantifying the impact of a life event towards the onset of bipolar disorder or illness recurrence has proven difficult due to methodological challenges in psychometric assessment and associated causality [1]. The Longitudinal Follow-Up Evaluation (LIFE) and other LIFE modification scales have predominantly been utilized in research studies, but have identified age, socioeconomic status, family status, race, anxiety, and disruptive comorbidity as associated risk factors for negative life events in childhood and adolescent bipolar disorder [2,3]. Additional factors to assess impact of stress in bipolar disorder include overall stress burden, patient perception of stressor severity, medical comorbidity, and time

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course of illness (i.e., premorbid, early-onset, established disease). Stress events also display variable degrees of kindling, or weakened association of stressors with mood episodes over time [4]. Emotional valence of the stressor, cumulative stress burden, and differences by bipolar subtype are not often systematically investigated.

There is increasing interest in molecular quantification of stress. Emerging evidence implicates DNA methylation in the regulation of human stress reactivity. Early traumatic stress demonstrates lasting impact on methylation patterns, particularly in genes related to hypothalamic–pituitaryadrenal (HPA) axis functionality [5,6]. As a biomarker, DNA methylation is ideal as dynamic, tissue-specific, sequence-context-dependent, and displays *trans*-generational inheritance [7]. Methylation biomarkers, in certain contexts, help lessen reliance on self-report and the impact of recall bias. For example, machine learning modeling of genome-wide methylomes enables classification of past and present nicotine use as well as estimates of pack per year history with high accuracy [8,9]. The ability to deconvolute stress impact from other environmental exposures and underlying inherited vulnerability would significantly advance the field.

As reviewed in our earlier work, astrocytic glutamate synaptic clearance is mediated primarily by excitatory amino acid transporter 2 (EAAT2) and the gene encoding for this transporter protein (i.e. *SLC1A2*) is a target for investigation of glutamate-glutamine cycling in bipolar disorder [10]. Polymorphisms in *SLC1A2* have been associated with suicide [11], lithium response [12,13], lifetime history of rapid cycling [14], susceptibility to bipolar disorder and schizophrenia [13,15-17], and nicotine initiation and dependence [18,19]. In comparison to controls, the *SLC1A2* promoter is hypermethylated in bipolar I (BD-I) adults without addiction; in contrast, the *SLC1A2* promoter is hypomethylated in BD-I patients with nicotine dependence and binge eating disorder (BED) [20]. This represents an epigenetic × environmental interaction where the functionality of the *SLC1A2* promotor region is altered to facilitate a change in synaptic glutamate. As *SLC1A2* is a convergence point for many processes impacting bipolar illness phenotype, it is a high yield target for investigating stress quantification.

In the current study, we aimed to 1) explore differences in self-reported recent stress between BD-I and bipolar II (BD-II) adults in a large-scale cohort using a valence-focused life stress questionnaire; 2) examine associations between recent stress burden and illness severity features; and 3) assess the feasibility of quantifying recent stress burden using the methylations status of the *SLC1A2* promoter.

2. Methods

2.1. Participants

Established in 2009, the Mayo Clinic Individualized Medicine Biobank for Bipolar Disorder recruited patients from Mayo Clinic, the Linder Center of HOPE/University of Cincinnati, and the University of Minnesota to investigate biomarkers for bipolar disease risk, pharmacogenomic treatment response, and illness quantification [21,22]. Study protocols were reviewed and approved by the Institutional Research Board at each participating institution. Procedures for participant recruitment, informed consent, clinical phenotyping, and biospecimen collection have been published in detail elsewhere [23]. Written informed consent was obtained from study participants. Adults (age 18–80) with bipolar disorder without active psychosis or suicidal ideation were eligible. Data were analyzed from the first 1225 Biobank enrollees for the current study.

2.2. Clinical phenotyping

The clinical phenotype of each enrolled participant was determined by the Structured Clinical Interview for DSM-IV (SCID), Modules A and D. The Bipolar Biobank Clinical Questionnaire (BiB-CQ), was administrated by the study team to assess bipolar illness course, psychiatric comorbidities (i.e., anxiety, drug and alcohol addiction, eating disorders), medical conditions, suicide attempts, medications, substance abuse, and stressors. For the current analysis, the following measures of illness severity were utilized: psychosis (current or historical, yes vs no), suicide attempts (1 or more requiring medical intervention), mood instability (sum of the lifetime presence of mixed episodes, rapid cycling, ultra- rapid/ultradian cycling, cycle acceleration over time, increased episode severity over time, anxiety disorder comorbidity (sum of the lifetime presence of six anxiety syndromes: posttraumatic stress disorder, generalized anxiety disorder, social anxiety disorder, obsessive–compulsive disorder, specific phobias, or panic disorder).

2.3. Stressor measurements

Participants reported stressors from a modified descriptive questionnaire, originally developed from the Stanley Foundation Bipolar Network (SFBN) [24]. The SFBN Life Stress Questionnaire evaluated stressors at multiple time points, recognizing that the impact of stressors may differ across the life span [25]. In a multinational study assessing more than 950 bipolar patients, stressors prior to last episode related to past psychosocial and family risk factors, as well as substance abuse comorbidity [25]. For this study, the questionnaire was modified to reflect 12 months prior to study participation (i.e., biobank enrollment, blood draw for genetic studies). The life events were developed to capture common stressors in adults. Eleven positive events were assessed and included: going away to school, graduated from school/college, engagement, marriage, birth of a child, adoption of a child, accepted a new job, job promotion, accepted a better job, retirement, bought a house, or moved (not including bought a house).

Fifteen negative events were assessed and included: problems with spouse or significant other, lack of family support, loss of important other by death (e.g., friend, relative, parent, spouse, etc.), lack of person the patient can trust and confide in, unemployment problems, problems meeting the demands of multiple social and/or occupational roles for which patient is responsible, problems with house (e.g., inadequate, homeless, etc.), educational problems (e.g., academics, discord with teachers/professors, illiteracy, etc.), occupational problems (e.g., threat of job loss, stressful work schedule, difficult work conditions, job dissatisfaction, job change, discord with boss or coworkers), miscarriage, financial problems, problems related to interaction with legal system/crime, problems related to other medical illness, inadequate health/mental health care coverage, problems with access to health care services (e.g., inadequate health services, difficulty getting health/mental health care facility, etc.), and other psychosocial problems. Subjects rated severity of stressor/impairment from each stressor as 0 = stressor not present, 1 = no impact, 2 = mild, 3 = moderate, and 4 = severe. Total stress score was calculated by summing all stressor severity scores.

2.4. High resolution melt analysis

Peripheral blood DNA from a subsample of 150 BD-I participants with and without addiction from the above cohort was previously analyzed by our group via high resolution melting polymerase chain reaction (HRM-PCR) [20]. The groups included: BD-I without substance abuse or BED (n = 30), BD-I with BED (n = 30), BD-I with alcohol abuse alone (n = 30), BD-I with nicotine dependence alone (n = 30), and BD-I with alcohol abuse + nicotine dependence (n = 30). HRM-PCR was performed on bisulfite-converted DNA using primer sequences that were designed to indiscriminately amplify both methylated and unmethylated bisulfite-converted DNA. Precision Melt AnalysisTM software (Bio-Rad, Hercules, CA, United States) was used to analyze PCR product melting temperatures. For the current analysis, we utilized the temperatures of the major melting peak of the region of the CpG island from –785 and –654 in the 5′ UTR of the *SLC1A2* promoter.

2.5. Statistical analysis

Frequency distributions or means with standard deviations were compared for demographic, illness severity features, and stress scores of BD-I and BD-II patients using Chi-square tests, two sample t-tests, or Wilcoxon rank sum tests. Logistic and linear regression models tested for association between stress score and illness severity features, stratified by valence and bipolar subtype. Models were adjusted for age and gender. Spearman's rank correlation coefficients (ρ) were calculated between melt peak temperatures and stress scores, stratified by valence and substance abuse status, in the BD-I subsample. A high stress indicator variable was formulated by dichotomization above the median to examine high vs low stress endorsement for each stress type (total, negative, positive). Multivariable models were constructed to determine the association between methylation temperature and the stress indicator (repeated for total, positive, and negative stress), adjusting for age, gender, and substance abuse (including the interaction with stress). No corrections were made for multiple comparisons due to the limited sample size (in which corrections would be overly conservative) and non-independent nature of the proposed comparisons. Analyses were conducted using SAS (version 9.4; Cary, NC, United States).

3. Results

Demographic, clinical characteristics, and last year stressor data are presented in Table 1. A total of 1225 patients were analyzed, including 863 subjects with BD-I (70.4%) and 362 with BD-II (29.6%). The BD-I and BD-II cohorts were both predominantly middle-aged (42.5 ± 15.0 years, combined average) and Caucasian (93.1%, combined average). The BD-I cohort contained more males than females (41.7 vs. 33.4% of the total sample, p = 0.007). More BD-I than BD-II patients endorsed lifetime psychosis (55.1 vs. 12.1%, p < 0.0001) and suicide attempt (35.7 vs. 26.6%, p = 0.002). BD-II patients displayed higher average mood instability scores (1.5 vs. 1.3, p = 0.004). The two groups did not differ significantly in lifetime substance abuse (e.g., alcohol use disorder, nicotine dependence, other substance use disorders), BED, or anxiety disorder sum. BD-II patients reported higher total stress burden in the last 12 months (20.7 vs. 18.6, p = 0.001), including higher total negative (17.4 vs. 16.1, p = 0.028) and positive stress (3.4 vs. 2.5, p < 0.0001).Table 2..

We tested for associations between recent reported stress burden and lifetime illness severity features. Comparison by valence reveals that associations, when present, are overwhelmingly related to the negative stress burden component. For BD-I and BD-II, increased negative stress was significantly associated with history of multiple suicide attempts (BD-I: OR 1.21, p = 0.008; BD-II: OR 1.51, p = 0.002), higher mood instability sum (BD-I: Est. 0.26, p < 0.0001; BD-II: Est. 0.22, p < 0.001), and higher anxiety disorder sum (BD-I: Est. 0.34, p < 0.0001; BD-II: Est. 0.38, p < 0.0001). For BD-I patients only, increased negative stress was significantly associated with alcohol abuse (OR 1.34, p < 0.0001), nicotine dependence (OR 1.34, p < 0.0001), other substance abuse (OR 1.42, p < 0.0001), and BED (OR 1.34, p = 0.007). BD-I patients additionally showed association between positive stress and BED (OR 1.72, p = 0.02). No associations were noted between psychosis and stress for either bipolar subtype.

To determine if recent stress burden was quantifiable using the *SCL1A2* promoter, we calculated the correlation between stress scores and promoter methylation in a subsample of BD-I patients (N = 150, Table 3). Given that substance abuse is a known mediator of *SCL1A2* methylation, we stratified the analysis by patients without any substance abuse or BED (n = 30), and those with comorbid BED (n = 30), comorbid alcohol abuse only (n = 30), comorbid nicotine dependence only (n = 30), and comorbid alcohol abuse + nicotine dependence (n = 30). There was no significant association between stress burden and promoter methylation in bipolar patients without substance abuse or binge eating disorder (Total: ρ −0.11, p = 0.19; Negative: ρ −0.10, p = 0.25; Positive: ρ −0.04, p = 0.59). However, in subjects with nicotine

Table 1
Demographic, illness severity features, and stress characteristics by bipolar subtype.

	Bipolar I (n = 863)	Bipolar II (n = 362)	
	Mean (SD); n (%)	Mean (SD); n (%)	p-value
Sociodemographic			
Age, years	42.8 ± 15.1	41.7 ± 14.6	0.33
Male	360 (41.7)	121 (33.4)	0.007
Caucasian	797 (92.4)	344 (95)	0.22
Illness severity features			
Psychosis[a]	463 (55.1)	43 (12.1)	<0.0001
≥ 1 suicide attempt[b]	304 (35.7)	94 (26.6)	0.002
Alcohol abuse[c]	330 (39.7)	137 (39.4)	0.91
Nicotine dependence	328 (39.4)	132 (38.4)	0.74
Other substance abuse	514 (60.9)	208 (58.9)	0.52
Binge eating disorder	84 (10.2)	26 (7.7)	0.19
Mood instability sum[d]	1.3 ± 1.3	1.5 ± 1.2	0.004
Anxiety disorders sum[e]	1.6 ± 1.5	1.5 ± 1.4	0.37
Stress score, last 12 months			
Total[f]	18.6 ± 12.9	20.7 ± 11.6	0.001
Negative	16.1 ± 10.5	17.4 ± 9.5	0.028
Positive	2.5 ± 4.4	3.4 ± 4.3	<0.0001

[a] Lifetime history of psychosis, regardless if psychosis occurred concurrent with an affective episode.
[b] Lifetime history of ≥ 1 serious suicide attempts requiring medical intervention (e.g., overdose or hospital admission).
[c] Substance use disorders in our study included DSM-IV-TR-defined alcohol abuse (AA), nicotine dependence (ND), and other substance abuse (OA).
[d] Calculated as the sum (range, 0–5) of the lifetime presence of mixed episodes, rapid cycling, ultra rapid/ultradian cycling, cycle acceleration over time, and increased episode severity over time.
[e] Calculated as the sum (range, 0–6) of the lifetime presence of six anxiety syndromes: posttraumatic stress disorder, generalized anxiety disorder, social anxiety disorder, obsessive–compulsive disorder, specific phobias, or panic disorder.
[f] Total stress score = sum of individual stressor severity scores. 0 = stressor not present, 1 = no impact, 2 = mild, 3 = moderate, and 4 = severe.

Table 2
Association of stress in the last 12 months to illness severity features by bipolar subtype.

		Bipolar I (n = 863)		Bipolar II (n = 362)	
Illness severity feature	Stress	OR[1]	p-value	OR[1]	p-value
Psychosis	Positive	0.98	0.91	0.41	0.08
	Negative	0.98	0.80	1.11	0.54
	Total	0.99	0.80	0.98	0.89
≥1 suicide attempt	Positive	0.72	0.08	1.68	0.08
	Negative	1.21	0.008	1.51	0.002
	Total	1.09	0.15	1.41	0.002
Alcohol use	Positive	1.37	0.06	1.07	0.79
	Negative	1.34	<0.0001	1.29	0.04
	Total	1.26	<0.0001	1.20	0.08
Nicotine dependence	Positive	1.18	0.31	0.70	0.20
	Negative	1.34	<0.0001	1.12	0.36
	Total	1.24	0.0002	1.02	0.85
Other substance use	Positive	1.11	0.54	0.83	0.49
	Negative	1.42	<0.0001	1.17	0.19
	Total	1.29	<0.0001	1.08	0.45
Binge eating disorder	Positive	1.72	0.02	1.14	0.78
	Negative	1.34	0.007	0.94	0.76
	Total	1.28	0.004	0.97	0.89
Illness severity feature	Stress	Est.	p-value	Est.	p-value
Mood instability sum	Positive	−0.04	0.67	0.02	0.92
	Negative	0.26	<0.0001	0.22	0.001
	Total	0.17	<0.0001	0.15	0.008
Anxiety disorders sum	Positive	0.21	0.07	0.23	0.18
	Negative	0.34	<0.0001	0.38	<0.0001
	Total	0.25	<0.0001	0.28	<0.0001

[1] Regression models were adjusted for age and gender.

Table 3
Spearman correlations (ρ) between stress in the last 12 months and SLC1A2 promoter methylation in Bipolar I patients by substance use disorder and multivariable model predicting methylation.

	Total Stress	Negative Stress	Positive Stress
Subgroup	*ρ*	*ρ*	*ρ*
No substance use (n = 30)	−0.12	−0.05	−0.12
Binge eating (n = 30)	−0.01	−0.14	0.26
Alcohol +/- nicotine (n = 60)	0.03	0.03	−0.11
Nicotine +/- alcohol (n = 60)	−0.37**	−0.28*	−0.31*
Alcohol only (n = 30)	0.34	0.34	0.24
Nicotine only (n = 30)	−0.44*	−0.42*	−0.17
Any substance use (n = 120)	−0.04	−0.04	−0.03
Multivariable Models[†] (n = 120)	Coef. (SE)	Coef. (SE)	Coef. (SE)
High stress (ref: Low stress)	−0.17 (0.08)*	0.07 (0.08)	−0.13 (0.07)
Substance abuse (ref: None)	−0.25 (0.05) **	−0.17 (0.05) **	−0.26 (0.05) **
Substance abuse * Stress	0.15 (0.08)	−0.08 (0.08)	0.15 (0.08)

* = $p < 0.05$; ** = $p < 0.01$.
[†] adjusted for age and gender (coefficients not shown).

dependence (with or without alcohol abuse/dependence), there was a significant negative correlation between stress burden and methylation status (Total: ρ −0.37, p = 0.0004; Negative: ρ −0.28, p = 0.03; Positive: ρ −0.31, p = 0.02). The strength of the correlation increased further when restricted to patients with nicotine dependence only (Total: ρ −0.44, p = 0.02; Negative: ρ −0.42, p = 0.02; Positive: ρ −0.17, p = 0.39). Alcohol abuse/dependence only, by contrast, is positively correlated with *SCL1A2* methylation (Total: ρ 0.34, p = 0.085; Negative: ρ 0.34, p = 0.09; Positive: ρ 0.24, p = 0.25).

A multivariable model was constructed to examine the association between methylation status and stress level (high versus low), adjusting for the main effects of age, gender, and substance use, as well as the interaction between stress and substance use (Table 3). The main effect of high total stress compared to low total stress was associated with significant *SCL1A2* hypomethylation ($p < 0.05$). High negative or positive total stress, however, was not significantly associated with *SCL1A2* methylation status. Substance abuse was significantly associated with hypomethylation of the *SCL1A2* promoter by total and stress valence subtypes ($p < 0.01$). The interaction between stress and substance use was not significant.

4. Discussion

These data would suggest that BD-I and BD-II patients may perceive and experience stress differently. In the current study, BD-II patients endorsed overall higher total, positive, and negative recent stress burden than BD-I patients. In both subtypes, stress in the last 12 months associated with lifetime suicide attempts, mood instability, and number of anxiety disorders. Unique to BD-I patients, however, was an association between recent stress and all forms of substance use (i.e., nicotine, alcohol, food, other drugs). For both BD-I and BD-II, negative stress associated with more illness severity features than positive or total stress. No association across stress valence or illness subtype was observed with psychosis, although prevalence (55.1%) was similar to published reports [26].

Patient stress perception and coping strategy are critical psychometric properties to evaluating differences in stress impact between bipolar subtypes. *Amann et al.* found that BD-II patients endorsed more negative and positive life events than BD-I patients, but only in BD-I patients did the number of life events associate with risk of depressive relapse [27]. Another study reported no differences in stress generation between subtypes, but a more pronounced influence of events on BD-I illness course [28]. In a smartphone self-monitoring investigation, BD-II participants endorsed chronically lower mood, less time in euthymia, and higher percentage of time with depressive symptoms compared to their BD-I peers [29]. BD-II individuals also display more negative cognitive and coping styles but reduced association between style and symptom expression [30,31]. BD-II individuals may thus experience persistent subclinical depressive symptoms that increase endorsement of stress but fail to reach a sufficient threshold to trigger full affective episodes. By contrast, evidence indicates that BD-I individuals experience continued affective lability, intensity, and reduced resilience into euthymia [32-34]. Deficits are described in the fronto-limbic and emotion regulation circuitry as well as attention and executive functioning in euthymic BD-I compared to BD-II, which may contribute to differences in stress generation and perception [35-37].

We found that negative stress associates strongly with illness severity features, consistent with prior studies [38-41]. Higher levels of negative affect and lower positive affect are observed in response to negative and positive daily stressors, respectively, in bipolar patients [42]. Meta-analysis suggests that cognitive deficits in euthymic bipolar illness extends to multiple domains that impact reward processing, including verbal learning, memory, abstraction, set-shifting, attention, and inhibition [43]. Bipolar youth report less positive life events, suggesting early and excessive downregulation of the dopaminergic fronto-striatal circuit [3]. We found a single positive association between positive stress and BED in BD-I. BED prevalence and clinical correlates do not differ between BD-I and BD-II subtypes [44], but higher levels of emotional reactivity and anxiety are reported in bipolar illness with comorbid BED [45]. The association between positive stress and BED in BD-I may reflect differences in emotional regulation between subtypes and/or secondary effects of the medications (e.g., antipsychotics, mood stabilizers) more frequently employed to stabilize BD-I manic illness that influence appetite [45].

Stress, substance abuse, and affective dysregulation exist in a devastating multidirectional feedback loop, each increasing vulnerability to the other and driving illness severity [46,47]. Patients with functional polymorphisms in *SLC1A2* show increased vulnerability to early life stress [48] and rats display reduced EAAT2 expression in response to prenatal stress [49], indicating a role for glutamate signaling in stress phenotype development. Our group previously reported that the *SLC1A2* promoter is hypermethylated in BD-I patients without substance use disorders compared to healthy controls but hypomethylated [20]. Our current results found no significant interaction between stress and substance abuse in predicting methylation of the *SLC1A2* promoter. After adjusting for age and gender, only high total stress versus low stress demonstrated a significant association with hypomethylation of the promoter, while the presence of substance abuse significantly associated with promoter hypomethylation regardless of valence subtype.

These results support the feasibility of quantification of high vs. low total stress burden using the *SLC1A2* promoter. We additionally observe that substance abuse may act as a potent independent stressor resulting in persistent hypomethylation of the *SLC1A2* promoter (increased transcription). As a result, the promoter may be less responsive to fluctuations in other types of stress. Whether this chronically increased transcription is neuroprotective or pathogenic remains to be determined. In rodent models, chronic stress is associated with conflicting patterns of observed EAAT2 expression [10]. Human investigations into the association between BD and *SLC1A2* are lacking, except for a single study indicating increased expression [50]. Of note, EAAT2 is known to undergo alternative splicing and a host of post-translational modifications that mediate receptor downregulation and desensitization [51]. Hypomethylation of the *SLC1A2* promoter may, therefore, not lead to obligate transcription and translation of functional EAAT2. As with methylation-based quantification of nicotine exposure and aging acceleration, quantification of stress in BD will most likely require a large-scale whole-methylome and machine learning approach to disaggregate the effects of stress from other environmental exposures [9,52].

Several limitations of the current study must be taken into

consideration. The study design is retrospective and cross-sectional rather than prospective and longitudinal. The stressor questionnaire assessed a fixed number of pre-designated "positive" and "negative" stress events without developmental scaling or consideration of potential cultural differences or chronicity. We do think the questionnaire captures real world life events and stressors for a patient group with mean age of 42, but may be less applicable to pediatric, adolescent, and geriatric populations. The cohort is also primarily Caucasian, which may limit generalizability. Furthermore, a given stressor can possess both positive and negative emotional valence, which here is acknowledged through examination of overall stress burden. The questionnaire is not clinically validated but produced by consensus of the Mayo bipolar working group consensus as existing tools only tested a more limited number of positive life events. This limited assessment of positive events as a veritable form of stress upon illness course. Stressor reports are subjective, raising potential for recall bias. Furthermore, the mediating effects of current or early trauma on stress perception were not assessed. Finally, gender and age of illness onset were not considered. Limitations, however, are balanced by robust clinical phenotyping and significantly larger sample size compared to prior studies. The feasibility analysis of quantification of stress at the *SLC1A2* promoter possesses its own limitations. Prior studies have established genome-wide association of variation in *SLC1A2* to multiple phenotypic aspects of bipolar illness, from susceptibility to treatment response, justifying use of a more limited sample of subjects for the current testing. However, the smaller sample may be underpowered to detect stress-induced methylation alterations by valence.

5. Conclusion

Our findings indicate unique stress profiles in BD-I and BD-II that associate to more illness severity features in BD-I. Sequential acquisition of stress-generative comorbidities, such as substance abuse and anxiety, may foster progressive illness instability with said comorbidities potentially capable of negatively influencing illness course independent of stress. The mechanism by which BD-II illness remains resistant to the development of a similar pattern of morbidities despite perceived higher stress intensity remains to be determined. Numerous combinations of allele × epiallele × environment × time interactions influence observed heterogeneity in symptomatology and likely underscore the inconsistencies observed in clinical testing of kindling and stress generation models to date.

CRediT authorship contribution statement

Adrienne Grzenda: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Marin Veldic:** Conceptualization, Methodology, Resources, Writing – review & editing. **Yun-Fang Jia:** Investigation. **Susan L. McElroy:** Writing – review & editing. **David J. Bond:** Writing – review & editing. **Jennifer R. Geske:** Methodology, Formal analysis, Software, Data curation, Visualization, Writing – review & editing. **Aysegul Ozerdem:** Writing – review & editing. **Balwinder Singh:** Writing – review & editing. **Joanna M. Biernacka:** Conceptualization, Supervision, Methodology, Formal analysis, Software, Data curation. **Doo-Sup Choi:** Investigation, Resources, Writing – review & editing. **Mark A. Frye:** Conceptualization, Supervision, Project administration, Funding acquisition, Resources, Methodology, Writing – review & editing.

Declaration of Competing Interest

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