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ARTICLE

Association between the epigenetic lifespan predictor GrimAge and history of suicide attempt in bipolar disorder

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Bipolar disorder (BD) has been previously associated with premature mortality and aging, including acceleration of epigenetic aging. Suicide attempts (SA) are greatly elevated in BD and are associated with decreased lifespan, biological aging, and poorer clinical outcomes. We investigated the relationship between GrimAge, an epigenetic clock trained on time-to-death and associated with mortality and lifespan, and SA in two independent cohorts of BD individuals (discovery cohort - controls ($n = 50$), BD individuals with ($n = 77$, BD/SA) and without ($n = 67$, BD/non-SA) lifetime history of SA; replication cohort - BD/SA ($n = 48$) and BD/non-SA ($n = 47$)). An acceleration index for the GrimAge clock (GrimAgeAccel) was computed from blood DNA methylation (DNAm) and compared between groups with multiple general linear models. Differences in epigenetic aging from the discovery cohort were validated in the independent replication cohort. In the discovery cohort, controls, BD/non-SA, and BD/SA significantly differed on GrimAgeAccel ($F = 5.424$, $p = 0.005$), with the highest GrimAgeAccel in BD/SA ($p = 0.004$, BD/SA vs. controls). Within the BD individuals, BD/non-SA and BD/SA differed on GrimAgeAccel in both cohorts ($p = 0.008$) after covariate adjustment. Finally, DNAm-based surrogates revealed possible involvement of plasminogen activator inhibitor 1, leptin, and smoking pack-years in driving accelerated epigenetic aging. These findings pair with existing evidence that not only BD, but also SA, may be associated with an accelerated biological aging and provide putative biological mechanisms for morbidity and premature mortality in this population.

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INTRODUCTION

Bipolar disorder (BD) is a severe and chronic psychiatric disorder with an estimated global prevalence of at least 1% [1, 2]. Aside from significant functional impairments in daily life [3], BD is associated with premature mortality likely due to comorbid medical conditions including cardiovascular diseases, diabetes mellitus, obesity, endocrine and thyroid diseases [4], and suicide [5]. On average, the lifespan of individuals with BD is 9–17 years shorter than that of the general population [6, 7]. The suicide rate for BD is 10–30 times higher than the general population, with 20–60% of these individuals attempting suicide at least once in their lifetime [8]. Additionally, suicide attempt (SA) is associated with decreased lifespan, which is accounted for by, besides an increased risk of suicide, comorbid medical conditions [9].

Emerging biomarkers of aging have begun to clarify the observed shortened lifespan in BD and, to a lesser extent, SA. Telomere length has been repeatedly shown to be reduced in the context of BD [10], with evidence of further shortening associated with a high number of previous SA [11] and suicidal ideation in patients [12]. DNA methylation (DNAm) varies with age across the lifespan (both globally and locally), involves both genetic and environmental contributions, and can reversibly modulate gene expression [13]. For these reasons, it is an appealing measure from which to derive estimates of aging more closely tied to biological development [14], contrary to the weaker predictor, time-since-birth [15]. Epigenetic clocks, which estimate age from DNAm patterns, offer biologically-derived age estimates which can be directly compared to chronological age to determine acceleration or deceleration of biological aging [16]. These clocks have traditionally been trained on chronological age data [17, 18] and have been associated with various neurodegenerative and psychiatric diseases [19, 20], including BD [21, 22]. As chronological age is not perfectly

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synonymous with lifespan, novel epigenetic clocks specifically trained on phenotypical age and lifespan data are beginning to emerge. GrimAge, a clock estimated with DNAm-based predictors of plasma proteins and a DNAm-based smoking pack-years estimate, was specifically trained on time-to-death and has outperformed others when predicting all-cause mortality [23]. GrimAgeAccel, a measure derived from the regression of DNAm-based age on chronological age, provides information on accelerated (positive) or decelerated (negative) epigenetic aging (EA). Of note, positive GrimAgeAccel has been associated with deficits in neurocognitive and neurostructural outcomes, such as lower cognitive ability and vascular brain lesions [24].

Although investigations of EA using first-generation clocks have revealed accelerated aging in BD [21, 22], the mortality-associated GrimAgeAccel has only recently focused on BD, with work by our group identifying higher GrimAgeAccel in BD as well as its relationship with cognitive impairment in patients [25]. Given the evidence that suicidal behavior, aside from the heightened mortality associated with suicide, also associates with premature mortality for natural causes [9], EA may be a plausible biological candidate for this risk elevation. Accelerated aging studies in suicidal behavior have documented acceleration across measures [11, 26], but the only study that investigated GrimAgeAccel in the context of suicide, comparing groups of high and low lethality SA, found no significant difference [27].

No study has considered GrimAgeAccel in BD or a matched-diagnosis reference group with and without SA, in contrast to existing studies focusing on telomere length [11, 12]. This consideration is critical to not only advance the understanding of suicide within BD specifically, but to clarify whether EA associated with SA is specific to SA vs. BD diagnosis. Furthermore, studying SA within BD allows for rigorous insights to uniquely associated biological mechanisms, as reference groups in previous studies have often been non-psychiatric controls. In this study, we investigated whether GrimAgeAccel differentiates individuals with BD and a history of SA (BD/SA) from those with BD and no history of SA (BD/non-SA). We leverage a richly phenotyped discovery cohort with an independent replication cohort to test whether GrimAgeAccel is especially pronounced in BD/SA.

MATERIALS AND METHODS

Discovery cohort

Sample recruitment for this study has been recently described [25]. Briefly, 144 BD (124 BD-I/20 BD-II) individuals were recruited alongside 50 non-psychiatric controls (CON) matched for age, sex, and race/ethnicity at the Center of Excellence in Mood Disorders, Houston, TX. BD diagnosis and features of illness severity (substance use comorbidity, total number of comorbidities, total number of psychiatric hospitalizations, age of onset of mood disorder, length of illness) were ascertained in the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), and a standardized method was used to collect demographic information. Young Mania Rating Scale (YMRS) [28] and Montgomery-Åsberg Depression Rating Scale (MADRS) [29] were administered. The criterion for suicidality was one or more documented actual, aborted, and interrupted SA assessed by the Columbia Suicide History Form (CSHF) [30] yielding subgroups of BD/SA ($n = 67$) and BD/non-SA ($n = 77$). Exclusion criteria included other medical conditions such as neurological disorders, traumatic brain injury, current pregnancy, schizophrenia, developmental disorders, eating disorders, and intellectual disability. CON ($n = 50$) had neither history of any Axis I disorder in first-degree relatives nor prescribed psychotropic medication. Clinical interviews were administered by trained evaluators and reviewed by a board-certified psychiatrist. All participants completed a urine drug screen to exclude current illegal drug use. Informed consent was obtained from all participants at enrollment and prior to any procedure, and the protocol for the study was approved by the local institutional review board (IRB, HSC-MS-09-0340).

Replication cohort

Subjects were recruited through the Iowa Neuroscience Institute Bipolar Disorder Research Program of Excellence (BD-RPOE), approved by the IRB of the University of Iowa (IRB#201708703) [31–37]. Participants between 18 and 70 years with the ability to consent and a confirmed DSM-IV diagnosis of BD-I were recruited. History of actual SA and number of lifetime attempts were recorded with the Columbia Suicide Severity Rating Scale [38], a rating scale which definitions of suicide behavior were based in the CSHF used in the discovery cohort. Illness severity features were ascertained with a standardized method to collect demographic information and the Mini-International Neuropsychiatric Interview [39]. YMRS [28] and MADRS [29] were administered. Exclusion criteria included a history of loss of consciousness for more than 10 min, seizure disorder, brain damage or other neurological problems, coronary or cerebral artery disease, alcohol or drug dependence within the past 3 months, current pregnancy, or contraindication for magnetic resonance imaging. The final replication cohort included 48 BD/SA and 47 BD/non-SA and did not include CON.

DNA extraction

Blood was collected by venipuncture in EDTA-containing vacutainers and stored at −80 °C. In the discovery cohort, buffy coat from fasting participants was isolated before storage, followed by DNA isolation with the DNeasy Blood & Tissue Mini Kit (Qiagen, Hilden, Germany) and quantification on NanoDrop (Thermo, Waltham, MA, USA). In the replication cohort, 1 mL of whole blood per sample from non-fasting participants was used with the Puregene Blood Kit with RNase A solution (Qiagen). Elution Buffer CDB-02 (Kurabo Industries Ltd, Osaka, Japan) was used instead of DNA Hydration Solution.

Methylation assay

Five hundred nanograms of DNA were bisulfite-converted with the EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA) and interrogated for genome-wide DNAm on the Infinium EPICMethylation BeadChip (Illumina, San Diego, CA, USA). Poor quality probes with detection p values < 0.01 were excluded using *minfi* [40]. From the DNAm data, estimates of white blood cell count proportions (CD8 + T-lymphocytes (CD8T), CD4 + T-lymphocytes (CD4T), monocytes (Mono), granulocytes (Gran), natural killer cells (NK), and B-lymphocytes (B cell)) using the Houseman procedure [41] and smoking scores using EpiSmokEr [42, 43] were retrieved. DNAm-based calculations of GrimAge and its components (i.e., DNAm surrogate markers of the plasma levels of plasminogen activation inhibitor 1 (DNAmPAI-1), growth differentiation factor 15 (DNAmGDF-15), leptin (DNAmLeptin), tissue inhibitor metalloproteinases 1 (DNAmTIMP1), cystatin C (DNAmCystatinC), adrenomedullin (DNAmADM), beta-2-microglobulin (DNAmB2M), and smoking pack-years (DNAmPACKYRS)) were performed using the New DNA Methylation Age Calculator (https://dnamage.genetics.ucla.edu/). The DNAm surrogates of these markers are denoted by the prefix "DNAm" to specify that they do not represent the DNA methylation of their coding genes, but rather a composite made of multiple CpG sites predicting their blood levels [44]. GrimAgeAccel and age-adjusted values for all GrimAge components were calculated by regressing the predicted epigenetic markers on chronological age and using their residuals as an acceleration index for GrimAge and age-adjusted surrogate markers, respectively. Positive and negative GrimAgeAccel values represent acceleration and deceleration of GrimAge, respectively, and will be analyzed as a continuous variable to allow for subtle differences to be detected within groups.

Genotyping

Samples from the discovery cohort were genotyped on the Infinium Global Screening Array-24 (Illumina). Principal components (PC) analysis was performed, and the first three PCs were retained for use as covariates of genomic ancestry (as continuous variables).

Statistical analyses

All variables included as outcomes in analyses were examined for normality with the Shapiro–Wilk test and, if necessary, log-normalized. For covariate analyses, genomic data were not available for the replication cohort. Instead, self-reported race (as a categorical variable) was used as a substitute covariate for PCs.

In both cohorts, the association between GrimAge and chronological age was assessed within each group using Spearman's correlation test (Figs. 1A, B and S1). For demographic (Table 1) and clinical (Table S1) variables, Wilcoxon rank sum test with continuity correction, independent samples t-test, one-way analysis of variance (ANOVA), and Kruskal-Wallis tests were applied to reveal between-group differences. A comparison of the two cohorts was conducted with Fisher's Exact Test and Welch Two

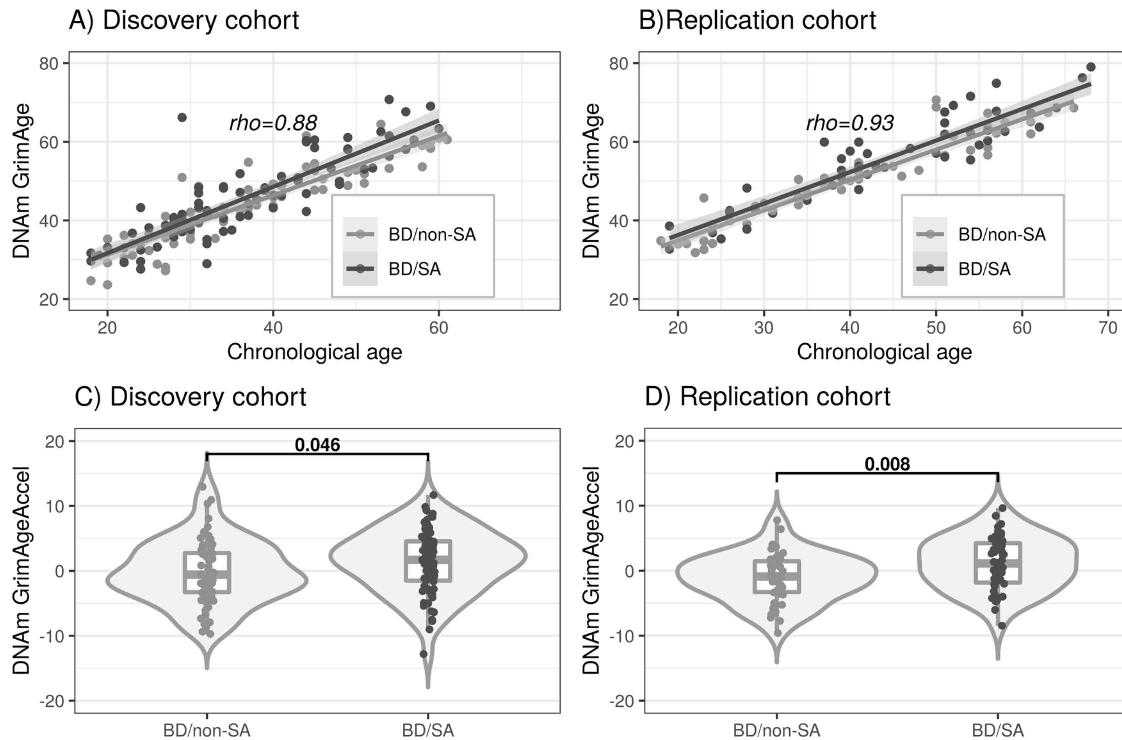

Fig. 1 Accelerated DNAm GrimAge and GrimAge in BD groups. Correlation between estimated GrimAge and chronological age in **A** the discovery cohort. Spearman's correlation analysis indicates a significant correlation ($p < 2.2E-16$) between DNA methylation (DNAm) GrimAge and chronological age in the entire sample, as well as in the subgroups (BD/non-SA: rho = 0.91, $p < 2.2E-16$; BD/SA: rho = 0.85, $p < 2.2E-16$). **B** In the replication cohort, Spearman's correlation analysis indicates a significant correlation ($p < 2.2E-16$) between DNAm GrimAge and chronological age in the entire sample, as well as in the subgroups (BD/non-SA: rho = 0.95, $p < 2.2E-16$; BD/SA: rho=0.90, $p < 2.2E-16$). Accelerated GrimAge (GrimAgeAccel, log-normalized) in BD/non-SA and BD/SA in **C** the discovery cohort ($n = 67$ BD/non-SA and $n = 77$ BD/SA) and **D** the replication cohort ($n = 47$ BD/non-SA and $n = 48$ BD/SA). p-values determined by independent samples t-tests. BD/SA, bipolar disorder with history of suicide attempt. BD/non-SA, bipolar disorder with no history of suicide attempt.

Sample t-test/Wilcoxon (Table S2). Analysis of covariance (ANCOVA) models were tested in the discovery cohort to predict GrimAgeAccel (as a continuous variable) from group (Table 2). In Model 1, covariates included age, sex, years of education, and GWAS PCs (or self-reported race/ethnicity in the replication cohort). In Model 2, covariates were age, sex, years of education, GWAS PCs, and body mass index (BMI). In Model 3, covariates were age, sex, years of education, GWAS PCs, BMI, and white blood cell count proportions. In Model 4, covariates were age, sex, years of education, GWAS PCs, BMI, white blood cell count proportions, and smoking score. Finally, given our previous results on the strong association between GrimAgeAccel and smoking in individuals with BD [25], we decided to include a model specifically controlling for smoking score as a sensitivity analysis (in the absence of other potential confounders, Model 5). p values < 0.01 were considered statistically significant after Bonferroni correction for multiple testing. Also in the discovery cohort, two models were tested to predict each DNAm-based, normalized, GrimAge subcomponent per group (Table S3). Model 6 was an ANOVA and in Model 7 (ANCOVA), age, sex, GWAS PCs, and years of education were included as covariates. This set of analyses was not repeated in the replication cohort due to the lack of a control group.

In both the discovery and the replication cohort, these models were tested to predict each DNAm-based, age-adjusted GrimAge subcomponent per group (BD/non-SA, BD/SA) (Table 3). Model 6 was repeated with Welch's independent samples t-test to compare the two groups. Finally, to investigate the potential mechanisms for elevated GrimAgeAccel in BD/SA compared to BD/non-SA, we tested six dichotomic logistic regression models to predict GrimAgeAccel in each cohort, including variables related to clinical variables as covariates (Table 4). As outcome in the analyses, data were divided by percentile of GrimAgeAccel into two groups (high and low GrimAgeAccel). Covariates included length of illness (in years), total number of psychiatric comorbidities, substance use comorbidity, age of onset of mood disorder, current lithium use (yes/no), and prescribed medication use (yes/no). All analyses were performed in R 4.1.1 [45], using *ggplot2* [46], *tidyr* [47], *dplyr* [48], *magrittr* [49], and *ggpubr* [50] packages, as well as SPSS 28 [51].

RESULTS

Demographic comparisons

In the discovery cohort (mean age of 36.9 years), BD and CON groups did not significantly differ in chronological age, sex, BMI, and race/ethnicity (Table 1). Their years of education and smoking scores were significantly different, with the BD/SA group having more years of education and higher smoking scores than BD/non-SA and CON ($p = 0.005$ and $p = 0.0001$, respectively). Of note, the reliability of the smoking scores in predicting current self-reported smoking status was confirmed in both cohorts (Fig. S5). Age, sex, BMI, race/ethnicity, and years of education were not significantly different between groups in the replication cohort (mean age of 41.9 years); however, BD/SA had a significantly higher smoking score ($p = 0.001$). Both cohorts included a similar proportion of male and female subjects, but differed for age, race/ethnicity, smoking scores, and years of education (Table S2). Finally, we found significant differences across the three groups regarding the levels of CD4T and NK cells in the discovery cohort, with both BD/SA ($p = 0.017$) and BD/non-SA ($p = 0.006$) groups showing reduced levels compared to CON, as well as a significant increase in Mono in BD/SA group compared to BD/non-SA ($p = 0.043$) in the replication cohort (Table 1).

GrimAgeAccel in BD and SA

In our primary analysis, the three groups in the discovery cohort significantly differed on GrimAgeAccel when covaried for age, sex, years of education, and GWAS PCs (Model 1: $F_{BD/SA}(2,176) = 5.931$, $p = 0.003$). Considering our sample size per group, an observed effect size (f) of 0.24, and a significance threshold of $\alpha = 0.05$, our calculated statistical power for this between-group comparison was 85%. The group effect remained statistically significant after

Table 1. Demographic information.

	Discovery cohort					Replication cohort		
	CON ($n = 50$)	**BD/non-SA ($n = 67$)**	**BD/SA ($n = 77$)**	***p* value[#]**	***p* value**	**BD/non-SA ($n = 47$)**	**BD/SA ($n = 48$)**	***p* value**
Demographic characteristics								
Female Sex, *n* (%)	34 (68.0%)	48 (71.6%)	55 (71.4%)	0.901[a]	1.000[a]	28 (59.6%)	34 (70.8%)	0.286[a]
Age, median (IQR)	34.0 (16.0)	34.0 (18.5)	34.0 (15.0)	0.756[e]	0.901[c]	37.0 (23.0)	42.5 (17.8)	0.080[c]
Race/ethnicity, *n* (%)				0.305[a]	0.417[a]			0.436[a]
White/Caucasian	13 (26.0%)	23 (34.3%)	35 (45.5%)			39 (83.0%)	35 (72.9%)	
Hispanic/Latinx	41 (20.0%)	10 (14.9%)	12 (15.6%)			4 (8.5%)	5 (10.4%)	
Black/African American	22 (44.0%)	23 (34.3%)	24 (31.2%)			0 (0%)	4 (8.3%)	
American Indian/Alaskan Native	0 (0%)	2 (3.3%)	0 (0%)			1 (2.1%)	1 (2.1%)	
Asian	-------	-------	-------			1 (2.1%)	0 (0%)	
More than one race	2 (4.0%)	6 (8.9%)	4 (5.2%)			1 (2.1%)	2 (4.2%)	
Missing	8					2		
Smoking score, median (IQR)	3.47 (0.69)	3.66 (0.96)	4.03 (2.02)	**1.12E-04[e]**	**0.003[c]**	0.79 (2.81)	3.20 (4.48)	**0.001[c]**
Years of education, median (IQR)	15.00 (2.00)	14.00 (3.00)	13.00 (3.00)	**0.005[e]**	**0.030[c]**	16.00 (2.00)	14.50 (3.00)	0.258[c]
Missing	1							
Body Mass Index, mean (SD)	29.5 (6.97)	30.0 (6.72)	30.1 (8.16)	0.899[d]	0.765[b]	30.3 (7.68)	31.1 (7.16)	0.564[c]
Missing	12							
White blood cells								
CD8T, median (IQR)	0.099 (0.101)	0.093 (0.077)	0.102 (0.101)	0.097[e]	0.206[c]	0.078 (0.051)	0.081 (0.061)	0.938[c]
CD4T, median (IQR)	0.149 (0.119)	0.127 (0.090)	0.121 (0.118)	**0.017[e]**	1.000[c]	0.108 (0.073)	0.119 (0.054)	0.428[b]
NK, mean (SD)	0.029 (0.059)	0.004 (0.032)	0.004 (0.029)	**0.006[e]**	0.355[c]	0.009 (0.019)	0.004 (0.013)	0.296[c]
Mono, median (IQR)	0.044 (0.054)	0.052 (0.042)	0.052 (0.037)	0.686[e]	0.321[c]	0.054 (0.032)	0.065 (0.029)	**0.043[c]**
B cell, median (IQR)	0.033 (0.056)	0.023 (0.034)	0.019 (0.047)	0.361[e]	0.846[c]	0.000 (0.010)	0.000 (0.011)	0.583[c]
Gran, median (IQR)	0.569 (0.209)	0.602 (0.152)	0.565 (0.217)	0.058[e]	0.154[c]	0.592 (0.129)	0.566 (0.086)	0.340[c]

BD/SA bipolar disorder with history of suicide attempt, *BD/non-SA* bipolar disorder with no history of suicide attempt, *B cell* B lymphocytes, *CD4T* CD4+ T-lymphocytes, *CD8T* CD8+T-lymphocytes, *CON* non-psychiatric controls, *Gran* granulocytes, *Mono* monocytes, *NK* natural killer cells

[a]Fisher's exact test.

[b]t-test.

[c]Mann-Whitney/Wilcoxon rank sum test with continuity correction.

[d]One-way analysis of variance (ANOVA).

[e]Kruskal-Wallis test.

[#]*p* represents the significance of the comparison between BD/SA, BD/non-SA, and control. *p* represents the significance of the comparison between BD/SA vs BD/non-SA. Nominally significant differences (unadjusted $p < 0.05$) are bolded.

Table 2. Adjusted analysis of differences in GrimAgeAccel between groups.

	Discovery cohort (3 groups[a])		Discovery cohort (2 groups[b])		Replication cohort[c]	
	$F_{BD/SA}$ (df, df)	*p* value	$F_{BD/SA}$ (df, df)	*p*-value	$F_{BD/SA}$ (df, df)	*p* value
Model 1	5.931 (2, 176)	**0.003**	5.126 (1, 128)	**0.025**	7.683 (1, 83)	**0.007**
Model 2	5.098 (2, 164)	**0.008**	3.896 (1, 122)	0.051	8.654 (1, 82)	**0.004**
Model 3	6.227 (2, 158)	**0.002**	4.137 (1, 117)	**0.044**	8.837 (1, 76)	**0.004**
Model 4	7.904 (2, 157)	**0.001**	5.527 (1, 116)	**0.020**	17.000 (1, 75)	**9.55E-05**
Model 5	6.457 (2, 188)	**0.002**	5.127 (1, 140)	**0.025**	10.040 (1, 91)	**0.002**

Model 1: covaried for age, sex, GWAS principal components (PCs), and years of education. Model 2: covaried for age, sex, GWAS, PCs, years of education, and body mass index (BMI). Model 3: covaried for age, sex, GWAS PCs, years of education, BMI, and white blood cell proportions. Model 4: covaried for age, sex, GWAS PCs, years of education, BMI, white blood cell proportions, and smoking score. Model 5: covaried for smoking score. Bonferroni-corrected $p < 0.01$ was taken as the significance threshold. Nominally significant differences ($p < 0.05$) are bolded.

[a]BD/SA (bipolar disorder with history of suicide attempt) vs. BD/non-SA (bipolar disorder with no history of suicide attempt) vs. controls.

[b]BD/SA vs. BD/non-SA.

[c]Used self-reported "race/ethnicity" (as a categorical variable) instead of genomic ancestral PCs since no GWAS data was available for the replication cohort.

further adjustment for BMI (Model 2: $F_{BD/SA}(2,164) = 5.098$, $p = 0.008$) and after additional adjustment for white blood cell count proportions (Model 3: $F_{BD/SA}(2,158) = 6.227$, $p = 0.002$) and smoking score (Model 4: $F_{BD/SA}(2,157) = 7.904$, $p = 0.001$). We also found an increased GrimAgeAccel among BD/SA compared to CON when adjusting for smoking score only (Model 5: $F_{BD/SA}(2,188)=6.457$, $p = 0.002$) (Table 2). Post-hoc comparisons revealed a significantly greater GrimAgeAccel in BD/SA compared to CON ($p = 0.004$, Fig. S2); based on mean values, this equates to ~3 years of accelerated GrimAge. Absolute mean (standard deviation) GrimAgeAccel (in years) was −1.41(4.77) in CON, −0.22(4.87) in BD/non-SA, and +1.42(4.84) in BD/SA (Table S3).

When focusing only on BD groups, GrimAgeAccel was significantly higher in BD/SA compared to BD/non-SA in both the discovery (1.6 years accelerated, $p = 0.046$) and replication cohorts (2.2 years accelerated, $p = 0.008$) (Fig. 1C, D; Table 2). Based on our sample sizes per group and the observed effect sizes obtained in our analyses, we had a statistical power of 53% and 74% to detect group differences between BD/SA and BD/non-SA in the discovery ($f = 0.17$) and replication ($f = 0.27$) cohorts, respectively. In the discovery cohort, adjusted models showed that BD/SA remained significantly associated with a greater GrimAgeAccel compared to BD/non-SA after adjusting for covariates in Models 1, 3, 4, and 5, but not in Model 2 ($p = 0.051$). On the other hand, differences between groups remained significant in all models in the replication cohort, with BD/SA consistently showing higher GrimAgeAccel compared to BD/non-SA (Table 2). No differences were observed between cohorts for each of the BD groups (Table S2).

Individual GrimAge-associated surrogate and smoking pack-years protein estimates

DNAm-based surrogate protein markers and DNAmPACKYRS significantly correlated with chronological age, with the exception of DNAmLeptin (Figs. S3 and S4). An advantage of the GrimAge clock is that each of its surrogate protein DNAm markers can be queried to investigate their role in accelerated aging. Age-adjusted DNAmPAI1 ($p = 0.027$) levels and DNAmPACKYRS ($p = 0.031$) were significantly higher in BD/SA compared to CON (Model 6, Table S3), even after further adjustment for age, sex, PCs, and years of education ($p = 0.023$ and $p = 0.017$, respectively) (Model 7, Table S3). DNAmPAI1 was also elevated in BD/non-SA compared to CON. Additionally, age-adjusted DNAmADM showed a nominally significant difference ($p = 0.039$) between BD/SA and CON after covariate adjustment (Model 7, Table S3).

When focusing on the two BD subgroups, no significant differences in plasma protein markers were detected in the discovery cohort (Table 3). However, in both cohorts, we found a nominally significant difference in BD/SA compared to BD/non-SA on age-adjusted DNAmPACKYRS in Model 6 (discovery $p = 0.046$; replication $p = 0.007$), which remained after covariate adjustment in Model 7 ($p = 0.034$ and $p = 0.006$, respectively). In the replication cohort, age-adjusted DNAmLeptin showed a nominally significant difference ($p = 0.024$) between BD/SA and BD/non-SA after covariate adjustment (Model 7).

GrimAgeAccel in BD and SA and clinical variables

BD/non-SA and BD/SA significantly differed for many clinical variables in both cohorts (Table S1), including MADRS scores, length of illness, total number of comorbidities, and total number of psychiatric hospitalizations. YMRS scores, substance abuse, and age at onset of any mood disorder were also different between groups in the discovery cohort, while current lithium use differed in the replication cohort (Table S1). To assess the influence of these clinical variables on the group differences previously identified, we used logistic regression models to test for the association of the diagnostic (BD-non/SA or BD/SA) with the categorical outcome (positive or negative GrimAgeAccel) while adjusting for these clinical variables (Table 4).

Initially, we found that BD/SA had 69% increased odds of having accelerated GrimAge relative to BD/non-SA, with an odds ratio (OR) of 2.362 (95% CI, 1.207–4.623). On the other hand, the BD/non-SA group had 19% lower odds of having accelerated GrimAge relative to BD/SA, with an OR of 0.451 (95% CI, 0.231–0.880). These associations remained significant after adjustment for total number of comorbidities (Model 9), comorbidities and length of illness (Model 10), comorbidities, length of illness, and current use of the medication (Model 11), and comorbidities, length of illness, medication, and current lithium use (Model 12). Although the model including substance abuse disorder was not significant (Model 13), the *p* value ($p = 0.056$) indicates a trend towards an increased odds of accelerated GrimAge for BD/SA compared to BD/non-SA. These associations were replicated in the replication cohort (Table 4).

GrimAgeAccel in BD and SA in younger and older individuals

To explore whether the SA-linked aging acceleration was different among younger or older individuals, we repeated the analyses after filtering our dataset to only younger (<25th percentile of chronological age) and older (>75th percentile of chronological age) individuals. In the discovery cohort, we found that GrimAgeAccel was significantly higher in BD/SA compared to BD/non-SA within the older subgroup ($p = 0.009$) when minimally adjusted in Model 1. BD/SA remained significantly associated with a greater GrimAgeAccel compared to BD/non-SA after adjusting for covariates in Models 2, 3, and 5, but not in Model 4 ($p = 0.132$,

Table 3. GrimAge Clock and its components in BD/non-SA and BD/SA.

	Discovery cohort							Replication cohort						
	BD/non-SA: $n = 67$	BD/SA: $n = 77$	Model 6			Model 7		BD/non-SA: $n = 47$	BD/SA: $n = 48$	Model 6			Model 7[a]	
	Mean (SD)	Mean (SD)	t (df = 141)	p value	Cohen's d	$F_{BD/SA}$ (df = 1, 127)	p value	Mean (SD)	Mean (SD)	t (df = 93)	p value	Cohen's d	$F_{BD/SA}$ (df = 1, 83)	p value
AgeAccelGrim	−0.217 (4.87)	1.42 (4.84)	−2.013	**0.046**	0.337	5.126	**0.025**	−1.10 (3.71)	1.06 (4.06)	−2.697	**0.008**	0.556	7.683	**0.007**
DNAmPACKYRS	−1.25 (12.5)	3.18 (13.7)	−2.013	**0.046**	0.338	4.599	**0.034**	−3.44 (12.0)	3.44 (12.4)	−2.736	**0.007**	0.564	8.030	**0.006**
DNAmCystatinC	−6331 (43894)	532 (40552)	−0.963	0.338	0.162	1.113	0.294	−1776 (1701)	1776 (1786)	−0.987	0.326	0.204	0.938	0.336
DNAmLeptin	67.7 (4246)	−166 (3895)	0.340	0.735	0.057	0.413	0.521	−619 (3440)	593 (4397)	−1.492	0.141	0.307	5.273	**0.024**
DNAmTIMP1	76.3 (875)	101 (854)	−0.167	0.868	0.028	0.021	0.886	9.38 (405)	−9.38 (425)	0.219	0.827	0.045	0.046	0.831
DNAmADM	2.28 (22.8)	0.944 (23.3)	0.346	0.730	0.058	0.357	0.551	−0.024 (18.0)	0.023 (14.5)	−0.014	0.989	0.003	0.000	0.985
DNAmB2M	5609 (134347)	7723 (145708)	−0.090	0.928	0.015	0.006	0.941	−8632 (9360)	8632 (8795)	−0.921	0.359	0.359	0.887	0.349
DNAmGDF15	−23.3 (244)	43.4 (271)	−1.546	0.125	0.258	2.298	0.139	−8.96 (117)	8.96 (150)	−0.645	0.520	0.133	0.45	0.504
DNAmPAI1	579 (2460)	17.9 (2755)	1.283	0.202	0.215	2.542	0.113	−324 (2312)	324 (2181)	−1.399	0.165	0.289	1.894	0.172

Model 6: Independent samples *t*-test. Model 7: covaried for age, sex, GWAS principal components (PCs), years of education.
[a]Used race instead of genomic ancestral PCs. Bonferroni-corrected $p < 0.005$ was taken as the significance threshold. Nominally significant differences ($p < 0.05$) are bolded. BD/SA, bipolar disorder with history of suicide attempt. BD/non-SA, bipolar disorder with no history of suicide attempt. The GrimAge components were calculated based on DNA methylation (DNAm) levels at multiple CpG sites and represent surrogate markers of the plasma levels of these proteins (and smoking pack years) in all subjects. These measures were age-adjusted by regressing their DNAm-predicted levels on chronological age and using their residuals for statistical analyses.

Table S4). Similar set of analyses found no difference between BD/SA and BD/non-SA within the younger subgroup of individuals ($p > 0.05$ for all models) in the discovery sample. Finally, in the replication cohort, we found no significant differences between groups after dividing the sample into younger and older individuals using the same strategy above (Table S4).

DISCUSSION

In this study, we extend prior work documenting an accelerated mortality-associated epigenetic clock in BD [25] with novel evidence that SA within BD is associated with even greater GrimAgeAccel. Specifically, we found that BD/SA shows an ~3 years higher GrimAgeAccel when compared to CON and at least 1.6 years higher GrimAgeAccel when compared to BD/non-SA. These differences are robust to a variety of potential confounds, including age, sex, years of education, BMI, white blood cell count proportions, genomic ancestry, and smoking. Altogether, these findings pair with existing evidence that BD and SA are associated with accelerated biological aging and provide putative biological mechanisms for premature mortality.

This is the first study to document higher GrimAge in SA compared to a non-psychiatric control group, although previous studies have investigated accelerated EA related to suicidal behavior [26, 27]. SA is typically associated (but not synonymous) with a more severe illness presentation [52–55], which may account for some of the observed relationships and cumulatively contribute to accelerated aging [19, 56–58]. Indeed, BD/SA showed longer length of illness and higher numbers of comorbidities and hospitalizations than BD/non-SA. We explored whether the relationship between SA and GrimAgeAccel was attenuated when covarying for these indices of severity (Table 4) and found that covarying for substance abuse slightly attenuated the observed difference between BD/SA and BD/non-SA. Although not a strong finding, this suggests a potential influence of this comorbidity on the excess GrimAgeAccel observed with SA, which should be explored in future studies with larger sample sizes given previous indications of an association between substance abuse and accelerated aging [59, 60]. In addition, SA itself or genetic liability for suicidal behavior may also directly contribute to premature aging, although recent genetic data suggest that EA is largely independent of genetic risk [61]. Future studies may also consider exploring the association of GrimAge with specific clinical subtypes in BD, such as suicide decedents in postmortem tissues, and self-injurious thoughts and behaviors. Previous studies have explored associations between accelerated aging and number of SAs [11] and SA lethality [27]. Medical comorbidities of BD such as cardiovascular disease [62], asthma [63], diabetes [64], and hypothyroidism [65] may also play a role in EA [66–69]. Integrating multi-level information in longitudinal studies may be key to uncovering novel understandings of the underlying pathophysiology as well as potential interventional targets.

When exploring GrimAge subcomponents, analyses revealed that the DNAmPAI-1 was elevated in BD/SA, but even greater in BD/non-SA compared to CON in the discovery cohort. PAI-1 is a physiological inhibitor of tissue plasminogen activator (tPA) in plasma and is increased in situations related to ischemic cardiovascular events and senescence [70, 71]. A lack of tPA or high levels of PAI-1 may disrupt brain-derived neurotrophic factor (BDNF) processing and result in abnormal neuronal function [72, 73] and reduced BDNF levels in BD [74], which is also observed in suicide attempters [75] and postmortem brains of suicide decedents [76, 77].

In the replication cohort, after covariate adjustment, DNAm-Leptin showed a significant elevation in BD/SA compared to BD/non-SA. An increase in leptin can increase acute inflammatory response [78] and contribute to chronic inflammation in obesity [79]. Indeed, violent SA has been associated with abdominal

Table 4. Phenotype-adjusted differences in GrimAgeAccel between BD/non-SA and BD/SA groups.

	Discovery cohort			Replication cohort		
	Wald $X^2_{BD/SA}$ (df = 1)	*p* value	*Odds Ratio (95% CI)*	Wald $X^2_{BD/SA}$ (df = 1)	*p* value	*Odds Ratio (95% CI)*
Model 8	6.302	**0.012**	2.36 (1.207–4.623)	1.663	**0.020**	1.15 (0.930–1.430)
Model 9	5.602	**0.018**	2.28 (1.152–4.515)	3.540	**0.060**	1.33 (0.990–1.800)
Model 10	6.267	**0.012**	2.64 (1.235–5.627)	6.592	**0.010**	1.80 (1.150–2.830)
Model 11	5.692	**0.017**	2.30 (1.160–4.565)	6.311	**0.010**	1.12 (1.030–1.220)
Model 12	5.107	**0.024**	2.25 (1.114–4.551)	5.853	**0.020**	1.03 (0.660–1.630)
Model 13	3.637	0.056	2.01 (0.981–4.154)	2.110	0.050	1.57 (1.060–2.330)

Model 8: dichotomic logistic regression. Model 9: covaried for total number of comorbidities. Model 10: covaried for total number of comorbidities and length of illness. Model 11: covaried for total number of comorbidities, length of illness, and current use of medication. Model 12: covaried for total number of comorbidities, length of illness, current use of medication (any), and current use of lithium. Model 13: covaried for total number of comorbidities, length of illness, current use of medication (any), current use of lithium, and substance use disorder. Nominally significant differences (unadjusted $p < 0.05$) are bolded.

obesity in BD individuals [80], although previous studies have also shown that plasma leptin levels are decreased in individuals with suicide behavior compared to CON [81, 82]. High leptin levels are associated with insulin resistance and a higher risk for metabolic syndrome and cardiovascular disease, which have all been previously associated with BD [83, 84] and may be related to evidence of accelerated vascular aging [85].

GrimAgeAccel shows a stepwise elevation from CON to BD/non-SA to BD/SA. Clinically, this suggests that interventions targeting factors associated with risk for biological aging may contribute to lowering the deleterious biological consequences of SA in BD. Indeed, recent clinical trials suggest that patterns of EA can be reversed with both pharmacological [86] and diet/lifestyle changes [87]. Although our study found no direct association between GrimAgeAccel and medication use, we cannot rule out their potential anti-aging effects given our lack of detailed information on medication adherence, treatment duration, or response in the individuals enrolled in these studies. In fact, lithium, which is a known anti-suicidal drug [88], has also been repeatedly shown to present significant anti-aging effects [89], although no specific effects on epigenetic aging have been demonstrated yet. Any potential effect of medications on treating individuals with suicidal behavior based on accelerated epigenetic aging remains speculative at this time. Moreover, our exploratory analyses also found that the difference between BD/SA and BD/non-SA was primarily detected within older individuals, with no clear evidence of a higher GrimAgeAccel associated with SA in younger individuals. These findings could possibly suggest that the GrimAge acceleration in individuals with a history of SA reflects an accumulation of lifetime stressor exposures, as older individuals with a greater cumulative exposure to stress might be most likely to show the SA-specific association. Nonetheless, in addition to being an unreplicated finding in the replication cohort, our age-specific exploratory analyses may also be statistically underpowered to definitively and directly address this issue, so we cannot rule out the possibility that accelerated EA may already be taking place in young individuals with BD based on our results (as suggested by other aging markers in this population [90].

Limitations of the study include the cross-sectional design and retrospective assessment of SA; lack of trauma and life stress severity variables; and the small size of the replication sample. Also, we do not have information on the severity of current substance use comorbidity (including alcohol) from the participants, limiting analyses on their previously suggested effects on EA in our sample [91, 92]. We also have limited information on the proportion of many blood cell types, including cells known to be associated with normal aging processes, such as naïve T lymphocytes [93, 94]. Finally, we cannot rule out the possibility of Type I errors given the large number of comparisons and the relatively small sample sizes per group. Strengths of our study include the assessment of an independent replication cohort, which validates initial findings and reduces the likelihood of false positives; robust covariates including genomic ancestry, multiple white blood cell count proportions, and an objective DNAm-based smoking score; the inclusion of three groups to tease apart influences of BD and SA; and a rich clinical dataset in both cohorts.

In conclusion, we found that GrimAge shows increasing acceleration from CON to BD/non-SA to BD/SA, with a robust differentiation between BD with and without SA. These relationships were resilient to many possible confounding influences and replicated in an independent cohort. Considering the severity of both BD and SA and the associated reductions in life expectancy, we propose GrimAge and EA as key targets for future studies focused on innovating biological tools to reduce morbidity and mortality in this population.

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AUTHOR CONTRIBUTIONS

Design and conceptualization of the study: CNCL, EHCK, MEG, and GRF. Sample collection and recruitment: EHCK, BMRA, JFR, AW, JAW, VAM, JGF, APD, JQ, and JCS. Data generation, processing, and statistical analyses: CNCL, EHCK, SM, ADFC, and APD. Scientific discussion and interpretation of results: CNCL, EHCK, SM, AW, JAW, VAM, APD, JQ, JCS, JGF, MEG, and GRF. Wrote the manuscript: CNCL, EHCK, SM, MEG, and GRF.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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