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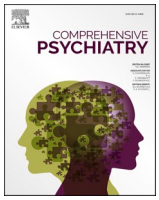

Blood-based DNA methylation profiles in major depressive disorder, bipolar disorder, and schizophrenia spectrum disorders

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ABSTRACT

Alterations in DNA methylation (DNAm) profiles have been implicated in affective and psychotic disorders. However, no comprehensive understanding of peripheral DNAm profiles associated with diagnostic groups, course of illness, and other clinical variables has emerged yet. In particular, studies exploring commonalities and differences across diagnoses are lacking. Here we conducted a systematic epigenetic characterization of the transdiagnostic German FOR2107 cohort, including individuals with major depressive disorder (MDD, $n = 342$), bipolar disorder (BD, $n = 99$), or a schizophrenia spectrum disorder (SSD, $n = 101$) and healthy controls (HC, $n = 339$). For 183 MDD cases and 178 HC, we assessed additional DNAm data from the two-year follow-up study visit. To explore DNAm differences between and across diagnostic groups, case-control and case-case methylome-wide association studies were performed. Our sample was further characterized using methylation risk scores (MRS) for MDD and SSD. Finally, epigenetic age acceleration was examined and compared to a measure of brain age acceleration. We identified few methylome-wide significant associations with diagnostic groups. MRS for MDD did not differ between diagnostic groups, and an increase in MRS for SSD in SSD compared to HC did not remain significant when adjusting for smoking behavior and BMI. An increase in epigenetic age acceleration was most evident for SSD compared to HC, which did not remain significant when adjusting for covariates. No correlation between epigenetic and brain age acceleration was observed. Our findings emphasize the relevance of potential confounding factors in epigenetics research in psychiatry and contribute to a growing body of studies on DNAm profiles across affective and psychotic disorders.

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1. Introduction

Major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia spectrum disorders (SSD), including schizophrenia (SCZ) and schizoaffective disorder, are severe mental disorders that contribute significantly to the global burden of disease [1]. They are characterized by affective and psychotic symptoms, with overlapping clinical presentations [2] and substantial cross-disorder genetic correlations [3]. While extensive research has been devoted to understanding the etiology and pathophysiology of these disorders, the molecular mechanisms underlying their development and progression remain incompletely understood. Emerging evidence suggests that epigenetic modifications, particularly DNA methylation (DNAm), might play a crucial role, given their capacity to integrate both genetic and environmental factors [4,5]. DNAm describes the addition of a methyl group to cytosine residues predominantly in CpG dinucleotides and has important regulatory effects on gene expression [6]. Due to their accessibility, epigenetic alterations in peripheral blood are of particular interest, making them a potential source for biomarkers of affective and psychotic disorders [7].

Previous studies in the field of affective and psychotic disorders have investigated DNAm in different cell types and tissues, particularly in post-mortem brain and peripheral blood samples [7,8]. Targeted approaches have identified disorder-associated DNAm differences in candidate genes, including *NR3C1*, *SLC6A4*, *BDNF*, *PDYN*, *COMT*, *RELN*, and *OXTR*, which are related to the hypothalamic-pituitary-adrenal axis stress response and different neural processes [7,9,10]. In addition, methylome-wide association studies (MWAS) investigating differential methylation across the genome have been conducted for MDD [11–14] and SSD [15–17]; for BD, large-scale MWAS were not yet available at the time of writing. Most studies detected a number of differentially methylated positions (DMPs) and differentially methylated regions between cases and controls. Despite some overlap between the findings, no comprehensive understanding has emerged within or across disorders, and transdiagnostic studies to elucidate commonalities and differences in peripheral DNAm profiles between MDD, BD, and SSD are lacking.

DNAm is dynamic in nature, and changes in response to exogenous or endogenous signals have been conceptualized as an epigenetic memory, especially since some changes exhibit long-term persistence [18]. Cigarette smoking and body mass index (BMI) are two prominent examples of modifiable health and lifestyle-associated factors with relevance to psychiatric conditions [19] for which characteristic DNAm signatures have been identified [20]. Moreover, changes in DNAm over time could potentially be associated with changes in disorder states, such as the presence or absence of mood episodes in MDD and BD. For the differentiation between state-dependent and trait-like characteristics, longitudinal study designs may prove particularly useful, e.g., by accounting for intraindividual variability [21].

One way to examine whether disorder-associated DNAm profiles in a given sample match DNAm profiles identified by previous MWAS is via methylation risk scores (MRS). MRS are weighted sums of CpG sites based on MWAS associations and they reflect the individual-level manifestations of a specific DNAm profile [22]. The concept of MRS is closely related to that of polygenic risk scores (PRS), which are calculated based on large-scale genome-wide association studies (GWAS). PRS are a frequently used research tool for summarizing individual-level genetic risk in complex disorders, such as MDD, BD, and SSD, that exhibit high polygenicity and considerable heritability [23]. As PRS only capture a static risk component, MRS may provide a complimentary view by capturing more dynamic aspects, which makes joint modeling of PRS and MRS interesting.

The disorders investigated in this study – MDD, BD, and SSD – are all associated with an increased mortality compared to the general population, which is not fully explained by suicide and other unnatural causes of death [24]. An accelerated aging has been hypothesized, which has motivated studies on biomarkers of aging in psychiatric conditions [25]. Epigenetic clocks, i.e., statistical models trained to predict chronological or biological age based on DNAm data [26–28], provide a valuable tool to examine the hypothesis of accelerated aging by quantifying the deviation of the estimated epigenetic age from the chronological age. First-generation epigenetic clocks, such as the Skin & Blood clock developed by Horvath et al. [26] (estimate hereinafter denoted as DNAm Age) were trained to predict chronological age. In contrast, second-generation clocks, such as the DNAm PhenoAge by Levine et al. [27] and the DNAm GrimAge by Lu et al. [28], were designed as biomarkers of biological aging and were shown to be associated with lifespan and mortality by the respective authors. Despite some inconsistencies in the results, mostly related to the usage of different epigenetic clocks, previous research has indicated an epigenetic age acceleration in SSD [29–34], BD [35,36], and MDD [37–41]. However, transdiagnostic characterizations, i.e. joint examinations of the three disorders within one study sample to enable direct comparisons, are rarely conducted. Moreover, while first studies (e.g. [42,43]) have started to investigate the relationship between epigenetic age acceleration and the so-called brain age gap, a biomarker of brain aging based on magnetic resonance imaging (MRI) data [44], little is known about this relationship in the context of the investigated disorders [30].

Against this backdrop, the present study describes a comprehensive exploration of methylome-wide DNAm profiles across affective and psychotic disorders within the German FOR2107 study. The FOR2107 study was previously established to investigate the neurobiology of affective and psychotic disorders and is characterized by its deep phenotyping and longitudinal design, which comprises a baseline assessment and a two-year follow-up study visit [45]. Within the scope of this study, we generated DNAm data for (1) a cross-sectional transdiagnostic sample including healthy controls (HC) and individuals with a diagnosis of MDD, BD, or SSD, and (2) a longitudinal sample including HC and individuals with a diagnosis of MDD. Based on this dataset, we pursued three main objectives. First, we aimed to identify DNAm differences associated with a lifetime diagnosis of MDD, BD, and SSD via case-control and case-case MWAS and with dynamic clinical variables via MWAS in the longitudinal sample. In the main MWAS models, covariates accounting for smoking behavior and BMI were included for a focus on effects directly related to the examined disorders. In addition, reduced models were fitted for a characterization of indirect effects of mental health-linked differences in lifestyle on DNAm profiles in affective and psychotic disorders. Second, we evaluated the manifestation of previously identified MDD- and SSD-associated DNAm profiles in our sample via a MRS approach, and compared the variance explained by MRS with the variance explained by corresponding disorder-specific PRS. Lastly, we aimed to test the hypothesis of epigenetic age acceleration and the correlation between epigenetic and brain age acceleration using three epigenetic clock models. Our study is innovative and unique in its transdiagnostic and multifaceted design, providing a broad comparative perspective across affective and psychotic disorders.

2. Methods

2.1. Sample

The present analyses were performed using data retrieved from the German FOR2107 cohort [45]. 881 FOR2107 participants were included in the present analyses, comprising HC ($n = 339$) and individuals with a lifetime diagnosis of MDD ($n = 342$), BD ($n = 99$), or SCZ/schizoaffective disorder (SSD, $n = 101$) derived from the German version of the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) [46]. Inclusion in the HC group required absence of any current or past psychiatric disorders according to DSM-IV. The MDD and BD groups comprised both individuals experiencing an affective episode at study visit and individuals in a euthymic mood state. For further details regarding study inclusion and exclusion criteria see [45]. All participants provided written informed consent during the recruitment process. Ethical approval was obtained from the local ethics committees

of the Universities of Marburg and Münster, Germany. For all 881 participants, data and DNA extracted from peripheral blood collected at the baseline study visit (T1) were retrieved for cross-sectional analyses. For 53 % of the participants of the MDD and HC groups ($n = 361$), data and DNA from the two-year follow-up study visit (T2) were retrieved for longitudinal analyses.

2.2. Genotyping, quality control, imputation, and PRS calculation

Genome-wide genotyping was previously performed using DNA extracted from peripheral blood and the Infinium PsychArray-24 BeadChip (Illumina, San Diego, CA, USA) [47]. Genomic positions were assigned relative to hg19. Data quality control (QC) for variants and samples was conducted using PLINK v1.9 [48]. First, variants were pre-filtered for a call rate < 98 % and a minor allele frequency (MAF) < 1 %. Samples were then filtered on the basis of the following criteria: (1) call rate < 98 %; (2) absolute autosomal heterozygosity coefficient > 0.2; (3) sex mismatch; (4) genetic duplicates and genetic relatedness ($\widehat{\pi}$ > 0.125); (5) non-European ancestry as inferred using KING [49]; and (6) genetic outliers with a distance from the mean of >6 standard deviations within the first ten principal components (PCs). After filtering, a sample of 859 individuals remained. Additional variants were removed in the event of: (1) non-autosomal position; (2) ambiguous alleles; (3) call rate < 98 %; (4) Hardy-Weinberg equilibrium test p value <1e-06; or (5) MAF < 1 %. Genotype phasing and imputation were conducted using Eagle 2.4.1 [50] and Minimac 4.1.2 [51] with the 1000 Genomes Phase 3 reference panel [52]. After imputation, variants were filtered for an imputation INFO score < 0.3 and MAF < 1 %. From the imputed genotype data, PRS for MDD and SSD were calculated using PRS-CS [53], with ϕ set to 1e-02 reflecting high polygenicity, and PLINK v1.9 [48] based on summary statistics of previous large-scale genome-wide association study (GWAS) meta-analyses [54,55], in which the FOR2107 sample was not part of the contributing cohorts. PRS were z-scaled for downstream analysis.

2.3. DNA methylation profiling

DNAm was profiled in genomic DNA extracted from peripheral blood using the Infinium MethylationEPIC v1.0 BeadChip (Illumina, San Diego, CA, USA). Samples were randomized across microarrays with respect to sex and case-control status. On each microarray, samples of the HC group and of at least one of the case groups were present. In respect to the latter, it should be noted that due to the different group sizes, it was unavoidable that individual diagnostic groups were not represented on every array. For the longitudinal measurements, both samples of a single individual were placed on the same methylation microarray to reduce technical noise. Data QC and processing were conducted in R (v3.6.1) [56] using the minfi (v1.32.0) [57] and ewastools (v1.7.2) [58] packages. Samples were excluded in the event of: (1) call rate < 98 % ($n = 5$); (2) failure in Illumina control metrics ($n = 0$); (3) sex mismatch ($n = 7$); or (4) single nucleotide polymorphism (SNP) fingerprint mismatch that could not be resolved ($n = 10$). For the latter, SNP fingerprints were generated based on 59 genetic markers present on both the MethylationEPIC array and the PsychArray, and checked for pairwise discordance between samples originating from different participants / concordance between samples from the same participant, as implemented in the ewastools package. In total, seven T1 samples and eight T2 samples were excluded. Of note, seven samples met two out of the four above listed exclusion criteria, therefore the sum of exclusions indicated for each criterion exceeds the total number of excluded samples. The methylation intensities of the remaining samples were normalized using the stratified quantile normalization approach, as implemented in minfi [59]. For downstream data analysis, M values were calculated from normalized data. After normalization, methylation probes were removed according to the following criteria: (1) call rate < 98 % ($n = 9913$); (2) non-CpG sites ($n = 2890$); (3) non-autosomal sites ($n = 18{,}704$); (4) sites with SNPs inside the probe body ($n = 27{,}754$); and (5) polymorphic (with MAF > 1 % in European populations, $n = 3588$) or cross-hybridizing probe binding sites ($n = 38{,}455$) [60].

To obtain the analysis sample, the quality-controlled dataset was filtered for the availability of high-quality genotype data and non-relatedness (cf. QC of genotype data described above). In case of participants for which baseline samples were missing, any respective follow-up samples were removed. In total, 854 individuals remained for analysis (Table 1). Of these, 349 individuals had data from both T1 and T2 (Table 2).

2.4. Data-derived covariates

Both the genotype and the methylation data were used to estimate covariates for the downstream analyses. Four ancestry principal components (aPCs) calculated from pruned pre-imputation genotype data using PLINK v1.9 [48] were used as covariates to account for effects of population stratification. To account for technical variation in the methylation data, 30 methylation principal components (mPCs) were calculated in R (v3.6.1) [56] from the intensity values of Illumina MethylationEPIC control probes (excluding negative control probes), following the Control Probe Adjustment approach described by Lehne and colleagues [61]. Blood cell type composition was estimated from normalized methylation data in accordance with Houseman et al. [62] and as implemented in minfi. mCigarette scores were calculated based on DNAm beta values according to Chybowska et al. [63] as a quantitative biomarker of smoking behavior. In addition, M values of the *AHRR* probe cg05575921, a well-established single-site smoking biomarker [64], were retrieved to adjust for residual effects of smoking behavior on DNAm unaccounted for by the mCigarette score (Supplementary Fig. 1 A-B). Both mCigarette and cg05575921 levels reflect current smoking and, to a lesser extent, past smoking history (Supplementary Fig. 1C-D).

2.5. MWAS

To test the association of DNAm with selected variables of interest, linear mixed models were fitted in R (v3.6.1) [56] using the limma (v3.42.0) package [65], with DNAm M values as dependent variables. Based on Mansell et al. [66], association p values <9e-08 were considered methylome-wide significant. Case-control and case-case MWAS as well as supplementary MWAS of smoking status and BMI were conducted using the combined dataset of all baseline and follow-up measurements to maximize power. For the case-control and case-case comparisons, the diagnostic group was included as independent variable of interest, together with a set of fixed effect covariates of no interest. The covariates included the sex, age (in years with two decimal places), estimates of cell type composition ($CD8^+$ T cells, $CD4^+$ T cells, natural killer cells, B cells, monocytes), four aPCs, 30 mPCs, the mCigarette score, M values of the *AHRR* probe cg05575921, and BMI. To account for the subset of longitudinally measured participants, the proband identity was included as a random effect, with the correlation between repeated measures estimated from 10,000 randomly selected CpG sites. Since the effect of the fixed effect covariates on DNAm was expected to be consistent across diagnostic groups, joint models were fitted including all available samples, and contrasts between diagnostic groups were specified to identify DMPs. In addition to the three case-control and the three case-case comparisons, a joint comparison of all individuals with a psychiatric diagnosis to the HC group was conducted. For the annotation of CpG sites, the IlluminaHumanMethylationEPICanno.ilm10b4.hg19 Bioconductor package was used, which is based on the Infinium MethylationEPIC Manifest file provided by Illumina. Statistical power was estimated using the pwrEWAS (v1.12.0, R version 4.2.2) package [67]. As supplementary analyses, reduced MWAS models were fitted, in which covariates related to modifiable lifestyle-related factors (mCigarette score, the *AHRR* probe cg05575921, and BMI)

Table 1
Characteristics of the cross-sectionally measured sample at the baseline FOR2107 study visit.

	HC	MDD	BD	SSD	Comparison
n	326	337	96	95	
%Female	63.19	63.80	52.08	43.16	p = 6.6e-04
Age	36.04 (13.19)	35.49 (13.09)	41.41 (11.97)	37.17 (11.25)	p = 8.6e-05 (HC < BD, p = 2.3e-04; MDD < BD, p = 6.8e-05)
BMI	24.12 (NA: 29; SD = 4.23)	25.33 (NA: 18; SD = 5.56)	26.85 (NA: 2; SD = 4.82)	28.38 (NA: 6; SD = 6.34)	p = 1.1e-11 (HC < BD, p = 3.6e-06; HC < SSD, p = 1.3e-09; MDD < BD, p = 1.6e-03; MDD < SSD, p = 3.9e-06)
Self-reported smoking status: ever / never / NA	78 / 245 / 3	121 / 207 / 9	55 / 37 / 4	62 / 33 / 0	p = 3.3e-16
mCigarette score	−0.4799 (0.0311)	−0.4736 (0.0424)	−0.4514 (0.0576)	−0.4322 (0.0669)	p = 4.2e-11 (HC < BD, p = 1e-04; HC < SSD, p = 3.7e-09; MDD < BD, p = 9.2e-04; MDD < SSD, p = 1.1e-07)
cg05575921	0.8738 (0.0436)	0.8564 (0.0685)	0.8226 (0.0993)	0.7837 (0.1160)	p = 1.7e-16 (HC > MDD, p = 1.1e-03; HC > BD, p = 1e-05; HC > SSD, p = 9.4e-16; MDD > BD, p = 2e-02; MDD > SSD, p = 1.8e-08; BD > SSD, p = 8.1e-03)
Mood state: euthymic / depressed / (hypo-) manic / mixed / NA	326 / 0 / 0 / 0 / 0	104 / 229 / 0 / 0 / 4	30 / 32 / 17 / 2 / 15	0 / 0 / 0 / 0 / 95	
$CD8^+$ T cells	0.0705 (0.0381)	0.0656 (0.0356)	0.0533 (0.0376)	0.0532 (0.0346)	p = 1.5e-06 (HC > BD, p = 1.2e-04; HC > SSD, p = 1.2e-04; MDD > BD, p = 7.5e-03; MDD > SSD, p = 7.5e-03)
$CD4^+$ T cells	0.1655 (0.0480)	0.1666 (0.0516)	0.1710 (0.0625)	0.1483 (0.0453)	p = 7.7e-03 (HC > SSD, p = 8.4e-03; MDD > SSD, p = 7.7e-03; BD > SSD, p = 2.2e-02)
Natural killer cells	0.0557 (0.0392)	0.0510 (0.0355)	0.0499 (0.0346)	0.0466 (0.0376)	p = 0.130
B cells	0.0534 (0.0194)	0.0543 (0.0219)	0.0519 (0.0191)	0.0520 (0.0206)	p = 0.833
Monocytes	0.0715 (0.0204)	0.0728 (0.0197)	0.0693 (0.0231)	0.0736 (0.0173)	p = 0.178
Granulocytes	0.5965 (0.0763)	0.6023 (0.0792)	0.6167 (0.0949)	0.6384 (0.0766)	p = 1.7e-05 (HC < BD, p = 2.5e-02; HC < SSD, p = 2.4e-05; MDD < SSD, p = 9.7e-04)

Characteristics of the sample remaining after quality control. Unless otherwise specified, values indicate the number of participants for categorical variables, and group means with SD for continuous variables. The age was recorded in years with two decimal places. For the methylation probe cg05575921, which represents a quantitative biomarker for smoking behavior, beta values (bound between 0 and 1) are provided. Proportions of six major leukocyte cell types ($CD8^+$ T cells, $CD4^+$ T cells, natural killer cells, B cells, monocytes, and granulocytes) were estimated from DNAm data using the Houseman approach as implemented in minfi. Group comparisons are based on Chi-squared tests for categorical variables (sex and self-reported smoking status), and Kruskal-Wallis tests with post hoc Dunn's tests for continuous variables (age, BMI, mCigarette score, cg05575921, and cell type proportions). BD, bipolar disorder, BMI, body mass index; HC, healthy controls; MDD, major depressive disorder; NA, data not available/applicable; SD, standard deviation; SSD, schizophrenia spectrum disorders.

were excluded, to obtain a general characterization of DNAm profiles associated with the investigated disorders. Moreover, supplementary MWAS of self-reported smoking status (ever versus never) and BMI, respectively, were conducted with the same covariates as in the reduced MWAS models, to enable direct comparisons with the results of the case-control and case-case MWAS.

For a focus on state-dependent DNAm differences, an MWAS of mood state (depressed versus euthymic) was conducted within the longitudinal subset of HC and participants with a diagnosis of MDD. Where possible, the mood state at study visit was derived from DSM-IV codes (Supplementary Table 1). All HC were classified as euthymic. Additional MWAS within the longitudinal subset were conducted for the number of depressive episodes and the number of psychiatric hospitalizations reported for the lifetime up to the first and second study visit, respectively.

All sets of DMPs resulting from the main MWAS with a p value <1e-05 were functionally characterized via Gene Ontology (GO) gene set enrichment analyses using the gometh function implemented in the missMethyl (v1.40.3, R version 4.4.3) package, which accounts for biases due to differences in the number of methylation probes per gene and due to probes mapping to multiple genes [68]. As background set, all probes present in the post-QC methylation dataset were used. All 22,298 GO gene sets available via the missMethyl package were included in the analysis. Enrichment p values were adjusted for multiple testing using the Benjamini and Hochberg method [69] for controlling the false discovery rate.

2.6. MRS calculation and analysis

MRS for MDD and SSD were calculated within the cross-sectional baseline sample in R (v3.6.1) [56] as weighted sums of M values, using weights from previously published studies that had no sample overlap with the FOR2107 cohort. To maximize the overlap between CpGs included in the published MRS models and our dataset, all methylation sites of good technical quality, which remained after the call rate-based filtering step (n = 855,946, see section 2.3), were considered for MRS calculation. For the MDD MRS, weights derived by Barbu et al. [13] from a LASSO penalized regression of MDD case-control status on blood DNAm were used; all 196 CpGs with non-zero weights were available in our dataset. For the SSD MRS, MWAS beta coefficients from Hannon et al. [15] of CpGs significantly associated with SCZ were used as weights; 938 out of the 1048 significant CpGs were available in our dataset. MRS were z-scaled across all baseline samples and compared between groups using the analysis of variance (ANOVA). In case of an observed p value <0.05 in the ANOVA, Tukey's Honest Significant Difference (HSD) post hoc tests were performed between all pairs of diagnostic groups. In order to set the inter-group differences in epigenetic risk in relation to the underlying genetic risk, the same statistical tests were performed on the PRS for MDD and SSD. Moreover, logistic regression models of case-control status of MDD and SSD, respectively, were fitted in the baseline samples of HC and the respective disorder group. Both the disorder-specific MRS and PRS were included as variables of interest, as well as sex, age (in years with two decimal places), the mCigarette score, and BMI as covariates. As a sensitivity analysis, the logistic regression was repeated with the same set of covariates included in the main MWAS model (sex, age, $CD8^+$ T cells, $CD4^+$ T cells, natural killer cells, B cells, monocytes, four aPCs, 30 mPCs, the mCigarette score, M values of the *AHRR* probe cg05575921, and BMI). Numerical variables were z-scaled to mean of zero and

Table 2
Characteristics of the longitudinally measured sample at the baseline and follow-up FOR2107 study visits.

	HC		MDD		Comparison
Study visit	Baseline	Follow-up	Baseline	Follow-up	
n	174	174	175	175	
%Female	62.64	62.64	66.29	66.29	$p = 0.799$
Age	37.00 (13.69)	39.19 (13.68)	36.17 (13.76)	38.38 (13.76)	$p = 0.058$
BMI	24.13 (NA: 14; *SD* = 4.22)	24.56 (NA: 3; *SD* = 4.03)	25.34 (NA: 6; *SD* = 5.36)	26.21 (NA: 6; *SD* = 5.61)	$p = 1.0e-02$ (HC1 < MDD2, $p = 6.5e-03$)
Self-reported smoking status: ever / never / NA	54 / 120 / 0	56 / 110 / 8	81 / 91 / 3	80 / 87 / 8	$p = 5.5e-04$
cg05575921	0.8713 (0.0485)	0.8705 (0.0462)	0.8483 (0.0786)	0.8452 (0.0795)	$p = 4.6e-03$ (HC1 > MDD2, $p = 1.7e-02$; HC2 > MDD2, $p = 3.9e-02$)
mCigarette score	−0.4766 (0.0330)	−0.4791 (0.0324)	−0.4683 (0.0481)	−0.4673 (0.0488)	$p = 0.416$
Depressive episodes			3.05 (NA: 9; *SD* = 3.13)	3.82 (NA: 9; *SD* = 3.36)	
Hospitalizations			1.41 (NA: 1; *SD* = 1.80)	1.82 (NA: 3; *SD* = 2.22)	
Mood state: euthymic / depressed / NA	174 / 0 / 0	174 / 0 / 0	59 / 114 / 2	106 / 69 / 0	
$CD8^+$ T cells	0.0668 (0.0364)	0.0655 (0.0369)	0.0665 (0.0350)	0.0671 (0.0386)	$p = 0.981$
$CD4^+$ T cells	0.1685 (0.0509)	0.1662 (0.0519)	0.1717 (0.0532)	0.1731 (0.0525)	$p = 0.566$
Natural killer cells	0.0569 (0.0378)	0.0538 (0.0354)	0.0500 (0.0355)	0.0461 (0.0340)	$p = 3.0e-02$ (HC1 > MDD2, $p = 3.0e-02$)
B cells	0.0557 (0.0202)	0.0555 (0.0205)	0.0527 (0.0190)	0.0554 (0.0213)	$p = 0.429$
Monocytes	0.0708 (0.0211)	0.0720 (0.0222)	0.0721 (0.0199)	0.0700 (0.0180)	$p = 0.852$
Granulocytes	0.5953 (0.0761)	0.6011 (0.0795)	0.5996 (0.0792)	0.6019 (0.0749)	$p = 0.777$

Characteristics of the sample remaining after quality control. Unless otherwise specified, values indicate the number of participants for categorical variables, and group means with SD for continuous variables. The age was recorded in years with two decimal places. For the methylation probe cg05575921, which represents a quantitative biomarker for smoking behavior, beta values (bound between 0 and 1) are provided. Proportions of six major leukocyte cell types ($CD8^+$ T cells, $CD4^+$ T cells, natural killer cells, B cells, monocytes, and granulocytes) were estimated from DNAm data using the Houseman approach as implemented in minfi. Group comparisons are based on Chi-squared tests for categorical variables (sex and self-reported smoking status), and Kruskal-Wallis tests with post hoc Dunn's tests for continuous variables (age, BMI, mCigarette score, cg05575921, and cell type proportions). BMI, body mass index; HC, healthy controls; HC1, HC sample at baseline study visit; HC2, HC sample at follow-up study visit; MDD, major depressive disorder; MDD2, MDD sample at follow-up study visit; NA, data not available/applicable; SD, standard deviation.

standard deviation of one prior to fitting the regression models. *P* values of regression coefficients were adjusted for the number of predictors in the respective model using the Benjamini-Hochberg procedure for controlling the false discovery rate. The incremental variance explained (ΔR^2) by MRS and PRS was calculated as the difference between R^2 of the full model and R^2 of the model without MRS or PRS, respectively. Finally, the hypothesis of a decreased MDD MRS and an increased SSD MRS in BD type 1 compared to type 2, analogous to the known genetic architecture of BD subtypes [70,71], was tested. BD cases were divided into BD type 1 ($n = 50$) and type 2 ($n = 46$), as based on DSM-IV codes, and MRS were compared using one-sided *t*-tests.

2.7. Age acceleration

Epigenetic age estimates based on the Skin & Blood clock (DNAm Age) [26], DNAm PhenoAge [27], and DNAm GrimAge [28] were calculated in R (v4.4.3) [72]. DNAm Age and DNAm PhenoAge were estimated using the methylclock package (v1.12.0) [73], and DNAm GrimAge was calculated based on R scripts and regression weights provided by the authors of the DNAm GrimAge model (equivalent to the implementation in the publicly available DNA Methylation Age Calculator webtool (https://dnamage.clockfoundation.org)). To maximize the overlap between CpGs included in the published epigenetic clock models and our dataset, all methylation sites of good technical quality, which remained after the call rate-based filtering step ($n = 855{,}946$, see section 2.3), were considered for epigenetic age calculation. The relative impact of probe missingness was estimated by calculating the proportion of absolute weights of the missing CpGs in relation to the sum of absolute weights of all CpGs in a clock model [74]. All 391 CpGs included in the Skin & Blood clock were present in our data, as well as 511 out of 513 CpGs in the DNAm PhenoAge model (0.4 % missing CpGs, 0.3 % missing weights) and 1017 out of 1030 CpGs in the DNAm GrimAge model (1.3 % missing CpGs, 0.2 % missing weights). For all three epigenetic clocks, residuals of the linear regression of epigenetic age on chronological age (in years with two decimal places) were considered estimates of epigenetic age acceleration, as there was a systematic deviation of epigenetic age estimates from chronological age.

Cross-sectional group differences in epigenetic age acceleration within the baseline sample were analyzed using ANOVA and Tukey's HSD post hoc tests in case of a *p* value <0.05 in the ANOVA. Linear regression models were fitted within the cross-sectional baseline sample to estimate the effect on MDD, BD, and SSD case status compared to HC when adjusting for sex, smoking as reflected by the mCigarette score, and BMI. Moreover, to disentangle the impact of individual covariates, a stepwise regression was fitted, in which the following covariates were added in a stepwise manner: (1) sex, (2) the mCigarette score, (3) BMI, and (4) five out of six estimates of cell type composition ($CD8^+$ T cells, $CD4^+$ T cells, natural killer cells, B cells, monocytes). As a sensitivity analysis, the linear regression was repeated with the same set of covariates included in the main MWAS model (sex, $CD8^+$ T cells, $CD4^+$ T cells, natural killer cells, B cells, monocytes, four aPCs, 30 mPCs, the mCigarette score, M values of the *AHRR* probe cg05575921, and BMI), except for the omission of age, as this was already accounted for in the calculation of the epigenetic age acceleration. Numerical variables were z-scaled to mean of zero and standard deviation of one prior to fitting the regression models. *P* values of regression coefficients were adjusted for the number of predictors in the respective model using the Benjamini-Hochberg procedure for controlling the false discovery rate.

To analyze the correlation between epigenetic and brain age acceleration, estimates of the brain age gap derived from MRI data at the

baseline study visit, which were previously calculated by Hahn et al. [44], were obtained for 843 out of 854 participants. For the remaining 11 participants, brain age estimates were not available due to missingness or insufficient quality of MRI data. The brain age estimation was based on an uncertainty-aware, transparent neural network model that was trained on the neuroimaging data of more than 10,000 individuals from the German National Cohort and which was shown to yield a low median absolute error in independent validation cohorts [44].

Following the approach described by Iftimovici et al. [75], a longitudinal analysis of epigenetic aging was conducted within the subset of HC and MDD for which epigenetic age estimates were available from both the baseline and the follow-up study visit. The change in epigenetic age (epigenetic age at follow-up minus epigenetic age at baseline) relative to the change in chronological age (chronological age at follow-up minus chronological age at baseline), hereafter referred to as slope of epigenetic aging, was taken as longitudinal measure of epigenetic aging. As the interval between baseline and follow-up study visit was approximately two years, chronological age in years with two decimal places was used for all calculations to improve the resolution compared to the commonly used age in full years without decimal places. Two-sided *t*-tests were conducted to compare the slope of epigenetic aging between the HC and MDD group. To account for the effect of covariates, linear regression models of MDD case-control status on the slope of epigenetic aging were fitted, including sex, chronological age at baseline, mCigarette scores at baseline, the change of mCigarette scores between baseline and follow-up, BMI at baseline, and the change of BMI between baseline and follow-up as covariates of no interest. Numerical variables were z-scaled to mean of zero and standard deviation of one prior to fitting the regression models. *P* values of regression coefficients were adjusted for the number of predictors in the respective model using the Benjamini-Hochberg procedure for controlling the false discovery rate.

3. Results

Post-QC, the dataset comprised 764,555 CpG methylation sites from 854 deeply phenotyped, unrelated individuals with a lifetime diagnosis of MDD, BD, or SSD, as well as HC (Table 1). For all of these individuals, high-quality genotype data were available. Longitudinal DNAm measurements were obtained for 175 MDD cases and 174 HC (Table 2).

3.1. MWAS

In the main case-control and case-case MWAS, only few methylome-wide significant DMPs were identified (Table 3, maximum of two significant DMPs between SSD and HC). In comparison, with a reduced MWAS model, in which the adjustment for smoking behavior and BMI was omitted, a larger number of DMPs reached methylome-wide significance (maximum of 28 significant DMPs between SSD and HC). Relative to the MWAS of smoking and BMI, yielding 267 and 307 significant DMPs, respectively, the amount of identified differential methylation between diagnostic groups was low, and the majority of disorder-related DMPs detected using the reduced model design overlapped with the DMPs identified in the MWAS of smoking or BMI. This corresponds to the recorded phenotypic information, which showed a significant overrepresentation of ever-smokers among the BD and SSD group compared to HC and MDD, as well as a significantly increased BMI in BD and SSD compared to HC and MDD (Table 1). The main MWAS of mood state, number of depressive episodes, and number of hospitalizations within the longitudinal subsample of individuals with MDD and HC yielded two, 19, and 37 methylome-wide significant DMPs, respectively. Supplementary Fig. 2 provides an overview of the identified methylome-wide significant DMPs across all conducted MWAS (main and supplementary), and depicts the overlap between sets of DMPs. The full list of methylome-wide significant DMPs is provided in Supplementary Table 2. QQ-plots including estimates of the inflation factor lambda (ranging from 0.97 to 1.06) for the main MWAS are shown in Supplementary Figs. 3–12. With regard to the statistical power of the case-control and case-case MWAS, power calculations (Supplementary Fig. 13) showed that there was 80 % power to detect mean methylation differences equal to or larger than 5 % in the best-powered model (MDD vs. HC) and differences equal to or larger than 17.5 % in the worst-powered model (SSD vs. BD). In the gene set enrichment analyses of DMPs with suggestive evidence of association ($p < 1e\text{-}05$), none of the GO gene sets were significantly enriched for DMPs after correction for multiple testing. Gene sets with nominally significant enrichment for DMPs of the main MWAS are listed in Supplementary Table 3.

3.2. MRS

To extend the epigenetic characterization of the present study sample based on previously identified DNAm profiles, MRS for MDD and SSD were compared between diagnostic groups. While no statistically significant inter-group differences were observed for the MDD MRS, an increase of the SSD MRS in SSD cases in comparison to HC (Tukey's HSD post hoc test $p < 0.05$) was identified (Fig. 1A). The distribution of PRS for MDD and SSD showed the expected significant differences between groups. When modeling the effect of the SSD MRS on SSD case-control status together with the SSD PRS and covariates such as BMI and biomarkers of smoking behavior, the association of the SSD MRS with case-control status was no longer significant (Fig. 1B, Supplementary Fig. 14). The variance explained by both the MDD MRS ($\Delta R^2 = 0.0007$) and SSD MRS ($\Delta R^2 = 0.0078$) was considerably lower than the variance explained by their genetic counterparts (MDD PRS $\Delta R^2 = 0.0433$; SSD PRS $\Delta R^2 = 0.1064$) in the respective case-control regression models depicted in Fig. 1B. For the examination of BD-related epigenetic signatures in the present study sample, no well-powered BD MWAS was publicly available at the time of analysis. Therefore, the available MDD and SSD MRS were used to explore the hypothesis of higher epigenetic similarity of BD type 1 cases with SSD and BD type 2 cases with MDD. When comparing the MRS between BD subtypes, no significant differences were observed (Fig. 1C).

3.3. Age acceleration

For all three epigenetic clocks, the correlation between epigenetic age estimates and chronological age was high ($R \geq 0.92$, Fig. 2A).

Table 3
Methylome-wide significant DMPs in case-control / case-case MWAS.

Contrast	CpG	Chr	Position (hg19)	Log Fold Change	Average M value	P value	Gene
MDD vs. HC	cg07524214	16	86,825,363	0.2856	2.0196	2.26e-08	
SSD vs. HC	cg00207441	2	3,175,600	−0.1956	3.3593	5.26e-08	
SSD vs. HC	cg11224906	16	70,453,427	0.0874	0.2791	6.33e-08	*ST3GAL2*
SSD vs. BD	cg27396293	9	21,028,665	0.2288	0.0642	5.37e-08	*HACD4*
Psych vs. HC	cg25330192	5	34,494,278	−0.1562	−0.9436	7.85e-08	

All differentially methylated positions (DMPs) with methylome-wide significant association (p < 9e-08) identified in the main case-control / case-case MWAS are listed, sorted by their p value. "Psych vs. HC" denotes the joint comparison of all psychiatric case groups against HC. Gene annotations are based on the UCSC database, as specified in the MethylationEPIC Manifest provided by Illumina. BD, bipolar disorder; Chr, chromosome; DMP, differentially methylated position; HC, healthy controls; MDD, major depressive disorder; MWAS, methylome-wide association study; SSD, schizophrenia spectrum disorders.

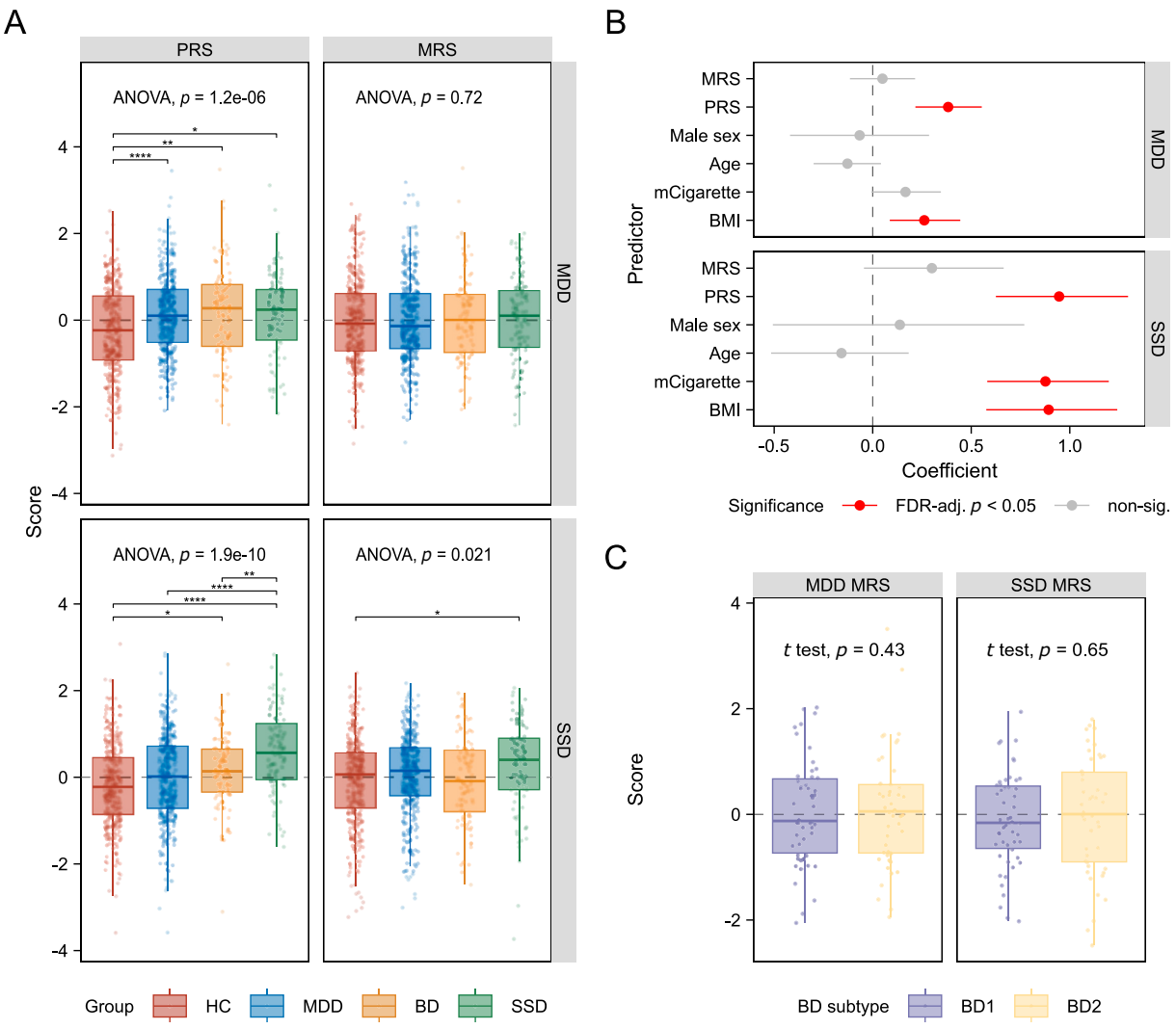

Fig. 1. MRS analysis.
(A) Distribution of polygenic and methylation risk scores for MDD and SSD within different diagnostic groups. Asterisks above pairs of groups indicate significant differences identified via Tukey's Honest Significant Difference post hoc test conducted in case of a p value <0.05 in the ANOVA. (B) Logistic regression of MDD MRS and MDD PRS on MDD case-control status (top) and SSD MRS and SSD PRS on SSD case-control status (bottom), with sex, age (in years with two decimal places), BMI, and the mCigarette score included as covariates. Numerical variables were z-scaled to mean of zero and standard deviation of one prior to fitting the regression models. *P* values of regression coefficients were adjusted for the number of predictors in the respective model using the Benjamini-Hochberg procedure for controlling the false discovery rate. (C) MDD and SSD MRS in cases with a diagnosis of BD type 1 (BD1, $n = 50$) and type 2 (BD2, $n = 46$). ANOVA, analysis of variance; BD, bipolar disorder; BMI, body mass index; FDR-adj., false discovery rate adjusted; HC, healthy controls; MDD, major depressive disorder; MRS, methylation risk score; non-sig., non-significant; PRS, polygenic risk score; SSD, schizophrenia spectrum disorders; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Similarly, pairwise correlations between the three epigenetic age estimates ranged between 0.91 and 0.93 (Supplementary Fig. 15). For the DNAm Age based on the Skin & Blood clock, no significant difference in epigenetic age acceleration was observed between diagnostic groups, whereas a highly significant increase in DNAm GrimAge acceleration was evident in BD and SSD compared to MDD and HC (Fig. 2B). For the DNAm PhenoAge, epigenetic age acceleration was significantly increased in MDD and SSD compared to HC. When adjusting for sex, smoking behavior, and BMI in a multivariable regression model, only an effect of SSD case status compared to HC on DNAm PhenoAge acceleration was observed (Fig. 2C). However, with additional adjustment for cell type composition, no significant effects of diagnostic group on epigenetic age acceleration remained (Supplementary Figs. 16, 17).

The brain age, which was estimated on the basis of neuroimaging data, showed high correlations with epigenetic age estimates for all three epigenetic clocks ($R \geq 0.86$, Supplementary Fig. 18 A). However, no correlation of the brain age gap with epigenetic age acceleration was observed (Supplementary Fig. 18B). There was no evidence for differences in the brain age gap between diagnostic groups (Supplementary Fig. 18C).

In the longitudinal analysis of epigenetic aging in MDD compared to HC, the slope of epigenetic aging between baseline and follow-up study visit (Supplementary Fig. 19 A) was significantly increased for the DNAm GrimAge estimates in MDD (*t*-test $p = 0.001$, Supplementary Fig. 19B). When adjusting for the effects of baseline age, smoking, and BMI, this effect did not remain significant (Supplementary Fig. 19C).

4. Discussion

The present study involved the epigenetic characterization of a subset of the transdiagnostic FOR2107 sample, which comprised individuals with a lifetime diagnosis of MDD ($n = 337$), BD ($n = 96$), or SSD ($n = 95$), as well as HC ($n = 326$). In the main case-control and case-case MWAS, only few CpG sites with methylome-wide significant

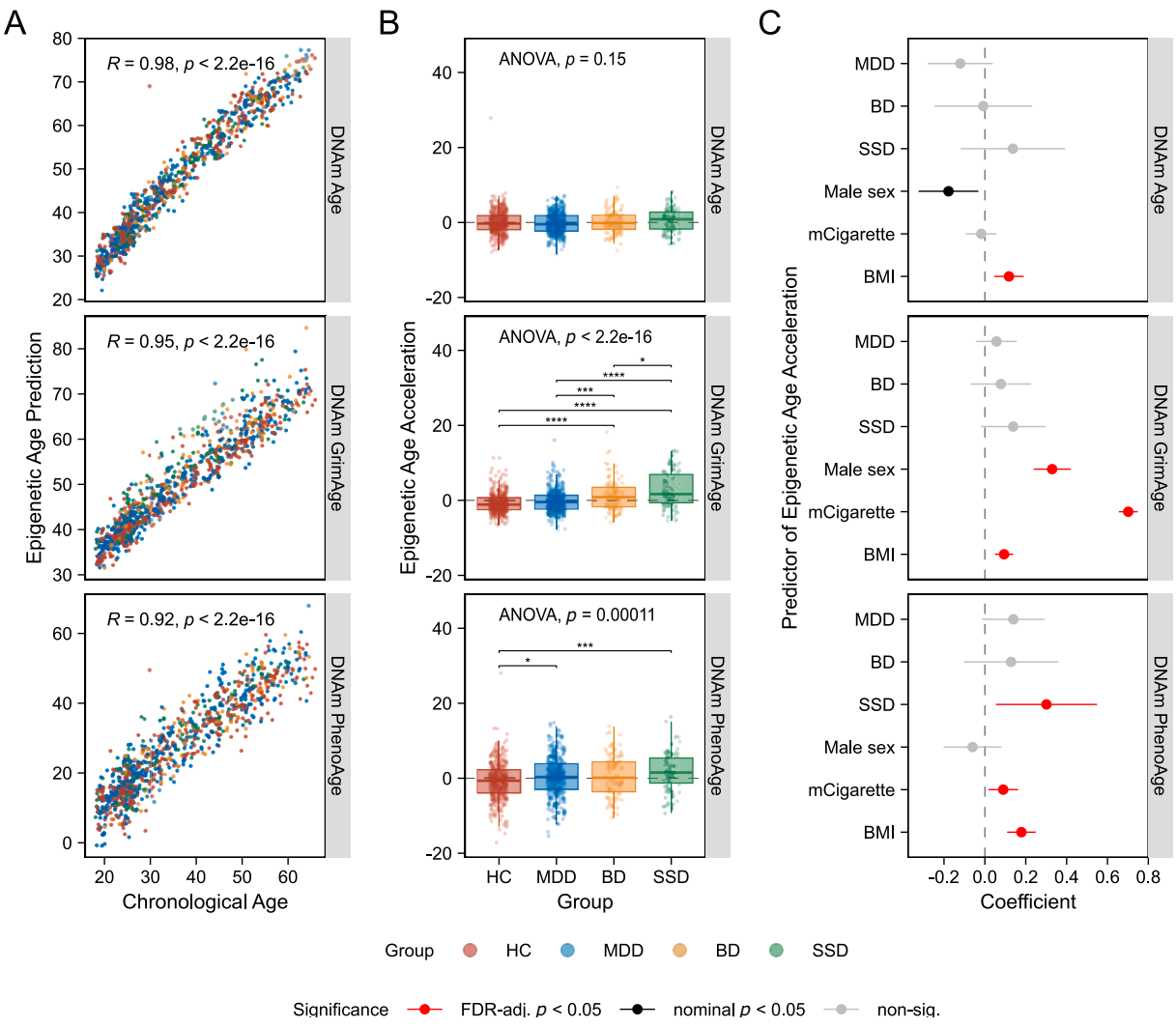

Fig. 2. Epigenetic age acceleration.
(A) For all three epigenetic clocks, the estimated epigenetic age was highly correlated with chronological age. (B) Significant differences in epigenetic age acceleration between diagnostic groups were identified for the DNAm GrimAge and DNAm PhenoAge. Asterisks above pairs of groups indicate significant differences identified via Tukey's Honest Significant Difference post hoc test conducted in case of a *p* value <0.05 in the ANOVA. (C) When adjusting for sex, the mCigarette score as proxy of smoking behavior, and BMI in a multivariable regression, the only remaining significant effect of a diagnosis on epigenetic age acceleration in comparison to HC was of SSD case status on DNAm PhenoAge acceleration. Numerical variables were z-scaled to mean of zero and standard deviation of one prior to fitting the regression models. *P* values of regression coefficients were adjusted for the number of predictors in the respective model using the Benjamini-Hochberg procedure for controlling the false discovery rate. ANOVA, analysis of variance; BD, bipolar disorder; BMI, body mass index; DNAm, DNA methylation; FDR-adj., false discovery rate adjusted; HC, healthy controls; MDD, major depressive disorder; non-sig., non-significant; *R*, Pearson correlation coefficient; SSD, schizophrenia spectrum disorders; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

differential methylation were identified. However, MWAS of smoking status and BMI demonstrated good data quality, and power calculations proved that the sample sizes were sufficient to detect large effects. In line with previous research, this indicates that blood-based DNAm profiles in MDD, BD, and SSD may not harbor uniform trait-related signals of large effect sizes, neither within nor across diagnostic boundaries.

The present findings are consistent, for example, with a previous study on DNAm in MDD by Barbu et al. [13], in which only a limited predictive value of blood-based DNAm profiles in regard to psychiatric diagnosis could be demonstrated. Importantly, signals of small effect size might still be present in blood-based DNAm profiles of individuals with affective and psychotic disorders, and more distinctive DNAm profiles may exist within disorder subtypes. In addition to increasing the total sample size, future studies may thus also need to focus on more homogeneous subgroups, e.g., with regards to symptom profiles, in order to identify stronger association signals [76].

Two out of the five CpG sites significantly associated with case-control or case-case status in the main MWAS mapped to protein-coding genes, namely *ST3GAL2* (annotated to cg11224906, significant difference between SSD and HC) and *HACD4* (annotated to cg27396293, significant difference between SSD and BD). Interestingly, *ST3GAL2* has previously been implicated in the context of schizophrenia based on transcriptional and proteomic signatures [77]. The protein encoded by *ST3GAL2*, a beta-galactoside alpha2–3 sialyltransferase, is involved in the sialylation of gangliosides and glycoproteins. While sialyation plays a role in a wide range of physiological and pathophysiological contexts, it appears to be of particular relevance to neuronal development and plasticity [78–81]. For *HACD4*, which encodes the enzyme 3-hydroxyacyl-CoA dehydratase 4 involved in fatty acid elongation pathways, no apparent functional link to affective or psychotic disorders could be identified.

Compared to the case-control and case-case MWAS, the MWAS of the number of depressive episodes and hospitalizations in MDD, conducted in the longitudinally measured subsample, revealed a slightly higher

number of CpG sites with significant differential methylation unrelated to smoking and BMI. Among the genes annotated to the 19 and 37 CpG sites significantly associated with the number of depressive episodes and number of hospitalizations, respectively, multiple genes were previously described to be involved in neurobiological processes or to be associated with psychiatric conditions. This includes *CRYBB2* [82–84], *GAS7* [85,86], and *HOXB3* [14] (DNAm associated with number of depressive episodes) as well as *DLGAP4* [87], *MRGPRF* [88,89], and *VAMP2* [90–92] (DNAm associated with number of hospitalizations). However, as no significant gene set enrichment was found in the present analyses, no global functional interpretation of these findings can be made at present.

Consistent with the limited findings from the case-control MWAS, the MRS analysis showed no significant effect of the MDD MRS on MDD case-control status, and only a weak effect of the SSD MRS on SSD case-control status, which did not remain significant when adjusting for smoking and BMI. In contrast, the genetic risk measured by PRS for MDD and SSD was significantly increased in cases compared to controls, respectively, attesting the validity of the diagnostic groupings in our sample. Given that sample sizes in the DNAm discovery studies employed by Barbu et al. [13] and Hannon et al. [15] were below 5000 individuals, while GWAS sample sizes have been well in excess of 100,000, this might be one explanation for the pronounced difference in variance explained between the respective disorder-specific PRS and MRS. Although it is unclear at present whether MRS may ever reach the predictive power of PRS, their predictive power might increase with growing discovery MWAS sample sizes via meta-analytical efforts. In the context of MDD, for example, a case-control MWAS meta-analysis is currently being conducted within the Psychiatric Genomics Consortium (see preprint by Shen et al. [93]), to which we are contributing with our data. Other possible explanations for the limited amount of variance explained by MRS include a lack of stability of disorder-associated methylation differences over time, insufficient generalizability of the DNAm profiles detected in the discovery studies to other samples, and a general absence of distinctive DNAm profiles associated with psychiatric diagnoses in peripheral blood. Concerning the similarity of DNAm profiles between BD type 1 and SSD or between BD type 2 and MDD, the currently available MRS preclude any conclusions, as the absence of observable differences might be due to the limited predictive power of the MRS and the limited target sample size.

Based on the results of the supplementary MWAS, the indirect contribution of the lifestyle-related factors smoking behavior and BMI to DNAm profiles observable in affective and psychotic disorders was strikingly apparent. This is consistent with previous knowledge on the relation between the investigated disorders and smoking/BMI and the effect of smoking/BMI on DNAm. While these indirectly disorder-associated profiles per se may not be diagnostically informative, they provide important clinical information on modifiable risk factors that might contribute to the increase in mortality [94]. The challenge of distinguishing lifestyle-related from directly disorder-associated signatures in MWAS highlights the need for comprehensively phenotyped samples, in which systematic differences between groups can be uncovered. The effect of exogenous factors on DNAm also relates to the epigenetic age acceleration observed in the DNAm GrimAge and PhenoAge model, since smoking behavior, for example, is known to increase mortality and was explicitly included in the design of the DNAm GrimAge model [28]. Our results are consistent with previous findings of epigenetic age acceleration in affective and psychotic disorders captured by second-generation epigenetic clocks [29,33–36,39,40] and emphasize that all contributions to an association signal should be dissected in order to derive insights of potential clinical relevance. Of note, while we did not observe a difference in epigenetic aging between any of the diagnostic groups based on the first-generation Skin & Blood clock, some previous studies had suggested a decrease in epigenetic aging in schizophrenia compared to controls [95,96] based on Horvath's first-generation multi-tissue clock [97]. However, almost 10 % of the training data for Horvath's multi-tissue clock consisted of individuals with schizophrenia (due to the inclusion of the publicly available schizophrenia case-control datasets GSE41037 and GSE41169), which diminishes the utility of this multi-tissue clock for the detection of SSD-related differences [98]. Moreover, Wu et al. [95] had also included GSE41037 and GSE41169 in their study sample, leading to considerable sample overlap between training and test data. Therefore, we do not consider our results on epigenetic age acceleration in SSD to be in contrast to prior evidence. The absence of correlations between estimates of epigenetic and brain age acceleration in the present FOR2107 sample is in line with a study by Teeuw et al. [30], and thus provides additional evidence that blood-based epigenetic clocks and brain age models might not capture equivalent aspects of biological aging.

The present study had four main limitations, which must be considered when interpreting the results. First, in the context of psychiatric disorders, blood represents only a surrogate tissue, and may not fully reflect any disorder-related DNAm profiles that are present in brain tissue. This, however, does not rule out the potential value of blood-based DNAm profiles as clinical biomarkers in psychiatry, and tools such as BECon [99], designed for interpreting DNAm findings from blood in the context of brain, may be used to generate hypotheses about causal mechanisms. Second, these initial analyses did not consider more specific factors, such as medication effects. Since previous research has suggested the existence of DNAm changes secondary to pharmacotherapy [100], this should be taken into account in future studies. Third, the present sample size was limited. If alterations in blood-based DNAm profiles do exist, but have only small effect sizes at the level of individual CpG sites, larger sample sizes are needed to obtain the statistical power that is required to detect these effects. Finally, it is conceivable that the mood state influenced the methylation results of the BD group. Due to the limited sample size, the present study did not conduct additional analyses in BD with regard to mood state. Future studies with larger samples should systematically investigate the association of mood state (e.g. euthymic, depressive, (hypo-)manic and mixed) with DNA methylation profiles.

5. Conclusion

The present study has added further evidence that no large-scale differences in blood-based DNAm profiles are directly associated with affective and psychotic disorders. Moreover, our study has confirmed that the disorder-associated DNAm signatures are strongly influenced by lifestyle-associated factors, in particular smoking behavior and BMI. Beyond the first line of analyses presented in this study, the generated DNAm dataset – in combination with the deep phenotype, genotype, and MRI data as well as other omics layers available for the FOR2107 cohort — represents a valuable resource for future multimodal studies and international meta-analyses of DNAm profiles in MDD, BD, and SSD.

CRediT authorship contribution statement

Friederike S. David: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Josef Frank:** Writing – review & editing, Resources, Methodology. **Frederike Stein:** Writing – review & editing, Resources, Data curation. **Susanne Meinert:** Writing – review & editing, Resources, Data curation. **Lea Zillich:** Writing – review & editing, Resources. **Lea Sirignano:** Writing – review & editing, Resources. **Fabian Streit:** Writing – review & editing, Resources. **Eva C. Beins:** Writing – review & editing, Investigation. **Lisa Sindermann:** Writing – review & editing, Investigation. **Paula Usemann:** Writing – review & editing, Resources, Investigation. **Janik Goltermann:** Writing – review & editing, Resources. **Elisabeth J. Leehr:** Writing – review & editing, Resources. **Sugirthan Sivalingam:** Writing – review & editing, Resources. **Stefan Herms:** Writing – review & editing, Resources. **Per Hoffmann:** Writing – review & editing,

Resources. **Tim Hahn:** Writing – review & editing, Resources, Funding acquisition. **Stephanie H. Witt:** Writing – review & editing, Resources, Funding acquisition. **Nina Alexander:** Writing – review & editing, Resources. **Tilo Kircher:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Udo Dannlowski:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Marcella Rietschel:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Markus M. Nöthen:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Andreas J. Forstner:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tim Hahn, Stephanie Witt, Tilo Kircher, Udo Dannlowski, Marcella Rietschel, Markus Nöthen, and Andreas Forstner report financial support was provided by German Research Foundation. Per Hoffmann reports a relationship with Life & Brain GmbH that includes: employment. Markus Nöthen reports a relationship with Life & Brain GmbH that includes: board membership, employment, and equity or stocks. Per Hoffmann reports a relationship with HMG Systems Engineering GmbH that includes: consulting or advisory. Per Hoffmann reports a relationship with Illumina Inc. that includes: speaking and lecture fees. Per Hoffmann reports a relationship with Beckman Coulter Inc. that includes: speaking and lecture fees. Markus Nöthen reports a relationship with HMG Systems Engineering GmbH that includes: board membership. Markus Nöthen reports a relationship with Deutsches Ärzteblatt that includes: board membership. Tilo Kircher reports a relationship with Servier that includes: funding grants. Tilo Kircher reports a relationship with Janssen that includes: funding grants. Tilo Kircher reports a relationship with Recordati that includes: funding grants. Tilo Kircher reports a relationship with Aristo that includes: funding grants. Tilo Kircher reports a relationship with Otsuka that includes: funding grants. Tilo Kircher reports a relationship with neuraxpharm that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.comppsych.2025.152629.

Data availability

The data presented in this study are available on reasonable request from the corresponding author. The data are not publicly available due to ethical and data protection considerations.

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