Dynamic Epigenetic Changes During Antidepressant Pharmacotherapy in Major Depressive Disorder

Svenja Müller[1,2,3], Josef Frank[2], Eric Zillich[2], Fabian Streit[2,4,5], Nicolai Koch[2], Miriam A. Schiele[6], Katharina Domschke[6,7], Andre Tadic[8,9], David P. Herzog[8,10], Stefanie Wagner[8], Marianne B. Müller[8,10], Giulia Treccani[11], Nils C. Gassen[12], Marcella Rietschel[2], Stephanie H. Witt[2,5,13], Klaus Lieb[8], Jan Engelmann[8,10*], Lea Zillich[2,6*]

[1] Department of Ergonomics, Leibniz Research Centre for Working Environment and Human Factors (IfADo), Dortmund, Germany

[2] Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.

[3] German Center for Mental Health (DZPG), partner site Bochum/Marburg, Germany

[4] Hector Institute for Artificial Intelligence in Psychiatry (HITKIP), Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

[5] German Center for Mental Health (DZPG), partner site Mannheim/Heidelberg/Ulm, Germany

[6] Department of Psychiatry and Psychotherapy, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany.

[7] German Center for Mental Health (DZPG), Partner Site Berlin/Potsdam, Berlin, Germany

[8] Department of Psychiatry and Psychotherapy, University Medical Center, Mainz, Germany

[9] Department of Psychiatry, Psychotherapy and Psychosomatics, Dr. Fontheim Mentale Gesundheit, Liebenburg, Germany.

[10] Translational Psychiatry, Department of Psychiatry and Psychotherapy & Focus Program Translational Neuroscience, University Medical Center, Mainz, Germany.

[11] Department of Systemic Neuroscience Institute of Anatomy and Cell Biology, Philipps University Marburg, Marburg, Germany.

[12] Neurohomeostasis Research Group, Department of Psychiatry and Psychotherapy, University Hospital Bonn, Bonn, Germany.

[13] Center for Innovative Psychiatric and Psychotherapeutic Research, Biobank, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

*Shared senior authorship: Jan Engelmann, Lea Zillich

Corresponding authors: Jan Engelmann; Lea Zillich

Abstract

Background: Although antidepressants remain the main pharmacological treatment for major depressive disorder (MDD), therapeutic response is highly variable, highlighting the need for reliable markers that predict and mechanistically explain treatment outcomes. Previous candidate-gene and cross-sectional epigenome-wide association studies (EWASs) have suggested DNA methylation signatures associated with antidepressant response; however, findings are inconsistent. To date, no longitudinal EWAS has systematically examined dynamic methylation changes across multiple time points during antidepressant treatment to identify early epigenetic markers of response.

Methods: Within the Early Medication Change trial, DNA methylation data was generated for 162 patients with MDD (81 responders, 81 non-responders) and 48 matched healthy controls. Patient samples were collected at four time points during eight weeks of standardized antidepressant treatment, and controls were assessed twice. Longitudinal EWASs were performed to identify differentially methylated positions (DMPs) and differentially methylated regions (DMRs) were identified using the comb-p algorithm. **Results:** Baseline methylation was associated with treatment outcome at day 56 through six DMRs (e.g., *TNRC6C*, *CAT*, *CCNY*), whereas no DMP reached significance. Longitudinal analyses identified one DMP linked to clinical improvement (*YLMP1*) and three DMRs with time effects (*GPR126, C20orf54*, *PM20D1)*. Combined patient-control analyses revealed additional DMPs and DMRs associated with diagnosis and temporal changes.

Conclusions: This study provides the first longitudinal evidence of dynamic, regionally coordinated methylation changes during antidepressant pharmacotherapy in MDD. These alterations occur in genes involved in neuroplasticity and inflammation and are linked to clinical response. Defining such methylation signatures as predictive or mechanistic markers could advance personalized treatment strategies for MDD, though replication and functional validation in larger cohorts remain essential.

Introduction

Major depressive disorder (MDD) affects over 300 million people globally and is a leading cause of disability worldwide (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018; Patel et al., 2016; Streit et al., 2023). Pharmacological treatment with antidepressants is recommended for moderate to severe episodes (APA, 2019; BÄK, 2022; NICE, 2022). However, treatment response is highly variable, often necessitating medication adjustments (Cipriani et al., 2018; Iniesta et al., 2016). Investigating the biological changes associated with antidepressant response may help clarify the underlying mechanisms of both depression and treatment efficacy, thereby facilitating the identification of novel therapeutic targets with improved efficacy.

MDD is increasingly understood as a disorder of dysregulated neuroplasticity rather than solely of neurotransmitter imbalance (Ferrarelli, 2022; Harmer, Duman, & Cowen, 2017). Research has emphasized the critical role of intracellular signaling, gene expression, and protein translation as key mediators of antidepressant action (Harmer et al., 2017). Antidepressants promote synaptic connectivity within and between neuronal networks that regulate depression-related behaviours (Price & Duman, 2020). Within this framework, epigenetic modification, particularly DNA methylation, has emerged as a crucial mechanism integrating genetic predisposition and environmental factors (e.g. medication) to modulate neuroplasticity, stress responsiveness and emotional regulation (Kanherkar, Bhatia-Dey, & Csoka, 2014; Klengel & Binder, 2015; O'Donnell & Meaney, 2020). Traditionally regarded as stable genomic marks, epigenetic modifications are now recognized as highly dynamic processes. These rapid epigenetic changes can occur even within minutes to hours, supporting short-term adaptations in neuronal function and highlighting their crucial role in experience-dependent plasticity (Herre & Korb, 2019). This underlines the potential role of DNA methylation as a marker of antidepressant response. Although DNA methylation is highly tissue-specific, peripheral DNA methylation can serve as a correlate for brain-related processes (Braun et al., 2019; Edgar et al., 2017) and is ideally suited for clinical translation.

Previous epigenetic investigations of antidepressant treatment response mainly focused on selected target genes like *BDNF, 5-HTT* and *FKBP5 (e.g.* Domschke et al., 2014; Gassen et al., 2015; Miller et al., 2013; Schiele et al., 2021; Tadić et al., 2014), or the serotonergic and noradrenergic pathways (see reviews: Dahrendorff, Currier, & Uddin, 2024; Webb et al., 2020). Beyond targeted candidate approaches, our group and others have conducted pre-treatment, single-time-point epigenome-wide association studies (EWAS) to explore predictive markers of pharmacological antidepressant treatment response. Among others, Ju

et al. identified several CpG sites differentially methylated between responders (N = 82) and non-responders (N = 95) after eight weeks of treatment, including a DMP within the *CHN2* gene that showed the strongest association with mRNA expression (Ju et al., 2019). In our EWAS comparing responders and non-responders (N = 40 each), no epigenome-wide significant DMPs were detected, but 20 DMRs were found near genes such as *SORBS2* and *CYP2C18* (Engelmann et al., 2022). Recently, Schiele et al. (Schiele et al., 2024) reported no genome-wide significant findings after six weeks of antidepressant treatment (N = 230), though suggestive associations emerged at CpG sites linked to genes including *TIMP2, VDAC1, and SORL1*. Despite the dynamic nature of DNA methylation patterns and evidence that antidepressants may exert their effects by modifying these epigenetic patterns (Lisoway et al., 2018; Vialou et al., 2013), longitudinal studies are rare and have only been conducted on candidate genes (Bruzzone et al., 2024; Kang et al., 2019). To date, no longitudinal, genome-wide studies have examined DNA methylation changes specifically in relation to antidepressant treatment response. Regarding psychotherapeutic treatment, recent research on trauma-focused therapies, comprising cognitive-behavioral therapy or Eye Movement Desensitization and Reprocessing (EMDR), reported 110 DMRs following treatment in individuals with treatment-resistant depression (Carvalho Silva et al., 2024). So far, a limited number of studies have investigated other non-pharmacological treatment regimes. For instance, Sirignano et al (Sirignano et al., 2021) identified a single differentially methylated site within *TNKS* associated with response to electroconvulsive therapy. However, to the best of our knowledge, there are currently no studies that have systematically and longitudinally investigated epigenome-wide methylation changes throughout pharmacological antidepressant therapy, encompassing the critical phases from treatment initiation to early response and eventual clinical remission.

To address this gap in the literature, we performed a comprehensive analysis of epigenetic alterations associated with antidepressant treatment response. We compared epigenetic profiles among responders, age- and sex-matched non-responders, and matched healthy controls by assessing differential DNA methylation prior to treatment initiation and at three additional time points throughout an eight-weeks standardized antidepressant treatment. All participants were recruited from the large, well-characterized Early Medication Change (EMC) cohort (Tadić et al., 2010, 2016). This longitudinal design allowed us to evaluate the predictive value of baseline methylation profiles for treatment outcome, and to capture dynamic changes in methylation patterns during treatment. By capturing both initial epigenetic states and their trajectories (measured three times after baseline), we aimed to identify markers reflecting treatment outcome. Additionally, comparing patients' methylation patterns to healthy controls (assessed twice) allowed the assessment of methylation

differences beyond treatment effects. Our study provides comprehensive insights into molecular signatures associated with antidepressant response, thereby advancing the discovery of biomarkers and precision medicine approaches for the complex treatment of MDD.

Methods and Materials

Study cohort

This investigation is a secondary analysis of 162 MDD patients from the 'Randomised Clinical Trial Comparing an Early Medication Change (EMC) Strategy with Treatment as Usual (TAU) in MDD' (ClinicalTrials.gov, NCT00974155). Out of 889 patients originally enrolled (between 2009 and 2014), genetic data was available for 560 (Müller et al., 2024) Of these, 81 responders and matched non-responders were selected based on their treatment response at day 28. Responders showed at least a 50% improvement in depressive symptoms, as measured by the 17-item Hamilton Rating Scale for Depression (HAMD-17), after four weeks of treatment according to study protocol. In addition, 48 sex and age-matched healthy controls were available and included in this investigation (Wagner et al., 2018). The detailed study design is shown in Fig. 1.

The EMC trial, conducted between 2009 and 2014, was a multicenter, randomized controlled clinical trial designed to evaluate whether patients who do not show improvement after 14 days of antidepressant treatment with escitalopram benefit from an early medication change. In this protocol, non-responders at day 14 were transitioned to venlafaxine. Those who continued to show no response at day 28 received an augmentation with lithium. This accelerated treatment strategy was compared against patients treated according to current guideline recommendations (treatment as usual; TAU), in which escitalopram was continued for an additional two weeks, and patients were only switched to venlafaxine in the case of non-response from day 29 onwards. All participants provided written informed consent after a complete and extensive description. The study was approved by the ethics committee of the Landesärztekammer Rheinland-Pfalz and was conducted in accordance with the Declaration of Helsinki. Details of the study protocol and the inclusion/exclusion criteria have been reported elsewhere (Tadić et al., 2010, 2016). The controls were recruited as part of a subproject of the EMC trial through posters in the study center.

Study procedures

Diagnoses were verified at screening using the German versions of the Mini International Neuropsychiatric Interview (M.I.N.I.) and the Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II). Socio-demographic and clinical data were obtained via patients‘ self-report. Depression severity was assessed weekly from baseline to day 56 using the HAMD17 by trained and blinded raters. If necessary, antidepressant premedication was washed out after inclusion and before the baseline visit. According to the study protocol, the antidepressant treatment was 20 mg of escitalopram from baseline to day 14 in all participants, followed by a predefined treatment algorithm. Other medications were administered to treat MDD-associated symptoms (e.g. insomnia) or adverse drug reactions (e.g. agitation or anxiety). Medication for the treatment of somatic comorbidities was continued unchanged.

- Figure 1 -

Quantification of DNA methylation

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). Blood samples were collected in fasting patients in the morning before medication intake at baseline (prior to start of study medication) and at day 14, 28 and 56 (see Fig. 1). For control participants, samples were obtained at baseline and after 28 days. All extracted DNA samples were stored at −20 °C until further processing. Responders and non-responders were matched by age and sex, and DNA from these matched pairs, along with samples from controls, was randomized and pipetted onto processing plates. A total of 500 ng of genomic DNA per sample was subjected to bisulfite conversion using the EZ-96 DNA Methylation-Gold Kit (Zymo Research, Irvine, USA). Genome-wide DNA methylation profiling was performed using the Infinium HumanMethylationEPIC BeadChip array, with scanning conducted on the Illumina HiScan system (Illumina, San Diego, CA) at the Genome Analysis Center at the Helmholtz Institute München.

Data preprocessing, QC, and filtering

All analyses were conducted using R version 4.4.1. (R Core Team, 2024) Methylation data were extracted from raw signal intensity files using an updated version of the CPACOR pipeline (Lehne et al., 2015, 2016). Standard sample- and probe-level quality control procedures were applied, including the exclusion of samples failing QC and probes with low reliability. After preprocessing, 164 patients (with baseline and at least one follow-up time point) and 51 control participants (43 of whom had data at both time points) were retained for analysis. A total of 642,148 CpG sites passed QC and remained for downstream analyses. Detailed preprocessing steps and QC thresholds are provided in Supplementary Information S1.

To account for technical variability and batch effects, principal component analysis (PCA) was performed on the internal control probes of the EPIC array. The first four principal components were included as covariates in all subsequent analyses. Cell-type heterogeneity was estimated using a reference-based deconvolution approach based on the method of (Houseman et al., 2012), which provides six proportional estimates summing approximately to one. To avoid multicollinearity in the EWAS, variance inflation factors were calculated for each estimate, and the granulocyte proportion - identified as contributing to collinearity - was excluded from further models.

Covariates

All analyses were adjusted for relevant covariates, including sex, age, smoking status (approximated via DNA methylation at *AHRR* cg05575921; Zeilinger et al., 2013), and body mass index (BMI). For two individuals with missing BMI values, group- and sex-specific mean imputation was applied. To control for cellular heterogeneity, estimated proportions of major blood cell types (CD8+ T cells, CD4+ T cells, monocytes, B cells, and NK cells) were included. In addition, two genotype-based principal components (PCs) were added to account for population stratification, and four control probe PCs were included to correct for batch effects. Samples lacking genotype information or excluded during genotype quality control (n = 2 patients, n = 3 controls) were removed from analyses. For details on genotype quality control, see Supplementary information S2. To evaluate the relative contribution of each covariate to the variance in DNA methylation and to justify their inclusion in downstream models, we performed variance partitioning using linear mixed models (implemented in the *variancePartition* R package; Hoffman & Schadt, 2016).

Epigenome-wide association study

The final dataset, comprising 162 patients and 48 controls after preprocessing and QC, was analyzed using linear models (LM) for the cross-sectional methylation analysis and linear mixed models (LMMs) implemented in the lme4 R package (Bates et al., 2015) for the longitudinal methylation analyses. Three separate models were used to address distinct research questions. All models accounted for symptom severity at baseline. Model 1 achieved this by including baseline HAMD, whereas Models 2 and 3 incorporated HAMD as a time-varying predictor. Model 1 used linear regression to predict HAMD scores at the final time point (day 56) based on methylation levels and HAMD scores at baseline. This approach (including 642,148 sites) allowed us to assess whether early methylation patterns and symptom severity could predict later clinical outcomes. Multiple testing correction was applied using the False Discovery Rate (FDR) approach according to Benjamini and Hochberg (Benjamini & Hochberg, 1995) and the resulting values are reported as q-values. CpG sites were annotated according to the manufacturer's manifest (Illumina MethylationEPIC v1 B5). Model 2 focused on patients (n=162) and included four time points. The model assessed the association between DNA methylation and symptom severity, while accounting for within-subject correlation across time via random intercepts. For each CpG site, the effects of time (day 14, day 28, and day 56 relative to baseline) and HAMD score (continuous) were estimated. Estimated marginal means (EMMs) were computed using the *emmeans* R package (Lenth et al., 2023) to derive average methylation levels at each time point. After model singularity checks, 606,546 CpG sites remained for this analysis. Model 3 examined longitudinal methylation changes from baseline to day 28 in patients (n = 162) and controls (n = 48). For each CpG site, the effects of time, HAMD score (continuous), and diagnostic group (patients vs. controls) were estimated. After model singularity checks, 562,713 CpG sites remained for this analysis. To assess bias and inflation in test statistics across all models, we calculated quantile-quantile (QQ) plots and estimated the inflation factor lambda (λ). Bacon correction using the Bayesian method implemented in the *bacon* R package (van Iterson et al., 2017), was applied for model 3 comparing patients and controls, where λ was elevated (λ = 1.55). Full methodological details of QC procedures and bacon correction are provided in the Supplementary Information S3.

Downstream analyses

Differentially methylated regions (DMRs) were identified using the comb-p function from the *ENmix* R package (Xu et al., 2016). This approach accounts for spatial autocorrelation among neighboring CpG sites by aggregating adjacent significant sites into regions of methylation enrichment (Pedersen et al., 2012). A sliding window was applied with a maximum inter-CpG distance of 750 base pairs and a bin size of 500 base pairs. Regions were initiated using a seed p-value threshold of 0.001. Multiple testing correction across identified regions was performed using the Sidak method. Only DMRs containing at least two CpG sites were retained for downstream analysis.

Gene set enrichment analysis was conducted using the gometh function from the *missMethyl* R package to identify overrepresented Gene Ontology (GO) biological processes among differentially methylated CpG sites (Phipson, Maksimovic, & Oshlack, 2016). The analysis was based on CpG sites with a nominal p-value < 0.001. The full set of tested CpG sites was used as the background, and the Illumina EPIC array annotation was specified.

Assessment of overlap with previously reported DMRs

To evaluate whether DMRs identified in our previous study (Engelmann et al., 2022) were also implicated in the current analysis, we compared the genomic positions of nominally significant DMPs and DMRs from all analyses in the present dataset with the coordinates of previously identified DMRs. CpG sites were considered overlapping if their positions fell within the boundaries of any previously identified DMR. DMRs in the present dataset were assessed for complete or partial overlap with prior DMRs.

Results

Sample characteristics

The clinical sample comprised 49.4% women, with a mean age of 41.7 years (SD = 11.4). Treatment responders were more likely to present with a first depressive episode, whereas non-responders had a higher prevalence of recurrent depression (p = 0.013). Detailed sociodemographic and clinical characteristics are presented in Table 1. Responder and non-responder groups showed significant differences in symptom severity at all time points, with the smallest difference observed at baseline and progressively larger differences at day 14,

day 28, and day 56 (see Figure 1). The control group consisted of 60.4% women, with a mean age of 38.3 years (SD = 12.8), a mean BMI of 23.7 (SD = 3.7), and 45.8% reported current smoking. Among sociodemographic variables, the only significant difference between patients and controls was BMI, which was higher in patients ($p < 0.001$).

Table 1. Clinical and sociodemographic data

	Total (n = 162)	Responders (n = 81)	Non-Responders (n = 81)	p-value (group comparison)
Age - yrs (SD)	41.71 (11.43)	41.79 (11.28)	41.63 (11.66)	0.929[a]
Sex female (%)	80 (49.4 %)	41 (50.6 %)	39 (48.1 %)	0.875[b]
BMI (SD)	27.04 (6.5)	26.47 (6.25)	27.62 (6.25)	0.262[a]
Smoking – yes (%)	50 (31.3 %)	30 (37.0 %)	20 (25.3 %)	0.153[b]
Age at onset – yrs (SD)	33.65 (12.58)	34.9 (12.37)	32.4 (12.7)	0.206[a]
Duration of current episode – weeks (SD)	32.29 (39.69)	34.15 (38.63)	30.43 (40.88)	0.553[a]
Course of disorder (%)				**0.013[b]**
First episode	56 (34.6 %)	36 (44.4 %)	20 (24.7 %)	
Recurrent	106 (65.4 %)	45 (55.6 %)	61 (75.3 %)	

Note: Continuous measures are presented as mean (standard deviation) and categorical measures as frequency (percent). M mean, SD standard deviation, yrs years. Response was defined as a decrease in HAMD17 from baseline to day 28 of ≥50 %; a=*t*-Test (or Welch test in case of variance inequality); b=$\chi 2$ test.

Baseline methylation associated with treatment outcome

In the model predicting treatment outcome at day 56, no epigenome-wide significant DMP emerged after FDR-correction for multiple testing. The regression coefficients corresponding to the 100 DMPs most strongly associated with treatment outcome are provided in Supplementary Table S1. Using regional analysis, we identified six DMRs whose methylation levels were associated with treatment outcome at day 56. The DMR showing the strongest (positive) association with treatment outcome was annotated to *Trinucleotide Repeat Containing 6C* (*TNRC6C)*, a protein-coding gene. Table 2 lists the DMRs and Fig. 2 depicts the Manhattan plot of this analysis with DMRs highlighted in green.

Table 2. Differentially methylated regions associated with treatment outcome after eight weeks of treatment.

chr	start	end	p	sidak p	N probe	Gene	Direction
17	76037035	76037563	6.97E-13	8.48E-10	6	*TNRC6C*	+
11	34460107	34460558	2.10E-11	2.99E-08	10	*CAT*	+
16	1583620	1584119	4.30E-09	5.53E-06	8	*TMEM204; IFT140*	+
10	81967195	81967667	1.74E-08	2.36E-05	7		+
10	35624244	35624602	2.61E-08	4.69E-05	4	*CCNY*	+
5	171057078	171057283	1.37E-05	0.0421	3		+

Note: Chr chromosome; + indicates that increased baseline methylation was associated with higher HAMD scores at day 56

- Figure 2 -

Longitudinal DNA methylation changes and associations with symptom improvement in patients

The linear-mixed model examined the effects of time (day 14, day 28, and day 56 relative to baseline) and continuous symptom change across the eight-week treatment period. Although the original sample was selected based on matched responder and non-responder groups, the model assessed longitudinal changes in continuous HAMD across all patients, thereby leveraging the repeated-measures design. A single significant DMP (cg15731816) associated with HAMD score was annotated to *YLP Motif Containing 1* (*YLPM1;* β = 0.0005, p = 1.25E-08, q = 0.008), with the positive beta indicating that increased methylation levels corresponded to higher HAMD scores over time. Importantly, over the course of treatment, both methylation at this site and HAMD scores generally decreased, suggesting that reductions in methylation were linked to clinical improvement. Figure 3 presents the volcano plot for symptom change, while Supplementary Figures S1-3 display the volcano plots for the effects observed for the different time points. The regression coefficients corresponding to the 100 DMPs most strongly associated with symptom change and the different time points are provided in Supplementary Table S2-5, respectively. Regional analyses revealed three DMRs in which methylation levels changed significantly over the course of treatment

from baseline to day 28, and one DMR from baseline to day 56. Of note, the same DMR – comprising five CpG sites – was detected at both day 28 and day 56 and mapped to *G protein-coupled receptor 126* (*GPR126)*, a protein-coding gene. A full list of DMRs is provided in Table 3.

- Figure 3 -

Table 3. Differentially methylated regions associated with DNA methylation changes over the course of four and eight weeks of treatment.

Time-point	chr	start	end	p	sidak p	N probe	Gene	Direction
day 28	1	205819179	205819407	1.33E-06	0.004	6	*PM20D1*	-
	20	749148	749621	1.51E-08	1.93E-05	8	*C20orf54*	+
	6	142623433	142623768	5.68E-09	1.03E-05	5	*GPR126; ADGRG6*	+
day 56	6	142623433	142623768	6.50E-09	1.18E-05	5	*GPR126; ADGRG6*	+

Note: Chr chromosome; + indicates increasing methylation levels over the specified time period; - indicates decreasing methylation levels

Epigenetic differences between patients and controls across two time points

In the linear-mixed model comparing patients and controls across two time-points, four CpG sites were identified as significantly differentially methylated for the main effect of group (patients vs. controls; table 4). The most pronounced difference was observed at cg17232014 in *Heme-binding protein 1* (*HEBP1; β* = 0.04, p = 2.6E-08, q = 0.015). No CpG sites showed statistically significant methylation differences for the effects of time point (day 28) or symptom change (continuous HAMD score from baseline to day 28) after multiple testing corrections. The regression coefficients corresponding to the 100 DMPs most strongly associated with group, time point, and symptom change are provided in Supplementary Table S6-8, respectively. The subsequent DMR analysis identified four DMRs associated with time point, one DMRs associated with HAMD score, but no one with group. The DMR showing the strongest association with time point were annotated to the protein-coding gene *Tenascin XB* (*TNXB)*. See table 4 for a list of the identified DMPs and DMRs.

Table 4. Differentially methylated sites/ regions associated with DNA methylation changes over the course of four weeks, symptom severity and group comparison (patients vs. controls).

A. effect (DMPs)	chr	position	p_corr	fdr p	probe id	Gene	beta
group	12	13153193	2.60E-08	0.014	cg17232014	*HEBP1; HTR7P1*	0,0396
	2	3142407	1.50E-07	0.028	cg08902894		-0,0249
	18	77106426	1.40E-07	0.028	cg27410534	*ATP9B*	-0,0226
	1	32739049	3.35E-07	0.047	cg10325497	*LCK*	-0,0201

B. effect (DMRs)	chr	start	end	p	sidak p	n probe	Gene	Direc-tion
HAMD	6	32847441	32847831	3.39E-09	4.89E-06	11	*PPP1R2P1*	+
day 28	6	32063403	32064162	1.81E-10	1.34E-07	19	*TNXB*	+
	15	66947167	66947618	2.31E-08	2.88E-05	6	*LINC01169*	+
	6	32847441	32847831	1.32E-08	1.91E-05	11	*PPP1R2P1*	+
	5	8457599	8458090	1.99E-07	0.0002	6	*MIR4458HG; LOC729506*	+

Note: chr Chromosome, + indicates higher methylation is associated with higher HAMD scores (upper part: HAMD), + indicates increasing methylation over the specified time period (lower part: day 28)

Gene-set enrichment analyses

In the gene-set enrichment analyses, several gene ontology categories showed nominally significant p-values across the different models. For example, in the second model, the biological process “regulation of microvillus assembly” at day 56 (p = 6.4E-04, q = 1) exhibited a strong nominal signal, alongside specific molecular functions related to transcription and protein modification (i.e. “RNA polymerase II core promoter sequence-specific DNA binding” at day 28 [p = 4.61E-05, q = 1]; “protein methyltransferase activity” linked to symptom severity [p = 3.7E-04, q = 1]). However, none of these findings remained statistically significant after correction for multiple testing. Results of the gene-set enrichment analyses are provided in Supplementary Tables S9-16.

Comparison of current findings with prior DMRs

No overlapping DMRs were identified between the current analyses and our previous investigation (Engelmann et al., 2022). Although a significant DMR was detected near *TNXB* in both datasets (model 3 in the present dataset), the regions did not overlap and correspond to distinct genomic loci. A comparison of the FDR-significant CpG sites from the current study with previously reported DMRs showed no overlap. However, overlaps with nominally significant sites, such as those annotated to *TNXB* (models 1 and 3), *SORBS2* (model 2), and *CYP2C18* (model 3), are presented in Supplementary Tables S17–S24.

Discussion

Previous epigenetic studies in major depression have primarily examined baseline DNA methylation as a predictor of antidepressant response, yielding mixed findings and offering limited insights into within-subject dynamics across treatment. Most prior work was cross-sectional or restricted to candidate genes, thus failing to capture the temporal trajectory of methylation during pharmacotherapy. Longitudinal methylome-wide investigations have mainly focused on non-pharmacological interventions, such as ECT or psychotherapy, leaving the dynamics of standardized antidepressant treatment largely unexplored. To address this gap, the present study profiled genome-wide methylation at baseline and at three follow-up time points over eight weeks of antidepressant treatment period, comparing responders, non-responders, and healthy controls in a randomized treatment framework.

Although the current EWAS did not identify any individual DMPs at baseline significantly associated with HAMD score at day 56, six DMRs were positively related to clinical outcome. The top DMR was annotated to *TNRC6C*, a key component of microRNA-mediated gene silencing, suggesting potential involvement of post-transcriptional regulation in antidepressant response, and previously implicated in psychiatric (Nemoda et al., 2015) and cardiovascular disease (Westerman et al., 2019). Prior EWAS have identified single-CpG associations with antidepressant response in genes such as *CHN2* (Ju et al., 2019). In our previous analysis, we identified 20 DMRs, notably in *SORBS2* and *CYP2C18*, which reappeared at nominal significance in the current dataset. Similarly, Schiele et al. (2024) found no epigenome-wide significant CpGs to predict response after six weeks of treatment but reported suggestive associations in genes such as *TIMP2*, *VDAC1*, and *SORL1*, genes linked to mitochondrial function and extracellular matrix remodeling. Taken together,

variability across pre-treatment EWAS likely reflects differences in analytical pipelines, cohort characteristics, and medication regimens. Future meta-analytic efforts integrating longitudinal EWAS and transcriptomic data with cell-type-resolved and functional annotation will be crucial in enhancing reproducibility and clarifying the mechanistic underpinnings of antidepressant response.

While identifying pre-treatment DNA methylation profiles for antidepressant response is crucial from a predictive perspective, longitudinal assessments provide complementary insights into methylation trajectories associated with treatment outcomes and may reveal potential mechanistic targets. Using a linear mixed model that accounted for repeated measures within individuals, we identified one CpG site (cg15731816, annotated to *YLPM1*) that was significantly associated with changes in depressive symptom severity throughout the entire treatment period. Specifically, higher methylation at this site was linked to higher symptom severity, while both methylation and HAMD scores generally decreased over time, suggesting that reductions in methylation may reflect or contribute to clinical improvement. Interestingly, a recent pan-ancestry analysis of sequencing data from three large biobanks identified core genes underlying a broad spectrum of human diseases, including *YLPM1*, which was implicated in psychiatric disease (Jurgens et al., 2024). Complementary evidence from cell type-specific eQTL analyses in MDD highlighted *YLPM1* as a candidate gene in oligodendrocytes, emphasizing the role of cellular heterogeneity in MDD pathogenesis (Liao et al. 2025). Moreover, a cross-ancestry rare variant study of obesity identified *YLPM1* as a gene expressed in both brain and adipose tissue, associated with obesity traits and neuropsychiatric phenotypes, pointing to potential pleiotropic effects on metabolism and mental health (Banerjee & Girirajan, 2025). Taken together, these converging lines of evidence support a role for *YLPM1* in modulating symptom trajectories in depression. In our study, methylation was measured in blood, but the correspondence with cell type-specific brain expression suggests peripheral methylation changes may reflect or serve as a proxy for processes relevant to symptom change. These findings highlight *YLPM1* as a potential target for stratified treatment approaches, although mechanistic studies are needed to establish causality.

The regional analysis identified dynamic DMRs, showing significant methylation changes relative to baseline at day 28 (three DMRs) and day 56 (one DMR), highlighting time-dependent effects during antidepressant treatment. One DMR annotated to *GPR126* was obtained across both time points, suggesting continuous, time-dependent epigenetic modifications throughout treatment. *GPR126*, also known as *Adhesion G protein-coupled receptor G6* (*ADGRG6),* is expressed in multiple tissues and regulates neural development

via G-protein- and N-terminus-dependent signaling (Li et al., 2024; Patra, Monk, & Engel, 2014). The observed methylation changes in *GPR126* across the course of treatment imply ongoing modulation of adhesion G protein-coupled receptor signaling, which may indirectly reflect neural development and synaptic remodeling, supporting the conceptualization of MDD as a disorder of dysregulated neuroplasticity (Ferrarelli, 2022; Harmer et al., 2017). Another DMR in *Peptidase M20 Domain Containing 1* (*PM20D1)* was detected at day 28, further confirming time-related effects in MDD patients. *PM20D1* has recently been highlighted in a cross-trait meta-analysis as functionally implicated in both MDD and type 2 diabetes (Baranova et al., 2025). Genes associated with both mood and metabolic disorders were also reported in our previous study (e.g., *SORBS2*; Engelmann et al., 2022). Altered methylation in these genes may contribute to comorbidity, potentially via inflammatory mechanisms (Gharipour et al., 2020). Together, these results highlight the value of longitudinal assessment and, to our knowledge, provide the first evidence of dynamic trajectory of DNA methylation during antidepressant therapy. Although no methylation changes were observed at day 14, modifications cannot be excluded, consistent with evidence that epigenetic changes are highly dynamic and can rapidly influence synaptic plasticity (Herre & Korb, 2019). Changes became evident from day 28 onward, emphasizing the importance of multiple time points to capture the full trajectory of epigenetic adaptation during antidepressant treatment treatment (Domschke et al., 2025).

To further disentangle disease-related modifications from treatment effects, we compared DNA methylation profiles between patients and healthy controls over time. In the case–control analysis, four CpG sites showed significant group differences, with the strongest effect observed at cg17232014 in *HEBP1*. This robust finding indicates a persistent epigenetic distinction between patients and controls, potentially reflecting disease-related alterations. *HEBP1* has been previously implicated in neuroinflammatory processes associated with Alzheimer's disease (Yagensky et al., 2019). Notably, in a separate methylome-wide association study of self-reported antidepressant use, *HEBP2* was identified among the top ten loci (Barbu et al., 2022). In a meta-analysis of two MDD case-control cohorts, Li et al. (2022) identified several CpG sites annotated to *TNNT3* that were associated with MDD, along with additional DMRs annotated to genes involved in neuronal synaptic plasticity, and inflammation. Although the specific sites differ from those in our study, the affected genes converge on key pathways, particularly inflammation. Regional analyses identified one DMR associated with symptom severity and four regions associated with time, with the most significant region mapping to *TNXB*, a gene involved in extracellular matrix organization and potentially stress-related signaling (Valcourt et al., 2015). These methylation changes, observed across all participants, likely reflect general temporal effects

or nonspecific biological processes. Notably, *TNXB* was previously reported as a differentially methylated region associated with antidepressant treatment response in our earlier study (Engelmann et al., 2022). Although the exact loci differed, these findings suggest potentially distinct but related roles of *TNXB* in temporal methylation dynamics and treatment response, highlighting the need for further investigation.

Although several gene ontology terms showed nominally significant p-values across the three EWAS models, none survived correction for multiple testing. These results should be interpreted cautiously, as they may reflect false positives common in high-dimensional EWAS (Campagna et al., 2021). Biologically, these nominally enriched pathways align with processes implicated in neuropsychiatric disorders, including signal transduction, vascular remodeling, and epigenetic regulation. Overall, these exploratory findings highlight plausible biological systems involved in depression-related methylation dynamics but require validation in larger, independent cohorts and complementary multi-omics approaches.

The EMC study provides a phenotypically well-characterized and homogeneous clinical cohort, with longitudinal DNA methylation data collected at four distinct time points during a predefined antidepressant treatment and including healthy controls measured at two time points. Nevertheless, several limitations should be noted: The sample size, although relatively robust for a longitudinal epigenetic clinical cohort, may still limit the power to detect small single-CpG effects. Although responders and non-responders already differed significantly in symptom severity at baseline, these differences were accounted for in all analyses. Nevertheless, baseline imbalances may still represent a potential confounding factor. All patients underwent the same treatment schedule during the first two weeks; however, later variations in medication regimes may have influenced DNA methylation patterns, potentially confounding treatment-response-related changes. The inclusion of healthy controls is an important strength, but future studies would benefit from more balanced patient-control ratios. DNA methylation was measured in peripheral blood, which may not fully reflect brain-specific processes related to MDD. Given the longitudinal design, repeated sampling of brain tissue in living participants would not have been possible, making peripheral blood the only feasible approach. DNA methylation patterns are dynamic, and antidepressants may partly exert their effects by modulating these marks. Future studies should integrate DNA methylation with gene expression to investigate functional consequences, and consider other epigenetic mechanisms, such as histone modifications (Chistiakov, Bobryshev, & Chekhonin, 2017; Ell et al., 2024).

In conclusion, this longitudinal EWAS provides novel evidence for dynamic DNA methylation changes during antidepressant treatment in patients with MDD. In addition to several

significant DMRs associated with treatment outcomes in the cross-sectional analysis, longitudinal models revealed several positions and regions, most notably in *YLPM1, GPR126*, *and HEBP1*, associated with symptom severity or time. *YLPM1* emerged as the only significant DMP significantly linked to symptom trajectories over time, with reductions in methylation corresponding to clinical improvement. This finding is supported by recent evidence implicating *YLPM1* in psychiatric disease and metabolic phenotypes, suggesting it may influence multiple processes relevant to the course of depressive symptoms. The dynamic methylation changes observed in *GPR126* are particularly noteworthy given its role in neural development and synaptic signaling, reinforcing the concept of MDD as a disorder of impaired neuroplasticity. Persistent methylation differences between patients and controls, such as at *HEBP1*, further point to stable, disease-related epigenetic signatures. Together, these results highlight the value of repeated-measures designs to disentangle temporal, treatment-related, and disease-specific effects on the methylome. Integrating cross-sectional, longitudinal, and case–control data strengthens confidence in the results, although pathway enrichment did not survive multiple-testing correction and should be interpreted cautiously. Future research should replicate these DMR-level associations in larger, independent cohorts and extend this work through harmonized meta-analyses and multi-omics integration, ultimately leading to the development of a DNA methylation marker of antidepressant response. Combining DNA methylation with functional genomic data will be essential to clarify causal mechanisms and elucidate how epigenetic regulation interacts with gene expression and clinical trajectories to shape antidepressant response and recovery.

Figures Legends

Figure 1. **Study design and Time Course of HAMD17 Scores in Responders and Non-Responders.** A. Overview of blood sampling and HAMD-17 assessment time points in patients and controls. B. Time course of depressive symptom severity measured by the Hamilton Depression Rating Scale (HAMD17) in responders and non-responders over 56 days. HAMD scores were significantly different between groups at all time points ($p < .05$). At Day 28, responders showed a ≥50% reduction in symptom severity compared to baseline, whereas non-responders displayed only a modest decline in symptom scores (<50%). Statistical significance is indicated as follows: $p < 0.05$ (**), $p < 0.01$ (**), and $p < 0.001$ (***).

Figure 2. Manhattan plot illustrating the association between DNA methylation and treatment outcome at day 56. Shown are $-\log_{10}$(p-values) across the genome for all tested CpG sites. DMRs identified in the regional analysis are highlighted in green.

Figure 3. Volcano plot illustrating the association between DNA methylation and symptom severity in patients. Each point represents a CpG site, with the x-axis showing the effect size (β) and the y-axis the $\log_{10}$-transformed p-value. CpGs with a positive association (increased methylation with higher symptom severity) are shown in red, and negative associations are shown in blue. Horizontal and vertical dashed lines indicate the thresholds for suggestive statistical significance ($p < 1E-05$) and effect size, respectively.

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Funding

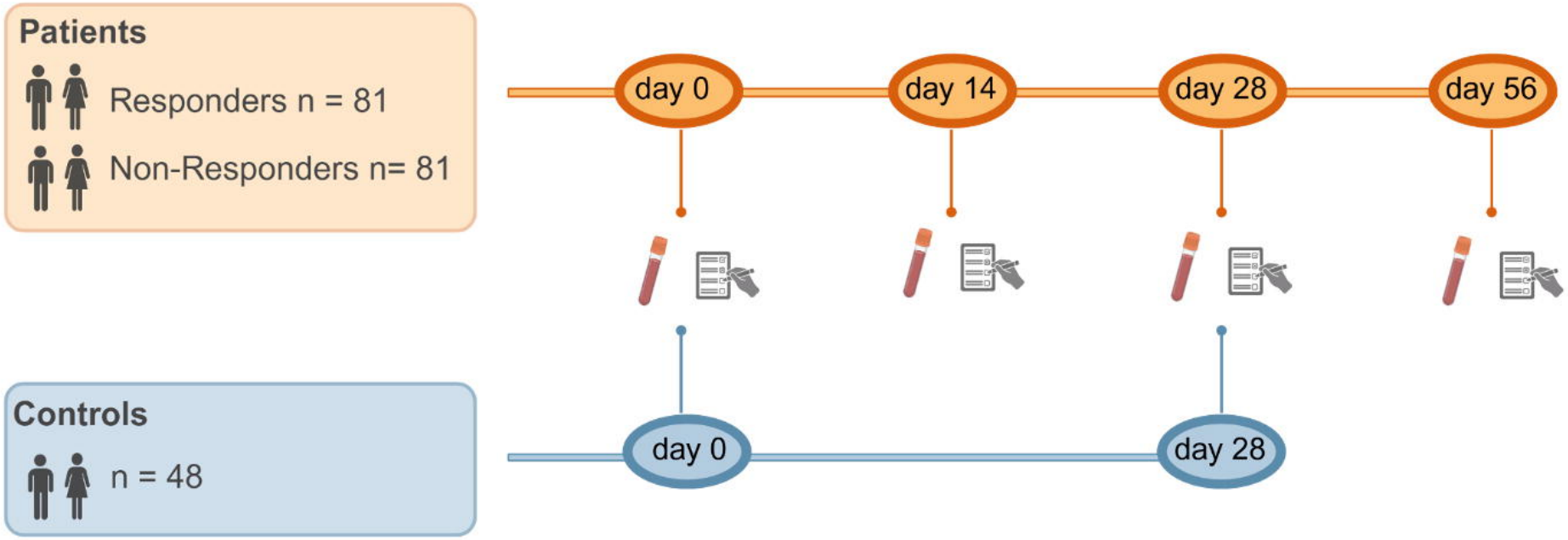
The EMC trial was funded by the German Federal Ministry for Education and Research (BMBF grant no: 01KG 0906; applicants: KL, AT, CH, ND, KK); the herein presented additional investigations are not part of the funding. The BMBF had no role in the conception of the study design, in the writing of the manuscript or the decision to submit the manuscript for publication. The recruitment of the control group was funded by the German Research Foundation ("Deutsche Forschungsgemeinschaft, DFG" (funding number: WA 2970/1-1). The DFG had no role in the conception of the study design, in the writing of the manuscript or the decision to submit the manuscript for publication. Further support was provided by the German Federal Ministry of Research, Technology and Space through ERANET NEURON (project "Clever"; grant no. 01EW2401B). The funding agency had no role in the conception of the study design, in the writing of the manuscript, or in the decision to submit the manuscript for publication.

Acknowledgement

The authors are grateful to the members of the EMC Study Group, which is currently involved in the acquisition of data. The EMC Study Group consists of the following members: Univ.-Prof. Dr. Klaus Lieb, Dr. André Tadić, Univ.-Prof. Christoph Hiemke, Dr. Nadine Dreimüller, Dr. Ömür Baskaya, Dr. Danuta Krannich, Dr. Sonja Lorenz, Annette Bernius, Tillmann Weichert, Dr. Markus Lorscheider, Dr. Dipl.-Psych. Stefanie Wagner, Dipl.-Psych. Isabella Helmreich, Dipl.-Psych. Karen Grüllich, Elnaz Ostad Haji (Laboratory), Yvonne Lober (study nurse), Danuta Weichert (study nurse), cand. med. Dr. Dr. Dietrich van Calker, Dr. Sonja Gerber, Dr. Claus M. Gross, Tobias Hornig, Dipl.-Psych. Rebecca Schneibel, Ritva Atmannsbacher (Freiburg); Prof. Dr. Dieter F. Braus, Dr. Julia Reiff, Dr. Christoph Kindler, Dr. Svenja Davis, Dr. Tina Eyalil, Dr. Thorgerdur Reynisdottir, Dr. Claudia Ginap, Dipl.-Psych. Julia Kraus, Dipl.-Psych. Sabine Kaaden, Dipl.-Psych. Jelena Janzen, Dipl.-Psych. Nina Löffler, Caterina Topaloglu (Wiesbaden). The authors wish to thank Susanne Wittmann and Peter Lichtner at the Genome Analysis Center at the Helmholtz Institute München for running the methylation arrays.

Disclosures

A Tadic is designated as inventor of the European patent number 12171541.1–2404 ‘Method for predicting response or non-response to a mono-aminergic antidepressant’. He has received during the last five years consultancy fees from Janssen and ROVI. K Lieb is designated as inventor of the European patent number 12171541.1–2404 ‘Method for predicting response or non-response to a mono-aminergic antidepressant’. K Domschke is a member of the Neurotorium Editoral Board, Lundbeck Foundation, and has received speaker’s honoraria by Janssen-Cilag. All other authors declare no competing interests.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Controls
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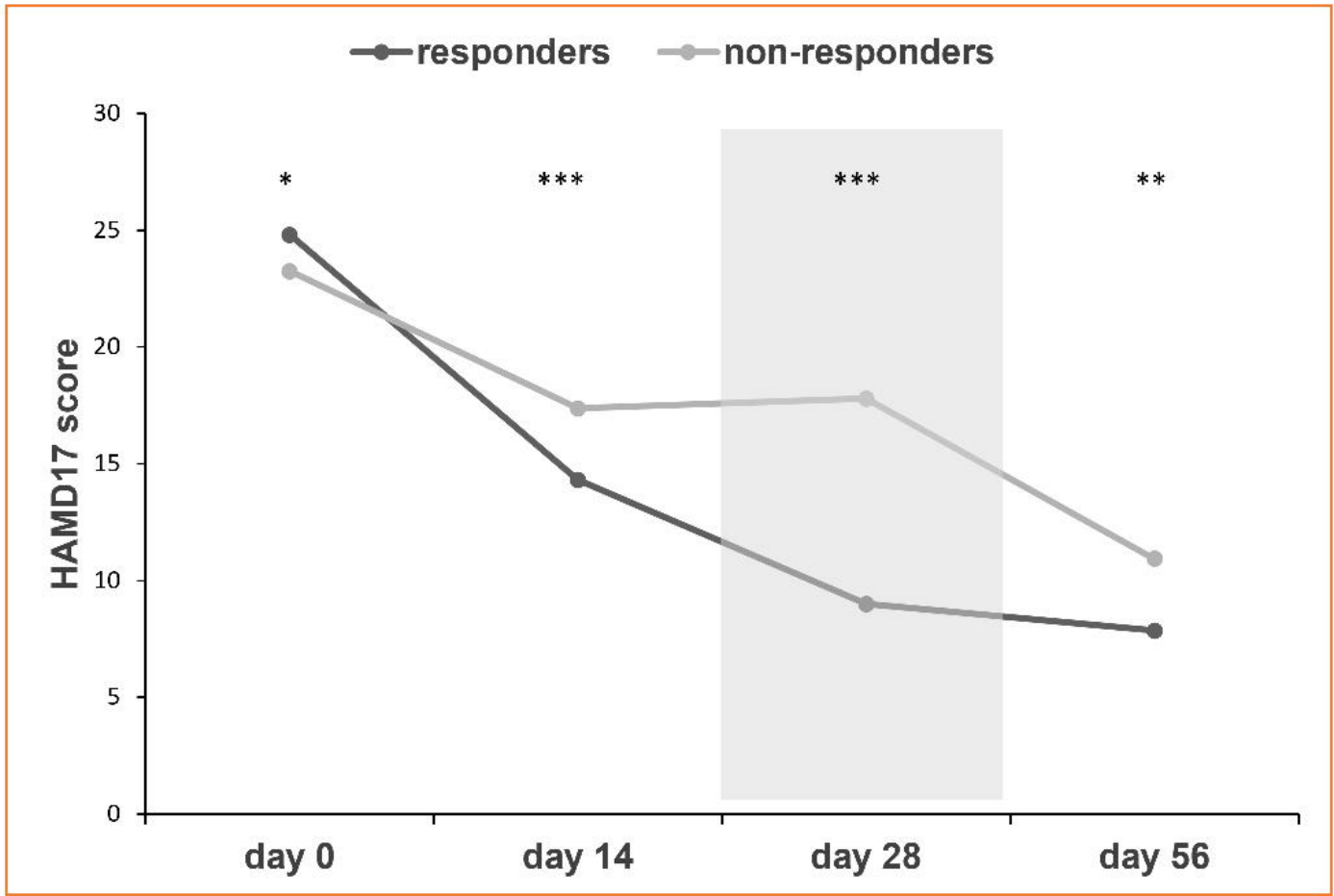
day 0
day 28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25
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15
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day 0
day 14
day 28
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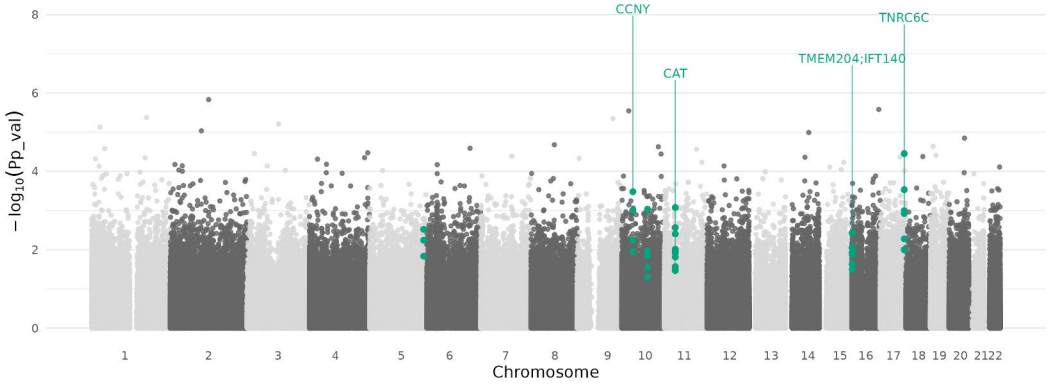
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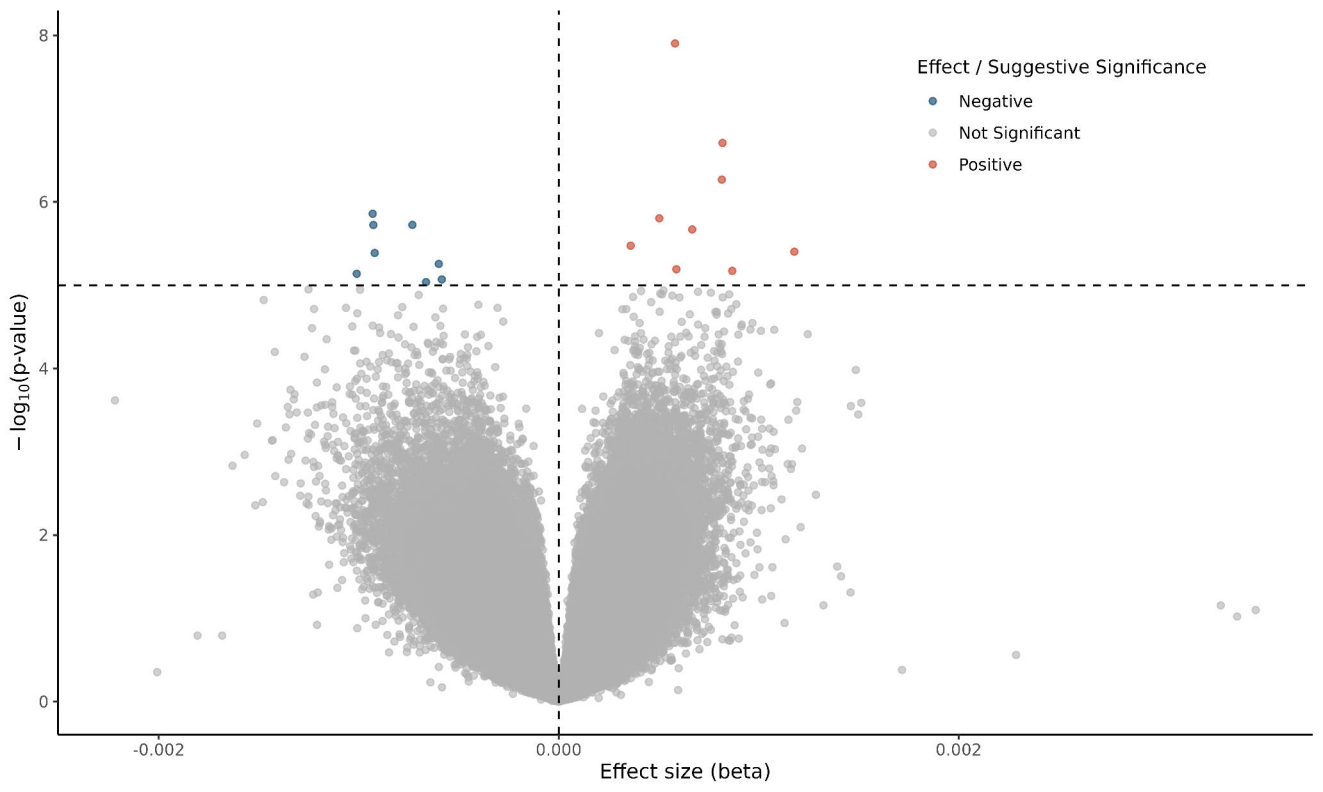
22
Chromosome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Effect size (beta)