Epigenetic Signatures in Antidepressant Treatment Response: a Methylome-wide Association Study in the EMC Trial

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Abstract

Although the currently available antidepressants are well established in the treatment of major depressive disorder (MDD), there is strong variability in the response of individual patients. Reliable predictors to guide treatment decisions before or in an early stage of treatment are needed. DNA-methylation has been proven a useful biomarker in different clinical conditions, but its importance for mechanisms of antidepressant response has not yet been determined. 80 MDD patients were selected out of >500 participants from the Early Medication Change (EMC) cohort with available genetic material based on their antidepressant response after four weeks and stratified into clear responders and age- and sex-matched non-responders (N=40, each). Early improvement after two weeks was analyzed as a secondary outcome. DNA-methylation was determined using the Illumina EPIC BeadChip. Epigenome-wide association studies were performed and differentially methylated regions (DMRs) identified using the comb-p algorithm. Enrichment was tested for hallmark gene-sets and in genome-wide association studies of depression and antidepressant response. No epigenome-wide significant differentially methylated positions were found for treatment response or early improvement. Twenty DMRs were associated with response; the strongest in an enhancer region in SORBS2, which has been related to cardiovascular diseases and type II diabetes. Another DMR was located in CYP2C18, a gene previously linked to antidepressant response. Results pointed towards differential methylation in genes associated with cardiac function, neuroticism, and depression. Linking differential methylation to antidepressant treatment response is an emerging topic and represents a step towards personalized medicine, potentially facilitating the prediction of patients’ response before treatment.

Introduction

Major depressive disorder (MDD) is one of the most common, burdensome, and costly mental disorders worldwide [1,2]. Although currently available pharmacological treatments of MDD are well established and safe, there is a strong variability in antidepressant treatment response and considerable number of depressed patients do not respond to the first antidepressant administered, requiring optimization of antidepressant pharmacotherapy [3-5]. Due to the unpredictable treatment outcome, there is a vital need to identify reliable predictors of antidepressant response to guide treatment decisions. In clinical studies, early improvement, defined as a decrease in depressive symptomatology after two weeks, is considered the most consistent clinical predictor of antidepressant response [6]. However, research has not identified any clinical and biological predictor of sufficient clinical utility to inform the selection of a specific antidepressant agent for an individual depressed patient to date [7,8].

MDD is moderately heritable, with heritability estimates from twin studies ranging between 30% and 40% [9]. In a recent genome-wide association study (GWAS), which investigate the association of common genetic variants with depression, 102 independent genome—wide significant variants contributing to disorder risk were identified, and the phenotypic variance explained by all investigated SNPs (i.e. SNP-heritability) was estimated to be 8.9% [10]. During the last few decades, scientific knowledge about the genetic background of depression has increased steadily and pharmacogenetic
approaches have broadly been investigated to identify genetic variation contributing to individual
treatment response in order to improve response prediction. A recent GWAS of antidepressant treatment
response in 5,151 depressed patients did not yield any genome-wide significant finding, but showed that
genetic variation explained around 13% of variance in the total meta-analysis and 20-40% of variance
within each cohort [11]. It has to be noted that the included samples showed high heterogeneity, and
because of the relatively small sample size compared to other GWAS in psychiatric genetics, analyses
were not stratified for diagnosis, drug, or drug dosage.

In addition to genetic variation, epigenetic alterations, specifically DNA-methylation, i.e. the addition of a
methyl-group to a cytosine nucleobase at the 5’ position in CpG dinucleotides, can influence gene
expression and may induce a wide range of changes at the cellular and systems function level [12,13].
The investigation of epigenetic variation represents a promising approach to not only investigate the
biological mechanisms underlying depression, but especially the response to antidepressant treatments.
Epigenome-wide association studies (EWAS) have investigated the association of DNA-methylation with
depression [14-17]), as well as with antidepressant use [18]. Only one of the mentioned
studies [17] yielded epigenome-wide significant results, although the two identified CpG-sites were not
annotated to nearby genes, which makes functional interpretation difficult. Previous epigenetic
investigation of antidepressant treatment response focused mainly on well-described candidate genes
like BDNF, NR3C1, and FKBP5 (reviews: [19-21]), but results are inconclusive. Additionally, candidate gene
approaches are based on already existing hypotheses, and limited to a small selection out of the ~20,000
genes in the human genome. EWAS represents a promising approach to identify new biological
mechanisms underlying individual differences in the context of antidepressant response, and identified
methylation signatures might guide clinical decisions in the future, by predicting therapy outcomes.

So far, only two studies have investigated genome-wide differences in baseline DNA methylation between
responders and non-responders to pharmacological antidepressant treatment. Ju and colleagues
revealed several CpG-sites differentially methylated between responders and non-responders to eight
weeks of escitalopram treatment, which were also associated with gene expression differences between
both groups [22]. The study highlighted a differential methylated position (DMP) located in the CHN2
gene, which was most significantly associated with mRNA expression and was replicable in an external
cohort with a similar treatment [22]. The second study by Martinez-Pinteno et al. [23] identified 21
differentially methylated DMPs between responders and non-responders to eight weeks of fluoxetine
 treatment in a cohort of depressed children and adolescents. The Ras Homolog Family Member J (RHOJ)
gene, encoding signaling molecules in the regulation of cytoskeletal organization, showed four
significantly hypermethylated CpG-sites in non-responders [23]. Due to the inconsistent findings of the
mentioned studies, further investigations of DNA methylation signatures between later responders and
non-responders are needed.

The primary aim of this study was to identify epigenetic signatures associated with antidepressant
treatment response, by testing baseline differential methylation between responders and non-responders
after four weeks of antidepressant treatment. Patients were enrolled from a large well-characterized
antidepressant trial, allowing us to carefully select clear responders and sex- and age-matched non-responders. As a secondary aim, we explores the DNA-methylation signatures of early improvement in the same sample.

Patients And Methods

Sample

This investigation is a secondary analysis of 80 MDD patients, who have participated in the “Randomized clinical trial comparing an early medication change (EMC) strategy with treatment as usual (TAU) in patients with Major Depressive Disorder (MDD) – the EMC trial” (ClinicalTrials.gov NCT00974155). A total of 889 depressed patients were enrolled between 2009 and 2014 in this trial. Genetic material at baseline was available for 560 patients, of which the 40 most clear responders and sex- and aged-matched non-responders were selected. The selection was based on their treatment response, measured with the Hamilton Rating Scale for Depression – 17 items (HAM-D17) to antidepressant study medication after four weeks. In addition, the course of depression severity was considered in weekly intervals from baseline to week 4. The non-responders showed no improvement in depressive symptomatology despite four weeks of antidepressant treatment; in the group of responders, depressive symptomatology decreased steadily and patients showed a complete remission after four weeks (see also Study Procedures). Details of the EMC study protocol have been described previously [24-26] and the treatment algorithm can be openly accessed by https://trialsjournal.biomedcentral.com/articles/10.1186/1745-6215-11-21. In summary, the EMC trial was a multi-center, randomized, controlled clinical trial investigating whether patients with non-improvement after 14 days of escitalopram treatment take advantage to an early medication change (EMC: change to venlafaxine from day 14 onwards followed by an augmentation with lithium after again non-response at day 28) compared to patients treated according to current guideline recommendations (TAU: continuing escitalopram for two more weeks and switching to venlafaxine at day 28). Study procedures were approved by the local ethics committee of the Landesärztekammer Rheinland-Pfalz and are compliant with the Code of Ethics of the World Medical Association (Declaration of Helsinki) in its current version.

Study Procedures

Diagnoses were based on the German Version of the Mini International Neuropsychiatric Interview (M.I.N.I. ; [27]) and the Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II;[28]). The socio-demographic and clinical characteristics, such as previously diagnosed cardiovascular or metabolic diseases, were assessed relying on patients’ self-reports. Depression severity was measured weekly from baseline to day 56 by the HAMD [29] by trained and blind raters [30]. Morning blood samples were obtained weekly. Antidepressant premedication was – if necessary – washed out after inclusion and before baseline visit. The antidepressant treatment according to study protocol was 20 mg escitalopram from baseline to day 14, followed by a predefined treatment algorithm. Other medications
were administered to treat depression-associated symptoms (e.g., insomnia) or adverse drug reactions (e.g., agitation or anxiety) with short-acting hypnotics (zolpidem or zopiclone), low potency antipsychotic drug pipamperone, histamine-receptor antagonist promethazine in standard doses as well as benzodiazepines in a dose-equivalent up to 15 mg diazepam per day was allowed. The main outcome parameters were: a) response, defined as decrease of 50% during four weeks of treatment, and b) early improvement, defined as a decrease of depression severity of at least 20% from baseline to day 14 [6].

**DNA-methylation**

DNA was extracted from whole blood using the QIAamp DNA Blood Midi Kit from Qiagen (Qiagen, Hilden, Germany). The genomic DNA samples were stored at -20°C. Responders and non-responders were matched based on age and gender and the DNA from the matched samples were randomized and pipetted on processing plates. 500ng genomic DNA were bisulfite converted using the EZ-96 DNA methylation gold kit (Zymo research, Irvine, USA). Epigenome-wide methylation levels were determined using the Illumina HumanMethylationEPIC Beadchip and Illumina HiScan array scanning systems (Illumina, San Diego, CA).

**Data preprocessing and quality control**

The R statistical environment, version 3.6.1, was used for all data preprocessing and analysis steps. We used an updated version of the CPACOR-pipeline to extract methylation data from raw intensity data and performed quality control [31]. Thresholds for sample removal were: (i) DNA quality was not sufficient (missing rate > 0.10) or (ii) a discrepancy between methylation-based and phenotypic sex emerged. Thresholds for probe removal were: (i) the call-rate was insufficient (< 0.95), (ii) SNPs with a minor allele frequency > 0.10 were located in the probe sequence, (iii) the probes were located on the X or Y chromosome. After quality control all 80 samples remained. After filtering, 706,677 out of 843,232 sites were available for analysis.

**Statistical Analysis**

Differences in clinical and sociodemographic characteristics between responders and non-responders were calculated by t-tests for independent variables or Chi²-tests, depending on the level of measurement.

Methylation values were log-transformed (base2) and included as dependent variables in the association analyses [32]. Principal component analysis was performed to extract signals of the internal control probes of the EPIC array and the resulting first ten principal components were included in all analyses to control for batch effects and technical quality. Additionally, the chip number and position on the chip were included. Cell-type heterogeneity was accounted for by estimating the cell counts based on the methylation data [33]. This approach results in six estimates, which roughly sum up to one. To avoid
multicollinearity in the EWAS, variance inflation factors were calculated for each cell count estimate. The estimated granulocyte count was subsequently removed from further analyses. For two participants, data on smoking was not available, and their smoking status was therefore estimated based on a validated set of sites [34].

*Epigenome-wide association analysis.* Tests of single site methylation differences between responders and non-responders were performed with linear models, adjusting for sex, age, smoking, standardized cell counts, and the first ten principal components of the internal control probes. Additionally, all analyses were run with early improvement after two weeks as a secondary outcome. Correction for multiple testing was applied using the Benjamini-Hochberg (FDR) correction and the resulting values are reported as $q$-values. CpG-sites were annotated using the manufacturer’s manifest (http://webdata.illumina.com.s3website-us-east-1.amazonaws.com/downloads/productfiles/methylationEPIC/infinium-methylationepic-v1-0-b4-manifest-file-csv.zip; downloaded on 10th of August 2018).

*Differentially methylated regions (DMRs).* The comb-p algorithm was applied to identify DMRs. Comb-p accounts for autocorrelation between tests of adjacent methylation sites and combines these sites, in a given window, to regions of enrichment [35]. In the present study the settings were: Seed-p value < 0.01, minimum of 2 probes, sliding window 500 bp. Correction for multiple testing was applied using the Šidák correction as implemented in comb-p.

*Gene-Set Enrichment Analysis.* missMethyl [36] was used for functional analysis to test differentially methylated CpG-sites overrepresented in hallmark gene-sets. Sites with a threshold of $p_{\text{nominal}} < 0.001$ were included and the Hallmark gene-set collection (MSigDB Version 7.1), which consists of 50 gene-sets representing specific well-designed biological states or processes, was used as reference [37]. missMethyl controls for several potential confounders, such as probe number bias, which is the increased likelihood of a gene being differentially methylated, if more probes cover the gene, and multi-gene bias, since probes can be annotated to more than one gene.

*GWAS-Enrichment-Analysis.* Gene-sets consisted of the genes to which CpG-sites with an uncorrected $p$-value < 0.001 in the EWAS were annotated to. Two gene-sets were created, one for early improvement and one for treatment response. Gene-set enrichment was tested in results of the two recent genome-wide association studies described in the introduction: one of antidepressant treatment response ($N_{\text{remission}} = 1,852$, $N_{\text{non-remission}} = 3,299$) [11] and one of major depressive disorder including PGC and UKB samples ($N_{\text{cases}} = 246,363$, $N_{\text{controls}} = 561,190$) [10]. This test was performed using Multi-marker Analysis of GenoMic Annotation (MAGMA) [38].

**Results**

The course of depression severity in responders and non-responders is shown in Figure 1. Mean age ($\pm SD$) was 41.5 ($\pm 11.1$) years, 58% of patients were women and depression severity at baseline was 22.3
(±4.1) points (HAMD\textsubscript{17}). As individuals were matched, there were no differences in age or sex distribution (all p ≥ 0.94). For details of the clinical and sociodemographic characteristics see Table 1.

Table 1
Clinical and Sociodemographic Data. Continuous measures are presented as mean (standard deviation) and categorical measures as frequency (percent).

|                                      | Total (N=80) | Responders (N=40) | Non-Responders (N=40) | p-value (group comparison) |
|--------------------------------------|--------------|-------------------|-----------------------|----------------------------|
| Age – yrs (SD)                       | 41.55 (11.09)| 41.45 (10.83)     | 41.65 (11.49)         | 0.94\textsuperscript{a}    |
| Female                               | 46 (57.5%)   | 23 (57.5%)        | 23 (57.5%)            |                            |
| Male – (%)                           | 34 (42.5%)   | 17 (42.5%)        | 17 (42.5%)            | 1.0\textsuperscript{b}     |
| Age at onset - yrs (SD)              | 33.48 (12.02)| 36.00 (12.13)     | 30.95 (11.50)         | 0.06\textsuperscript{a}    |
| Duration of current episode – wks (SD)| 30.38 (41.94)| 28.15 (40.87)     | 32.60 (43.39)         | 0.64\textsuperscript{a}    |
| 1\textsuperscript{st} episode recurrent – (%) | 28 (35%)     | 19 (48%)          | 9 (23%)               | 0.034\textsuperscript{b}   |
| Hamilton Scores                      |              |                   |                       |                            |
| Baseline (SD)                        | 22.33 (4.15) | 22.83 (3.88)      | 21.82 (4.4)           | 0.28\textsuperscript{a}    |
| Day 14 (SD)                          | 12.69 (8.83) | 4.57 (2.26)       | 20.8 (4.21)           | 0.00\textsuperscript{a}    |
| Day 28 (SD)                          | 12.61 (10.50)| 2.63 (1.76)       | 22.60 (3.97)          | 0.00\textsuperscript{a}    |
| Smokers – (%)                        |              |                   |                       | 0.35\textsuperscript{b}    |
| yes                                  | 28 (36%)     | 16 (42%)          | 12 (30%)              |                            |
| no                                   | 50 (64%)     | 22 (58%)          | 28 (70%)              |                            |
| Cardiovascular Disease – (%)         |              |                   |                       | 0.0504\textsuperscript{b}  |
| yes                                  | 16 (20%)     | 4 (10%)           | 12 (30%)              |                            |
| no                                   | 64 (80%)     | 36 (90%)          | 28 (70%)              |                            |
| Metabolic Disease – (%)              |              |                   |                       | 0.735\textsuperscript{b}   |
| yes                                  | 10 (12.5%)   | 4 (10%)           | 6 (15%)               |                            |
| no                                   | 70 (87.5%)   | 36 (90%)          | 34 (85%)              |                            |
| Early Improvement – (%)              |              |                   |                       |                            |
| yes                                  | 36 (45%)     | 39 (97.5%)        | 5 (12.5%)             |                            |
| no                                   | 44 (55%)     | 1 (2.5%)          | 35 (87.5%)            |                            |

\textsuperscript{a} t test; \textsuperscript{b} \chi\textsuperscript{2} test; SD = standard deviation, wks = weeks; yrs = years

Epigenome-wide association study. No epigenome-wide significant differentially methylated positions emerged for either treatment response or early improvement after controlling for multiple testing. The strongest association with response was observed with hypermethylation of cg02107110 in WDR47 (β =
0.17, \( p = 9.59 \times 10^{-7}, q = 0.57 \). For early improvement, the strongest association was observed for cg04568295, which was annotated to \( SIRT7 \) and \( MAFG \) (\( \beta = 0.11, p = 1.58 \times 10^{-6}, q = 0.86 \)). Regression coefficients for the 100 DMPs showing the strongest association can be found in Supplementary Table S1 for response and S2 for early improvement.

**Differentially methylated regions (DMRs).**

The DMR analysis identified twenty differentially methylated regions associated with treatment response and eleven with early improvement, Table 2 lists the DMRs and Figure 2 depicts the Manhattan plot of this analysis; DMRs are highlighted; results for early improvement are listed in Supplementary Table S3. The DMR showing the strongest association for both response to treatment and early improvement was annotated to \( \text{Sorbin And SH3 Domain Containing 2 (SORBS2)} \), a protein coding gene. The DMR consists of eight CpG-sites, hypermethylated in the responder group, seven of which are part of an enhancer region of \( SORBS2 \), pointing towards a potential functional relevance.
| Chr | Start              | Ende              | N probes | P       | Sidak P | Gene            | Direction |
|-----|--------------------|-------------------|----------|---------|----------|-----------------|-----------|
| 4   | 186732837          | 186733061         | 8        | 5.84E-16| 1.75E-12 | SORBS2         | +         |
| 6   | 32016214           | 32016427          | 8        | 2.49E-12| 8.26E-09 | TNXB           | +         |
| 1   | 174844397          | 174844561         | 5        | 1.19E-09| 5.11E-06 | RABGAP1L       | +         |
| 8   | 1713005            | 1713013           | 3        | 2.74E-08| 2.42E-03 | LOC101927752;CLN8 | +         |
| 10  | 96442621           | 96442675          | 3        | 4.59E-08| 6.01E-04 | CYP2C18        | +         |
| 3   | 23244051           | 23244131          | 6        | 4.84E-08| 4.28E-04 | UBE2E2-AS1;UBE2E2 | +         |
| 19  | 996220             | 996374            | 2        | 5.30E-08| 2.43E-04 | +               |           |
| 12  | 25801455           | 25801622          | 5        | 5.41E-08| 2.29E-04 | LMNTD1         | +         |
| 22  | 50528213           | 50528299          | 4        | 6.20E-08| 5.10E-04 | MOV10L1        | +         |
| 3   | 52099522           | 52099562          | 3        | 7.73E-08| 1.37E-03 | LINC00696      | -         |
| 22  | 50585229           | 50585401          | 4        | 1.58E-07| 6.49E-04 | MOV10L1        | +         |
| 1   | 108023366           | 108023487         | 5        | 2.65E-07| 1.55E-03 | NTNG1          | +         |
| 3   | 48632568           | 48632724          | 4        | 5.57E-07| 2.52E-03 | COL7A1         | +         |
| 2   | 128366514          | 128366595         | 2        | 5.90E-07| 5.13E-03 | MYO7B          | +         |
| 3   | 49459909           | 49460112          | 6        | 8.09E-07| 2.81E-03 | AMT;NICN1      | +         |
| 20  | 30225681           | 30225852          | 4        | 8.45E-07| 3.49E-03 | COX4I2         | +         |
| 6   | 116886276           | 116886350         | 2        | 9.22E-07| 8.76E-03 | +               |           |
| 6   | 32121355           | 32121523          | 8        | 1.44E-06| 6.05E-03 | PPT2;LOC100507547 | +         |
| 17  | 36997563           | 36997732          | 4        | 3.75E-06| 1.56E-02 | C17orf98       | +         |
| 10  | 45719880           | 45720041          | 2        | 5.02E-06| 2.18E-02 | -               |           |

Chr = chromosome, - = hypomethylation of DMR in responders, + = hypermethylation of DMR in responders
Gene-Set Enrichment Analysis. Results from the EWAS were most strongly overrepresented in the Hallmark gene-sets “apical surface” \( (p = 0.001, q = 0.078) \) and “myogenesis” \( (p = 0.006, q = 0.142) \), although none of the terms remained significant after multiple testing correction. Results of the gene-set enrichment analysis can be found in Supplementary Table S4 for response and S5 for early improvement.

GWAS-enrichment analysis. No significant enrichment of genes implicated by GWAS of MDD and antidepressant treatment response was observed \( (\forall p \geq 0.12) \). Detailed results are listed in Table 3.

Table 3

| Outcome               | GWAS   | N Genes | Beta  | SE   | \( P \) |
|-----------------------|---------|---------|-------|------|---------|
| Response              | ADR     | 560     | 0.01  | 0.036| 0.388   |
|                       | Depression | 557     | 0.027 | 0.041| 0.259   |
| Early Improvement     | ADR     | 451     | 0.028 | 0.041| 0.125   |
|                       | Depression | 448     | 0.06  | 0.049| 0.12    |

ADR = antidepressant treatment response, SE = standard error.

Exploratory Analysis. Genetic Variation in \textit{SORBS2} has repeatedly been associated with cardiovascular and metabolic diseases. Therefore, we performed follow-up analyses to investigate whether there was an association between previously diagnosed cardiovascular diseases, such as high blood pressure or arrhythmia, metabolic diseases, such as diabetes or obesity, and treatment response. Descriptively, patients who responded to antidepressant therapy were less likely to have a previous diagnosis of a cardiovascular disease, but a chi-square test was not significant \( (\chi^2(1) = 3.83, p = 0.0504) \). No association between metabolic diseases and treatment response was observed.

When we included previously diagnosed cardiovascular diseases in the EWAS regression model as a covariate, associations between treatment response and methylation of the DMR in \textit{SORBS2} remained significant. Also, in a separate analysis, previously diagnosed cardiovascular diseases did not predict methylation in \textit{SORBS2} \( (\forall p > 0.46) \).

Discussion

The aim of the present study was to identify differential methylation signatures before treatment initiation associated with antidepressant treatment outcome in 80 MDD patients, who were part of a large randomized controlled trial. We focused our analyses on antidepressant response after four weeks and
additionally investigated early improvement after two weeks of treatment. For both outcomes several differentially methylated regions at baseline were observed, which may point to possible underlying mechanisms of differential response to antidepressant pharmacotherapy.

While the epigenome-wide association study did not yield findings remaining significant after correction for multiple testing on the single site level, the region based analyses highlighted several CpG-sites as potentially relevant in antidepressant treatment response. The most strongly associated CpG-site for response was observed with hypermethylation of cg02107110 in the *WDR47* gene. *WDR47* is a microtubule-associated protein and plays a role in neuronal regulation, brain development and brain connectivity [39]. Regarding early improvement, the strongest associated CpG-site was found in cg04568295. DNA-methylation at this site has been associated with HbA1c-levels in type 1 diabetes [40].

The first epigenome-wide association study of antidepressant response by Ju and colleagues identified three DMPs before treatment between later responders (N = 82) and non-responders (N = 95). One DMP located in the *CHN2* gene could be replicated in a second cohort receiving the same antidepressant treatment [22]. However, the three CpG-sites highlighted in *CHN2* were not available for analysis in the present study after quality control had been performed. It also has to be taken into account that the reported results of Ju and colleagues were not corrected for major drivers of differential methylation such as smoking [34]. The second study by Martinez-Pinteno and colleagues investigated baseline differences in DNA methylation between responders and non-responders (N = 11, each) and reported 21 significantly differential methylated CpG-sites associated with response to fluoxetine in adolescents. Within the two genes *RHOJ* and *OR2L13* (*Olfactory Receptor family 2 subfamily L member 13*), four and three DMPs were found between responders and non-responders [23]. These results were not replicated for antidepressant response in adults in the present study.

The significant DMRs were found in genes associated with a variety of domains, such as psychiatric, skeletal, immunological and metabolic traits. For example, genetic variation in *RABGAP1L* has been associated with the psychiatric traits ease of getting up in the morning [41], depressive affect [42] and neuroticism [43], but also metabolic traits such as BMI [44]. The strongest association was observed for a region in *SORBS2 (ARGBP2)*. This DMR was differentially methylated between responders and non-responders, as well as between patients who showed an early improvement after two weeks of treatment, and those who did not. *SORBS2* is a protein-coding gene, which encodes the sorbin and SH3 domain containing 2 protein. Interestingly, the identified DMR is in an enhancer region, which provides evidence for a potential functional mechanism. Genetic variation in *SORBS2* has previously been implicated in cardiovascular diseases [45], type II diabetes [46], and educational attainment [47] in European ancestry populations. A pharmacogenetic GWAS suggested an association of *SORBS2* in response to lithium treatment [48] and in the recently published EWAS by Zhu and colleagues, *SORB2* was found as significant DMR associated with lifetime history of MDD in monozygotic discordant twins [15]. In addition, a review by Gharipour et al. highlighted *SORBS2* as one of three overlapping genes between mood disorders and obesity and formulated the hypothesis that hypermethylation in *SORBS2* might play a role in the co-occurrence of both syndromes due to inflammation processes [49]. Based on the findings
on SORBS2 in cardiovascular and metabolic diseases, we performed additional exploratory analysis, including previously diagnosed cardiac and metabolic comorbidities of our MDD patients. Even after taking the comorbidities into account, the association between response and differentially methylation of SORBS2 remained significant. Mechanistically, the SORBS2 splice variant neural Abelson-related gene-binding protein 2 (nArgBP2) could be of particular interest, because it is specifically expressed in neurons. The nArgBP2 protein is enriched at dendritic spines where it acts as a cytoskeletal adaptor protein [50]. Due to the specificity of nArgBP2 to excitatory synaptic inputs, dysregulation results in an excitatory/inhibitory imbalance that could contribute to the disease course in mood disorders [51] and also to the response to antidepressants.

Another DMR in CYP2C18 identified in the present study is of particular interest as CYP2C18 belongs to the cytochrome P450 super family, which is involved in metabolism of many drugs, and genetic variation in this gene has previously been associated with escitalopram treatment response [52]. The pharmacogenetic study by Braten and colleagues investigated novel CYP2C-haplotypes to improve genetic prediction of escitalopram metabolism. The presence of the two SNPs (i.e., rs2860840 (C>T: CYP2C18, 3'UTR) and rs11188059 (G>A: CYP2C18, intron5) was associated with a significantly lower serum concentration of escitalopram [54].

None of the gene-sets were significantly enriched for genetic variation identified in recent GWAS. This could be because the cut-off of p < 0.001 resulted in a relatively large gene-set, but a restriction to genes implied by DMR analysis did not yield significant findings either. Also, the GWAS of antidepressant treatment response, which is the most relevant for the present analysis, is relatively small in comparison to other GWAS on psychiatric phenotypes and therefore limited in statistical power [11].

Overall, there is a wide correlation between the two investigated outcome parameters (response and early improvement) and the majority of differentially methylated CpG-sites were implicated in both outcomes. This can be simply explained from the patient sample investigated here in which 97.5% (N = 39) of responders at week 4 also showed an early improvement to antidepressant treatment at week 2. However, this is also in line with convergent evidence in literature from our own studies as well as a number of additional investigations that early improvement, defined as a 20% decrease of depressive symptomatology within the first two weeks, is one of the most consistent clinical predictors of later response to antidepressants [6, 53, 54], and that biological predictors of antidepressant treatment response may be identified already in the early course of treatment.

The major strength of our study is the selection of the investigated patients from the large well-characterized EMC trial, which enabled to choose clear responders and non-responders and to match the two groups according to several criteria, such as age and sex. In addition, we were able to control for potentially influencing factors such as smoking and to include concomitant cardiovascular and metabolic diseases in our analysis.

Several limitations apply to the present study. The sample size is relatively small compared to case-control EWAS of MDD and therefore our results need to be confirmed in larger well-characterized MDD
samples. Secondly, gene expression patterns could not be investigated in our sample, as the respective biomaterial is not available, limiting the possibility to draw conclusions about functional mechanisms. Thirdly, DNA methylation was assessed at baseline. While from a prediction perspective, it is important to identify pretreatment biomarkers of later therapy response, a longitudinal assessment could provide important insights into methylation changes associated with antidepressant treatment outcomes. Furthermore, methylation was assessed in peripheral blood samples and may potentially not reflect methylation in the brain of depressed patients.

In conclusion, we identified differential methylated regions associated with pharmacological antidepressant response in a well-characterized MDD study sample before treatment initiation. The DMR showing the strongest association was annotated to SORBS2, which has previously been described as an overlapping gene between mood disorders and obesity. SORBS2 may therefore be a potential target gene enabling better understanding of mood disorders and additionally antidepressant treatment response, but confirmation in larger samples is needed. In summary, our results provide further evidence for the role of DNA methylation in patients’ response to antidepressant treatment. Exploring DNA methylation in larger and clinically well-characterized samples may lead enable the stratification into different response subtypes.

Declarations

Author Contributions
SHW, MRiet, FS, KL, MRiem and MBM planned the investigation. JE and DPH performed the DNA extraction. SS, MRiem and CS performed the methylation analysis. SHW, MRiet, LZ were responsible for generating genome-wide methylation data. LZ, JF, JCF and FS developed the analysis plan. LZ and JF performed all statistical analyses. LZ, JE, SW, JCF, LS, MR, FS, DPH and SHW reviewed the literature for the paper. LZ, JE, FS and SHW drafted the manuscript. All authors contributed, revised, and edited the final manuscript critically. All authors agreed to the publication of the final version of the manuscript.

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Data Availability
Raw data and summary statistics for all analyses are available on request.

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**Figures**
Figure 1

Mean Hamilton Rating Scale for Depression – 17 items (HAMD$_{17}$) Score by response to treatment, light gray represents responder, dark gray non-responder. Error bars represent standard deviations.
Figure 2

Differentially methylated CpG-sites and regions (green) associated with treatment response after four weeks.

Supplementary Files

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- EMCMethylationSupplement.xlsx