Methylation findings from major depressive disorder overlap in blood and brain and replicate in independent brain samples

Supplementary notes

Blood samples for the MWAS discovery phase

For this study, 1,200 individuals were selected from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing, longitudinal, multi-center, observational cohort study designed to investigate the long-term course and consequences of depression and anxiety disorders.[1-3] In total, NESDA involves 2,981 participants (18-65 years). This includes patients with a current or lifetime diagnosis of depression and/or anxiety disorder and controls (without any lifetime depressive disorder and/or anxiety disorder). Participants were recruited from the general population, general practices, and mental health organizations in order to reflect various settings and the entire range of psychopathology. Presence of MDD was ascertained with the DSM-IV based Composite International Diagnostic Interview (CIDI version 2.1) assessed by specially trained research staff.[4] Exclusion criteria were: a) clinically overt primary diagnosis of other psychiatric conditions, e.g. psychotic, obsessive compulsive, bipolar, or severe substance use disorder, and b) not being fluent in Dutch. Depression severity was measured in cases and controls with the 30-item IDS self-report version.[5] The ethical committees of all participating centers approved the study, and participants provided written informed consent.

Quality control of methylation data and CpG score calculation

We assayed the methylome using an optimized protocol for methyl-CG binding domain sequencing (MBD-seq) that provides almost complete coverage of all 28 million common CpGs in the genome.[6] In short, with ultrasonication we sheared 1 ug of genomic DNA

into, on average, 150 bp fragments and captured the methylated fraction of the genome with MethylMiner™ (Invitrogen). The captured fragments for each sample were used to create a barcoded sequencing library, which were pooled in equal molarities and sequenced on a NextSeq500 instrument (Illumina). To ensure consistency in the sample preparation, enrichment and library construction were performed using Biomeck NxP robotics (Beckman-Coulter).

After alignment we performed thorough quality control of reads, samples, and CpGs[7] using the RaMWAS Bioconducter package,[8]which is specifically designed for large-scale methylation studies. Of the 1,200 selected NESDA samples, 34 were excluded because the methylation enrichment (*n*=16) or library construction (*n*=18) failed. Reads aligning to loci without CpGs (non-CpGs) represent "noise" caused by, for example, alignment errors or imperfect. Using a threshold of 0.05 to remove samples with high "noise" levels (*n*=10), left an average non-CpG to CpG ratio of 0.01 (SD=0.005) in the remaining samples. For 10 samples, sequence variants called from the methylation data did not match the genotype information obtained from a previous GWAS of these samples.[9] This indicated that a sample swap or sample contamination may have occurred. As it was not possible to determine whether the problem was caused by the GWAS or MWAS data, we conservatively excluded all 10 samples from further analysis. Finally, to identify multidimensional outliers we used the R 'mvoutliers' package (function 'pcout' with the upper boundary for outlier detection set to 15, the scaling constant set to 0.5, and the boundary for final outliers set to 0.2) with the first 15 principal components of the methylation data as input. Fourteen samples were identified as multidimensional outliers and omitted. This left a sample of 1,132 subjects for statistical analysis.

The mean number of reads for the 1,132 samples was 59.4 million (SD=11.2 million) of which, on average, 99.1% aligned. Aligned reads were subjected to further

quality control. Although reads often map to multiple genomic locations, in most cases, a single alignment can be selected because it is clearly better than other alignments. In the case of multi-reads, multiple alignments receive equally good alignment scores. When Bowtie2[10] encounters multi-reads, it uses a pseudo-random number generator to select a single primary alignment. Duplicate-reads are reads that start at the same nucleotide positions. When sequencing a whole genome, duplicate-reads typically arise from artifacts in template preparation or amplification. However, in the context of sequencing an enriched genomic fraction, duplicate-reads are increasingly likely to occur because reads originate from a smaller fraction of the genome. Therefore, only when more than 3 (duplicate) reads start at the same position, we reset the read count to 1 implicitly assuming these reads are tagging a single clonal fragment. This left an average of 48.7 million reads per sample (=81.9% of all reads).

To identify all common CpGs, we combined reference genome sequence (hg19/GRCh37) with SNP information from the European super-population on the 1000 Genomes project (Phase 3). To avoid analyzing sites that are CpGs in only a very small proportion of subjects, we excluded CpGs created/destroyed by SNPs that had a minor allele frequency <1%. This resulted in 27,916,990 CpGs. CpGs in loci prone to alignment errors, e.g., in repetitive regions, were eliminated prior to the analysis. To identify these CpGs, we used RaMWAS to perform an *in silico* alignment experiment outlined elsewhere that aligns all possible reads to the reference.[7] Only 1.3% of the CpGs showed evidence of alignment problems (defined as 15% or more reads from this locus not aligning properly) and these CpGs were removed from further analyses. Finally, akin to filtering SNPs with low minor allele frequency, we eliminated 5,682,206 CpGs that were only methylated in a few participants (average read coverage <0.3). These sites may lack statistical power and create false positive MWAS findings due problems

associated with analyzing sparse data. After all quality control, 21,869,561 CpGs remained for statistical analysis.

Methylation scores (sometimes referred to as coverage in earlier literature) were calculated by estimating the number of fragments covering the CpG using a non-parametric estimate of the fragment size distribution.[11] These CpG scores provide a relative measure of the amount of methylation for each individual at that specific site.

Genotype information from NESDA participants

The NESDA participants were genotyped as previously described.[12] In short, the majority (95.2%) of DNA samples from the NESDA study were genotyped on Affymetrix 6.0 Human SNP array, while the remaining samples were genotyped on Perlegen-Affymetrix 5.0 array. In the quality control (QC) process, samples were excluded based on the following criteria: Affymetrix contrast QC < 0.4; missing rate > 10%; excess genome-wide heterozygosity or inbreeding levels (F<-0.075 or >0.075); genotypes inconsistencies with reported gender; mendelian error rate > 5 standard deviations from the mean of all samples; non-European/non-Dutch ancestry as indicated by principal component analysis.

SNPs were excluded for the following reasons: probes mapped badly against NCBI Build 37/UCSC hg19; minor allele frequency (MAF) <0.005; missing rate >5%; deviation from Hardy–Weinberg equilibrium (HWE) $P< 1\times10^{-12}$;

SNPs present in both arrays were cross-imputed using GONL reference panel.[13] After imputation SNPs were converted to best guess genotypes using Plink 1.90[14] and were removed if meeting the following more stringent criteria: a significant association with a single genotyping platform as compared to the other ($P<1\times10^{-5}$); an allele frequency difference >10% with the GONL reference set; HWE ($P<1\times10^{-5}$), Mendelian error rate > 5 standard deviations (N>40); imputation quality $R^2< 0.90$. The resulting data

were then imputed using 1000G Phase 3 all ancestries reference panel via the Michigan Imputation Server.[15] Among the imputed SNPs, those retained for the present analyses met the following criteria: allele frequency difference <5 standard deviations of the mean of all SNPs with the reference set; HWE $P>1\times10^{-5}$, Mendelian error rate <5 standard deviations; MAF >0.01; $R^2>0.5$.

Permutation of MWAS to study the null distribution in the discovery sample

Using permutations, we tested if the lambda observed for the discovery MWAS was caused by associations to the outcome variable, or if it was caused by uncontrolled artifacts. Using exactly the same dataset as used in the discovery phase we performed MWAS for 100 permutations of the MDD outcome variable and recorder the lambdas. Our results show average lambda of 0.9987 with a standard deviation of 0.0304. The 95% confidence interval ranged from 0.9927 to 1.0048. Thus, our permutations from this dataset show an average lambda that is not significantly different from 1 and therefore the slightly larger lambda observed for the discovery MWAS is likely to reflect associations to the MDD outcome variable.

Postmortem brain samples

In our study we used the following brain collections:

[MWAS primary brain samples] Postmortem brain tissue from 32 MDD cases and 32 matched controls were obtained from the Victorian Brain Bank Network, Australia.[16] For MDD cases, DSM-IV diagnoses were confirmed postmortem by two psychiatrists, using clinical case histories and the Diagnostic Instrument for Brain Studies (DIBS).[17] The controls had no history of psychiatric symptoms or substance abuse (as determined by both information from relatives and medical records) and were age/sex matched to the cases. As several brain regions may be of potential importance for MDD

etiology we obtained two tissue samples from each individual. The first sample was from the prefrontal cortex (Brodmann Area, BA10) and the second from the subgenual cortex (BA25).

[Independent replication set A (BA10)] The first set of independent replication samples involved BA10 samples from four collections that were analyzed together in a mega-analysis. These collections were grouped together as they were all sequenced on SOLiD Wildfire. Two collections were obtained from the Stanley Medical Research Institute (SMRI)[18]. The first consisted of 24 MDD cases (with or without psychosis) and 12 controls. The second collection comprised 12 (non-psychotic) cases and 15 controls. The SMRI uses DSM-IV diagnoses made by two senior psychiatrists on the basis of medical records and, when necessary, telephone interviews with family members. Diagnoses of unaffected controls are based on structured interviews by a senior psychiatrist with family member(s) to rule out Axis I diagnoses.

The third collection included 9 cases and 9 controls from the Netherlands Brain Bank[19]. Reports by family members of a lifetime diagnosis of MDD was confirmed postmortem by a certified psychiatrist on the basis of the medical records following DSM-IV criteria. Controls never received any psychiatric diagnosis or long-term psychotropic medication.

The fourth collection included 3 cases and 4 controls from the Harvard Brain Bank[20]. Family members initially reported diagnoses at the time of death and next of kin were asked to complete a questionnaire/participate in a phone interview to provide further details. A staff psychiatrist then reviewed the clinical records and family questionnaires to confirm or correct the psychiatric diagnosis.

[Independent replication set B (BA10)] The second independent replication collection included BA10 samples from a collection of 40 cases and 15 controls obtained from the Douglas-Bell Canada Brain Bank (Douglas Institute).[21] A trained interviewer

conducted the Structured Clinical Interview for DSM-IV Psychiatric Disorders (SCID-I) with one or more informants of the deceased. SCID-I assessments, case reports, Coroner's notes, and medical records are then reviewed by a panel of clinicians to obtain a consensus diagnosis. To compensate for the imbalance between the number of cases and control, we included an additional set of 10 controls from the Harvard Brain Bank together with these samples. To check for batch effects and ensure that results were not a function of using samples from different brain banks we performed a number of analysis. This included association testing where all cases were excluded and the two control groups were tested against each other, as well as case-control analysis with and without the additional control samples from Harvard. No major batch effects were detected. However, as described below, to further guard against these effects a covariate indicating the origin of the sample collection was included in the analysis. This replication sample set was analyzed separate of the sample collections included in replication sample A because there were sequenced on the newer NextSeq 500 platform.

MBD-seq data from postmortem brain samples

The MBD-seq data for brain was generated using similar protocols as was used for blood. In short, the fragmentation and the enrichment was performed following the same protocols as were used for blood. However, the brain MWAS samples from BA10 and BA25 and independent replication collection A were sequenced using the SOLiD 5500xl Wildfire instrument. Thus for those samples a barcoded sequencing library was manually created for each methylation capture using the 5500 SOLiD Fragment 48 Library Core Kit library preparation kit (Applied Biosystems). Labeled sequencing-fragment libraries were pooled in equal molarities, sequenced on a SOLiD 5500xl Wildfire instrument (Life Technologies) with 50bp reads and aligned with Cushaw3.[22] As for blood the

independent replication collection B was sequenced using the NextSeq500 platform and therefore followed the same protocol as was used for blood. The aligned MBD-seq data from brain was processed and analyzed using RaMWAS.[8]

Quality control of MBD-seq data from postmortem brain

Methylation data processing and quality control for the postmortem brain tissues largely followed the procedures used for the blood samples. In summary, samples were excluded for one of the following reasons: (i) failed methylation enrichment, (ii) failed library construction, (iii) a low (<15 million) number of sequencing reads, and (iv) a low (<7.5 million) number of reads remaining after quality control of reads, (v) the methylation data suggesting a different sex as what was recorded in the phenotype information. The number of remaining samples is reported in **Table 1**.

The mean number of reads for the remaining brain samples after sample quality control was 53.0/58.8 million (SD = 14.3/6.2 million) for collections sequenced on the SOLiD Wildfire/NextSeq 500 platform (MWAS samples and Replication sample B/Replication sample C). The average alignment rate was 77.0/99.2%. After removing multi- and duplicate-reads, an average of 23.6/46.5 million reads (SD = 7.0/5.3 million reads) per sample remained.

To identify regions showing alignment problems, we conducted an *in silico* alignment experiment using appropriate settings (50bp reads aligned with CUSHAW3[22] for SOLiD Wildfire and 75p reads aligned with Bowtie2[10] for NextSeq 500). With these settings, 7.9/1.3% of the CpGs were removed from further analyses. After excluding the CpGs with average coverage less than 0.3, 17.5/22.0 million CpGs remained for statistical analysis.

Quality control parameters are better for the NextSeq 500 versus SOLiD Wildfire. The reason is that the NextSeq is a newer platform, which employs longer reads and

improved sequencing chemistry. This discrepancy is therefore expected and it should be noted that although more reads are discarded for the SOLiD platform, the reads remaining after quality control are of high quality.

MWAS of postmortem brain

The MWAS for postmortem brain were performed as described for blood using study specific sets of covariates. Thus, in addition to a set of assay-related variables (e.g., sample batch and peak location), we included age and sex. Tissue specific covariates such as postmortem interval and pH where not significantly associated with major principal components. Furthermore, due to the slow turnover of brain cells that may be viable for decades,[23] the risk of confounding due to cell type heterogeneity is low. To avoid losing statistical power due to an increased number of "degrees of freedom", only significant covariates were used in the final MWAS. To control for possible unmeasured confounders, we included the first principle component that explained 3.55%/3.27% of the methylation variation for BA10/BA25 that remained after regressing out significant covariates.

Permutation based enrichment testing of biological features

The blood-brain MWAS overlap was tested for enrichment using shiftR with 100,000 permutations against a number of biological features including eQTLs, genetic features and chromatin states (**Table S7**). The overlap information and the tracks for the tested biological features are all bivariate datasets (either an overlap/feature is present or not) and therefore only one threshold exists. Thus, with the exception of using only one threshold for each test, the analyses were performed as described for the blood-brain MWAS overlap.

Biological features often overlap. This creates a risk of false positives due to confounding and interpretation problems. Choosing a proper reference category against which the features are contrasted will mitigate these problems. For this purpose we apply a set of rules that selects a reference category based on the magnitude of the overlap. i) When a feature is nested in another feature, the use of the non-overlapping part as the reference category avoids confounding by the higher level unit. Thus, when testing for enrichment of association findings in exons, using the non-exonic part of the gene as the reference avoids that the enrichment is driven by genes in general rather than the exons in specific. ii) When a feature comprises multiple categories, the reference category could be a "neutral" state or a category most similar to the majority of other categories. Examples involve the use the quiescence state when studying histone marks. iii) When two features show partial overlap, we propose to create mutually exclusive categories and then use one of those categories as the reference. For example, genes partially overlap with conserved regions. Using genes in conserved regions as the reference category, we can test whether the enrichment is caused by genes (through the part that is not in conserved regions) or conservation status (through the part that is not in genes). The status of features is determined empirically by calculating the overlap in terms of base pairs. For features that do not overlap with other features, rather than taking the rest of the genome as the reference category use the part of the genome that is not included in the set of features that is tested. This ensures that effect sizes can be compared across features and avoids that when a specific feature is enriched/depleted it affects the results of all other tested features.

The background used for the tested tracks are described in Table S7. The tested tracks of biological features were prepared as follows:

[eQTLs] Expression quantitative trait loci (eQTLS) data were obtained for frontal cortex (BA9), anterior cingulate cortex (BA24), and whole blood from the GTEx Project

v7 Release. Significant variant-gene pairs were processed to identify unique autosomal eQTL chromosome-position loci. Non-significant (null) loci were identified and filtered at MAF ≥ 1% from each complete set of tissue-specific variant-gene associations to generate backgrounds for each tissue. For enrichment testing a +/- 150 bp flank (the approximate fragment size obtained by MBD-seq) was applied to each eQTL to account for the localized effect of methylation not directly overlapping an exact eQTL coordinate. Median CpG-eQTL mapping interval was ~42.5 bp across each enrichment test.

[Genetic features] We used tracks from the UCSC genome browser and from ENCODE[24, 25] for major genetic features such as genes, exons, introns, untranslated regions, CpG islands, conserved regions, transcription factor binding sites, repeats and pseudogenes. Tracks for, for example, potential gene promoters as defined by 8kb upstream of a transcription start site and 2 kb CpG island shores were curated from the downloaded information. To account for the hierarchical and nested structure of the genetic features, the tracks were put on relevant backgrounds. For example, while genes were put on a background that include the rest of the genome, exons (which are fully contained within genes) were put on a background that included the rest of the genes only. This way the overall effect of the genes are not influencing the results when testing the exons.

[Chromatin states] We used the Roadmap Epigenomics Project chromHMM Core 15-state model chromatin tracks[26] using *Quiescent/Low* as the reference state. To study overlap between the 15 histone states across brain regions, we first calculated Cohen's kappa coefficient between each pair of regions. Kappa is a statistic which measures agreement between classifications and is generally thought to be a more robust than simple percent agreement calculations as it takes into account the possibility of the agreement occurring by chance. The pairwise Kappa statistics were then

assembled into a matrix that was subjected to principal component analysis followed by a promax rotation. The adult brain regions (*E067 Brain Angular Gyrus, E068 Brain Anterior Caudate, E069 Brain Cingulate Gyrus, E071 Brain Hippocampus Middle, E072 Brain Inferior Temporal Lobe, E073 Brain Dorsolateral Prefrontal Cortex, E074 Brain Substantia Nigra*) all loaded on factor 1 with loadings in the range of 0.69-0.79. The fetal brain tissues and developmental germinal matrix all loaded on factor 2, with loadings between 0.74-0.83. All cross loadings were smaller than 0.1 and the correlation between the two factors was 0.6. These finding suggested that histone states cluster in two groups and are fairly consistent within groups. To create a "consensus" track, we identified regions that had the same histone state in the majority of tissues (4 out of 7 for adult brain tissues and 2 out of 3 for fetal/developmental tissues). In addition to simplifying the interpretation of analysis of the data, combining data from multiple regions can reduce measurement errors in the data, especially in heterogeneous tissue like brain. These consensus tracks from adult brain were then used for enrichment testing with the blood-brain MWAS overlap.

Supplementary tables and figures

Table S1. Top five MWAS findings in blood

Chr.	Position (bp)	*T* statistic	*P* value	Q value	Gene
3	183,778,732	5.6613	1.91E-08	0.1482	HTR3C
1	51,641	-5.6159	2.47E-08	0.1482	
3	183,778,741	5.6157	2.48E-08	0.1482	HTR3C
5	154,662,498	-5.5996	2.71E-08	0.1482	
1	51,648	-5.5130	4.39E-08	0.1920	

Note: Chr. is chromosome. Negative T statistic indicates that the methylation measure is lower in cases than in controls. Genes located within 275 bp of the CpGs are indicated.

Table S2. (See separate file) MWAS findings in blood with P <1.00e-5

Table S3a. (See separate file) MWAS findings in blood of sex interaction analysis with P <1.00e-5

Table S3b. (See separate file) MWAS findings in blood of MDD status when controlling for antidepressants, with P <1.00e-5

Table S3c. (See separate file) MWAS findings in blood of antidepressant use among MDD cases with P <1.00e-5

Table S4. (See separate file) MWAS findings in BA10 brain with P <1.00e-5

Table S5. (See separate file) MWAS findings in BA25 brain with P <1.00e-5

Table S6. Overview of enrichment tests between MDD related MWAS

Datasets	Number of CpGs mapped	Number of CpGs tested at the 5% threshold	Number of CpGs tested at the 1% threshold	Optimal thresholds	Odds ratio	Corrected *P*	Number of sites followed up*
Blood/BA10	17,100,150	855,008	171,002	5%/1%	1.04	0.0054	9,085
Blood/BA25	17,100,150	855,008	171,002	5%/1%	1.00	0.8267	NA
BA10/BA25	17,536,447	876,822	175,364	5%/5%	1.05	<0.00001	NA
Blood/B-T	21,869,561	1,093,478	218,695	1%/5%	239.0	<0.00001	NA
Blood/Treat	21,869,561	1,093,478	218,695	1%/1%	1.00	0.7500	NA

B-T is blood MWAS where treatment was regressed out. Treat is blood MWAS where antidepressant use was tested in MDD cases only. *All 9,085 sites in the significant overlap between blood and BA10 were followed up in BA25 and in two independent collections of BA10 brain samples.

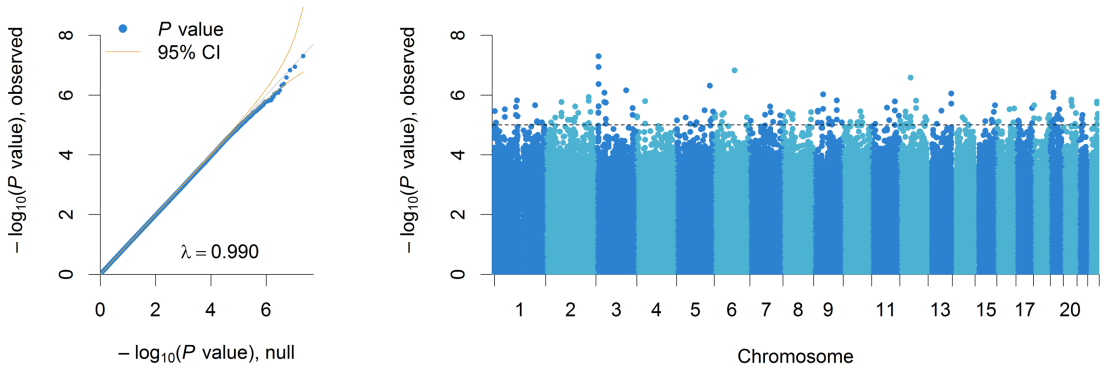
Table S7. (See separate file) Overlapping CpGs among top 1% BA10 brain MWAS results and top 5% blood MWAS results

Table S8. (See separate file) Enrichment testing of the blood-brain overlap and various biological features

Table S9. (See separate file) Overrepresented gene ontology terms with P <0.01 among genes detected from the significant blood-brain overlap

Table S10. (See separate file) Look-up replication of overlapping CpGs in a second brain tissue (BA25) and in two independent BA10 brain collections

Figure S1. QQ-plot and Manhattan plot for MWAS results of MDD status in blood testing for sex interaction



a. Quantile-Quantile plot of the MWAS in blood when testing for sex interaction. The observed *P* values, on a $-\log_{10}$ scale, are plotted against their expected values (grey main diagonal line) under the null hypothesis assuming none of the CpGs have an effect. Yellow lines indicate the 95% confidence intervals (CI). The lambda (λ) is close to one, indicating that markers that are not associated behave as expected under the null hypothesis. **b.** Manhattan plot of the MWAS in blood. The plot shows the MWAS *P* values on a $-\log_{10}$ scale (y-axis) by their chromosomal location (x-axis). The dashed line marks $P = 1 \times 10^{-5}$.

Figure S2a. QQ-plot and Manhattan plot for MWAS results of MDD status in blood when controlling for antidepressant

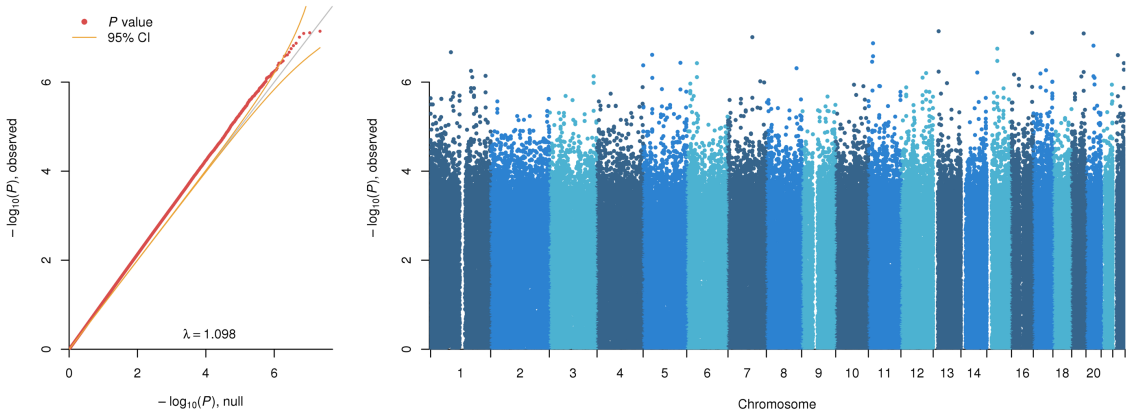
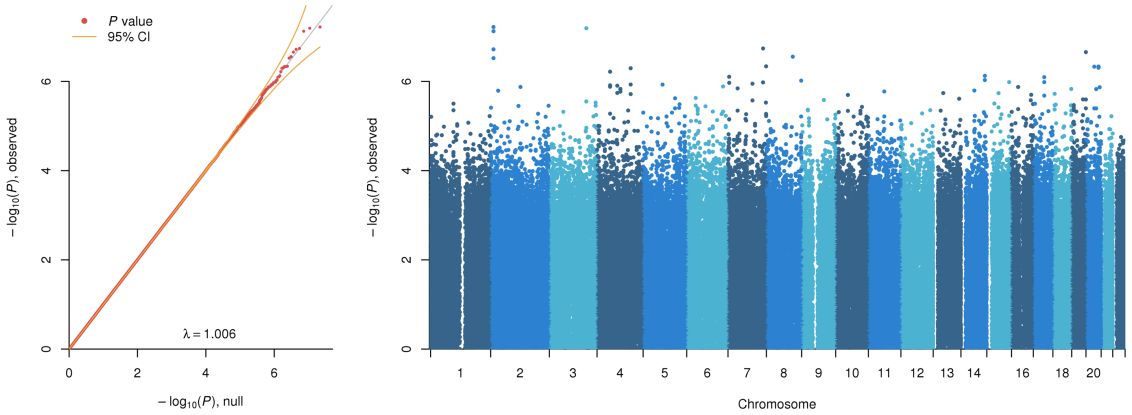
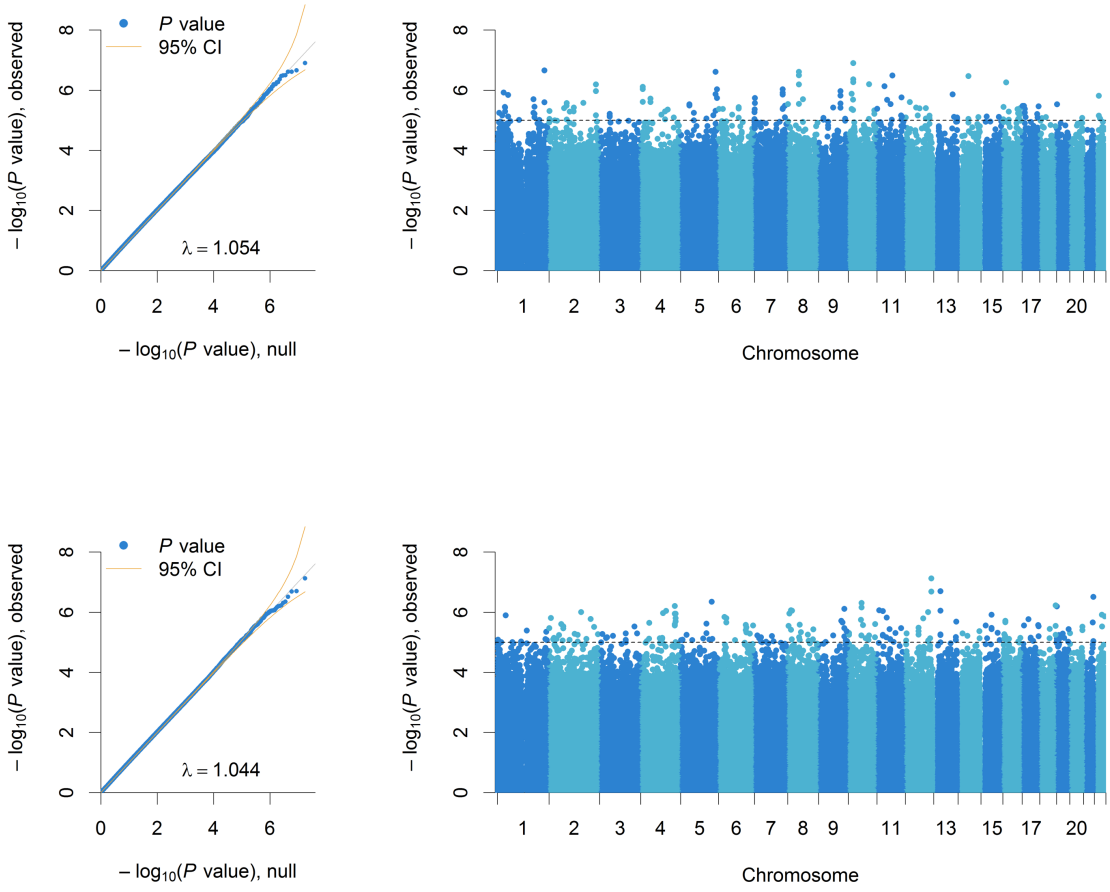


Figure S2b. QQ-plot and Manhattan plot for MWAS results of antidepressant use among MDD cases



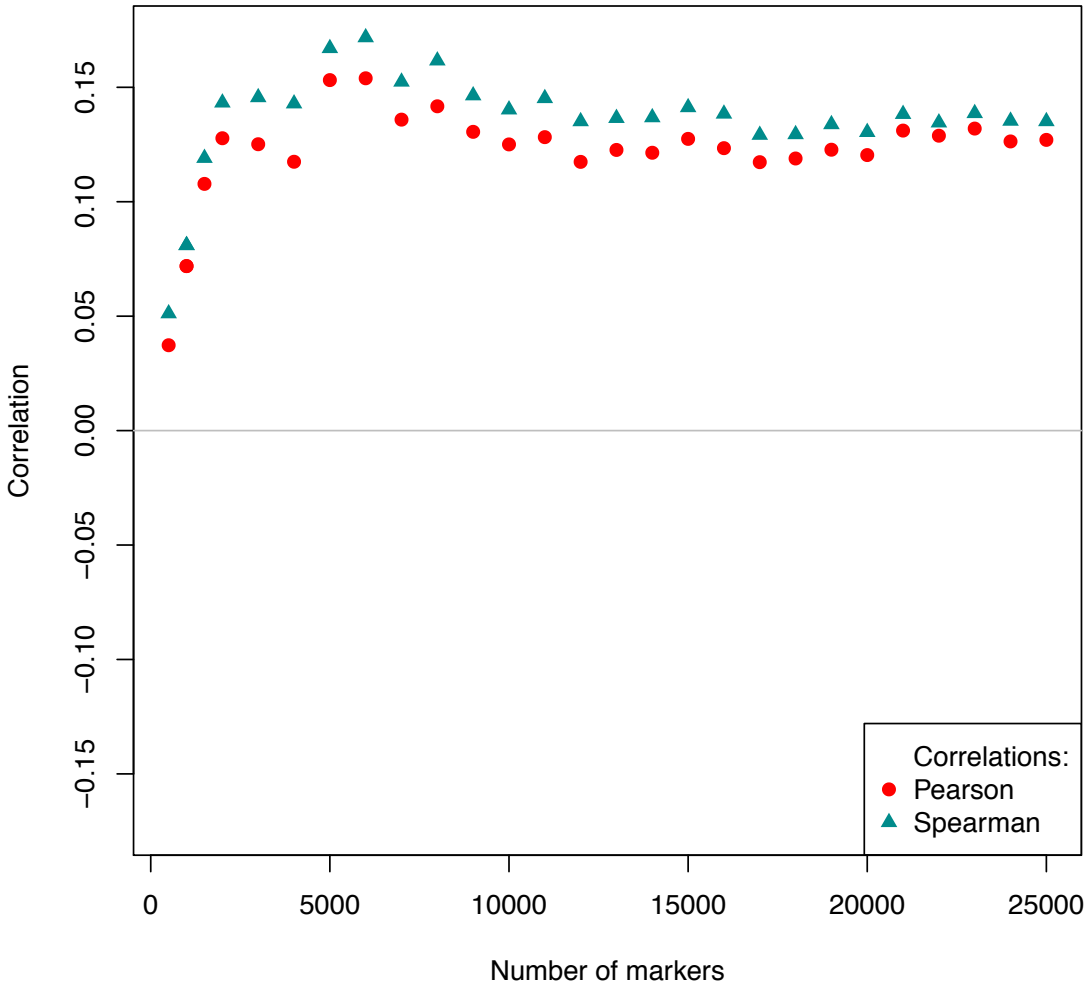
Left. Quantile-Quantile plot of the MWAS results. The observed *P* values, on a $-\log_{10}$ scale, are plotted against their expected values (grey main diagonal line) under the null hypothesis assuming none of the CpGs have an effect. Yellow lines indicate the 95% confidence intervals (CI). The lambda (λ) is close to one, indicating that markers that are not associated behave as expected under the null hypothesis. **Right.** Manhattan plot of the MWAS results. The plot shows the MWAS *P* values on a $-\log_{10}$ scale (y-axis) by their chromosomal location (x-axis).

Figure S3. QQ-plot and Manhattan plot for MWAS results of MDD status in BA10 and BA25 brain tissues



a/c. Quantile-Quantile plot of the MWAS in brain BA10/BA25. The observed *P* values, on a $-\log_{10}$ scale, are plotted against their expected values (grey main diagonal line) under the null hypothesis assuming none of the CpGs have an effect. Yellow lines indicate the 95% confidence intervals (CI). The lambda (λ) is close to one, indicating that markers that are not associated behave as expected under the null hypothesis. **b/d.** Manhattan plot of the MWAS in brain BA10/BA25. The plot shows the MWAS *P* values on a $-\log_{10}$ scale (y-axis) by their chromosomal location (x-axis). The dashed line marks $P = 1 \times 10^{-5}$.

Figure S4. Overview of methylation risk score (MRS) in blood using different numbers of top markers from brain.



The correlation of the methylation risk score (MRS) (y-axis) is shown for up to 25,000 CpG markers (x-axis). Only markers that were in the top of the brain MWAS were used to create the prediction model in the blood samples. After including approximately 5,000 CpGs the point where the predictive power reaches a stable plateau is reached, suggesting this selelection contains the majority of markers with effects in both brain and blood.

References

1. Boomsma DI, Willemsen G, Sullivan PF, Heutnik P, Meijer P, Sondervan D *et al.* Genome-wide association of major depression: Description of samples for the GAIN major depressive disorder study: NTR and NESDA Biobank Projects. *European Journal of Human Genetics* 2008; **16:** 335-342.

2. Penninx B, Beekman A, Smit J. The Netherlands Study of Depression and Anxiety (NESDA): Rationales, Objectives and Methods. *International Journal of Methods in Psychiatric Research* 2008; **17:** 121-140.

3. Sullivan P, de Geus E, Willemsen G, James MR, Smit JH, Zandbelt T *et al.* Genomewide association for major depressive disorder: a possible role for the presynaptic protein piccolo. *Molecular Psychiatry* 2009; **14:** 359-375.

4. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. Fourth Edition edn. American Psychiatric Association: Washington, DC, 1994.

5. World Health Organization. *Composite International Diagnostic Interview (CIDI), Version 2.1*. World Health Organization: Geneva, Switzerland, 1997.

6. Chan RF, Shabalin AA, Xie LY, Adkins DE, Zhao M, Turecki G *et al.* Enrichment methods provide a feasible approach to comprehensive and adequately powered investigations of the brain methylome. *Nucleic Acids Res* 2017; **45**(11)**:** e97.

7. Aberg KA, McClay JL, Nerella S, Xie LY, Clark SL, Hudson AD *et al.* MBD-seq as a cost-effective approach for methylome-wide association studies: demonstration in 1500 case--control samples. *Epigenomics* 2012; **4**(6)**:** 605-621.

8. Shabalin AA, Hattab MW, Clark SL, Chan RF, Kumar G, Aberg KA *et al.* RaMWAS: Fast Methylome-Wide Association Study Pipeline for Enrichment Platforms. *Bioinformatics* 2018.

9. Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt T *et al.* Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. *Mol Psychiatry* 2009; **14**(4)**:** 359-375.

10. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012; **9**(4)**:** 357-359.

11. van den Oord EJ, Bukszar J, Rudolf G, Nerella S, McClay JL, Xie LY *et al.* Estimation of CpG coverage in whole methylome next-generation sequencing studies. *BMC Bioinformatics* 2013; **14**(1)**:** 50.

12. Mbarek H, Milaneschi Y, Hottenga JJ, Ligthart L, de Geus EJC, Ehli EA *et al.* Genome-Wide Significance for PCLO as a Gene for Major Depressive Disorder. *Twin Res Hum Genet* 2017; **20**(4)**:** 267-270.

13. Fedko IO, Hottenga JJ, Medina-Gomez C, Pappa I, van Beijsterveldt CE, Ehli EA *et al.* Estimation of Genetic Relationships Between Individuals Across Cohorts and Platforms: Application to Childhood Height. *Behav Genet* 2015; **45**(5)**:** 514-528.

14. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* 2015; **4:** 7.

15. Das S, Forer L, Schonherr S, Sidore C, Locke AE, Kwong A *et al.* Next-generation genotype imputation service and methods. *Nat Genet* 2016; **48**(10)**:** 1284-1287.

16. Gibbons AS, Brooks L, Scarr E, Dean B. AMPA receptor expression is increased post-mortem samples of the anterior cingulate from subjects with major depressive disorder. *J Affect Disord* 2012; **136**(3)**:** 1232-1237.

17. Keks N, Hill C, Roberts S, Dean B, Opeskin K, MacKinnon A *et al.* Diagnostic instrument for brain studies (DIBS). 1997; **24:** 34.

18. Torrey EF, Webster M, Knable M, Johnston N, Yolken RH. The stanley foundation brain collection and neuropathology consortium. *Schizophr Res* 2000; **44**(2)**:** 151-155.

19. Alt SR, Turner JD, Klok MD, Meijer OC, Lakke EA, Derijk RH *et al.* Differential expression of glucocorticoid receptor transcripts in major depressive disorder is not epigenetically programmed. *Psychoneuroendocrinology* 2010; **35**(4)**:** 544-556.

20. Deep-Soboslay A, Benes FM, Haroutunian V, Ellis JK, Kleinman JE, Hyde TM. Psychiatric brain banking: three perspectives on current trends and future directions. *Biol Psychiatry* 2011; **69**(2)**:** 104-112.

21. Dumais A, Lesage AD, Alda M, Rouleau G, Dumont M, Chawky N *et al.* Risk factors for suicide completion in major depression: a case-control study of impulsive and aggressive behaviors in men. *Am J Psychiatry* 2005; **162**(11)**:** 2116-2124.

22. Liu Y, Popp B, Schmidt B. CUSHAW3: sensitive and accurate base-space and color-space short-read alignment with hybrid seeding. *PLoS One* 2014; **9**(1)**:** e86869.

23. Gage FH, Temple S. Neural stem cells: generating and regenerating the brain. *Neuron* 2013; **80**(3)**:** 588-601.

24. Myers RM, Stamatoyannopoulos J, Snyder M, Dunham I, Hardison RC, Bernstein BE *et al.* A user's guide to the encyclopedia of DNA elements (ENCODE). *PLoS Biol* 2011; **9**(4)**:** e1001046.

25. The ENCODE (ENCyclopedia Of DNA Elements) Project. *Science* 2004; **306**(5696)**:** 636-640.

26. Roadmap Epigenomics C, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A *et al.* Integrative analysis of 111 reference human epigenomes. *Nature* 2015; **518:** 317.