A methylome-wide mQTL analysis reveals associations of methylation sites with **GAD1** and **HDAC3** SNPs and a general psychiatric risk score

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**INTRODUCTION**

Genome-wide association studies have shown that the level of DNA methylation, probably the best-studied epigenetic mark, is partly associated with nearby single-nucleotide polymorphisms (SNPs). An increasing number of SNPs have been associated with the pathogenesis of psychiatric disorders. In this context, genetic variants in various genes, such as **APOE** and **APOC** are repeatedly highlighted. However, the exact mechanisms behind the interplay between methylation levels and polymorphisms are poorly understood. A further elucidation of the interaction between genetic and epigenetic mechanisms may allow a better understanding of the mechanistic role of genetic polymorphisms in development of complex psychiatric diseases.

Brain maturation occurs during adolescence through processes such as synaptic pruning. Normal brain development and function are highly dependent on DNA methylation as stable chemical modification of cytosines in CpG dinucleotides. The importance of these reactions was demonstrated for several cognitive processes, such as learning and memory and neuronal activity. This includes a previous study that showed a relationship between differential DNA methylation and genes involved in γ-aminobutyric acid (GABA)-ergic pathways. In 2013, Domschke revealed that, compared with healthy controls, **GAD1** methylation levels were lower in adults with panic disorder. Interestingly, a previous study showed that individuals homozygous for the major allele of rs4680 (within **COMT**) showed stress-related methylation changes, which was not observed in heterozygotes. Higher levels of DNA methylation repress transcription by inhibiting the binding of transcription factors or by changing microRNA expression. These findings indicate that changes in DNA methylation may contribute to the compensation and/or modulation of interindividual genetic variation and may be able to induce alterations in the complex regulatory landscape of gene expression levels in psychiatric disorders.

For the first time, our study specifically investigates to what extent 37 psychiatric disease-related SNPs are connected to methylation changes (methylation quantitative trait loci (mQTL) analyses) in 223 adolescent individuals showing a differently strong risk for the development of psychiatric illnesses, as evaluated by the Development and Well-Being Assessment (DAWBA) band score. In this context, we further scrutinize whether differentially methylated regions associated with genetic variations influence gene expression, as well as to what extent they modulate the risk for disease development.

**MATERIALS AND METHODS**

**Subjects**

**Discovery data set.** The present study included a total of 130 adolescent volunteers aged between 14 and 16, recruited between November 2012 and January 2013. The included subjects were randomly selected from public school in Uppsala County, with the aim to investigate potential psychiatric risk factors in youth. Self-reported information regarding basic...
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physiological parameters, for example, height, age and medication, was provided by the participants. In addition, body weight was measured for body mass index calculation. This data set served as discovery data set in our study.

Replication data set. In the replication stage, 93 samples from the same study, but characterized later in the time frame 2013–2014, were evaluated. The selection criteria included individuals with a risk for psychiatric diagnoses higher than 15%, aged between 14 and 16 years. The same parameters as in the discovery group were recorded for these individuals. Both studies were approved by the Regional Ethics Committee in Uppsala, and all participants gave their written informed consent.

Expression data set. Eleven healthy male volunteers aged between 18 and 40 years were recruited from the region of Uppsala, Sweden, between 2013 and 2014. Blood analyses were performed before and after a meal intake. The analyses regarding methylation and expression profiles were corrected for proportions of different cell types. More details about this healthy group as well as about preprocessing of the methylation specimens and expression patterns in this cohort can be found elsewhere.

Phenotype assessment

The risk for psychiatric diseases was assessed by performing a DAWBA web-based interview designed for individuals in the age range 5–17 years to generate Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and International Classification of Diseases (ICD)-10-based psychiatric diagnoses. DAWBA consists of two versions of individual standardized questionnaires, administered to adolescents and their parents. Computer-generated diagnostic predictions were only used in the present study. The average of the ‘probability bands’ was computer-assisted generated referring to several diagnoses such as anxiety disorders, depression, post-traumatic stress disorder, autism, separation anxiety disorder and obsessive compulsive disorder. DAWBA diagnoses are given in the range of less than 0.1% to over 70% probability that the individual could experience one of the mentioned DSM-IV or ICD-10-based diagnosis. A more detailed description of DAWBA-level bands and details about validation steps are given in Goodman et al.

Genotyping and DNA methylation

All study participants were genotyped using the Illumina Golden Gate array at the SNP&SEQ SciLife Platform at Uppsala University. On the basis of the literature search regarding associations of risk variants with psychiatric disorders, that is, eating disorders, panic disorder, obsessive-compulsive disorder, major depression and bipolar disorder, and on their inclusion in the microarray-assisted genotyping approach, we investigated a set of 37 SNPs with minor allele frequency higher than 5%. All SNPs were checked for Hardy-Weinberg equilibrium using \( \chi^2 \)-test. SNPs were considered in disequilibrium if \( P < 0.01 \). Each SNP was coded as 0 (homozygous major allele), 1 (heterozygous genotype) or 2 (homozygous minor allele). A dominant model was assumed for the SNPs. Genome-wide DNA methylation analysis was carried out using the Illumina Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA, USA, 450k). Description of experimental procedures can be found in Supplementary File 1.

DNA methylation preprocessing

Preprocessing of methylation data included background correction, probe exclusion, adjustment of type I and type II probes and removal of batch effects. The robust pipeline also included outlier detection by principal component analysis and white blood cell correction (Supplementary File 2). These analysis steps were performed using the lumi, sva, limma, washermelon and FactoMineR packages available in Bioconductor and operable in the R version 3.1.3 software (R is freely available under the GNU General Public License). The experimental flow of the discovery set is presented in Figure 1.

Criteria of sample exclusion

Samples were excluded based on the following criteria: samples that were shown to be outliers in any of the five quality-control plots generated by MethylIAId\(^{19}\) (rotated M versus U plot, overall sample-dependent control plot, bisulfite conversion control plot, overall sample-independent control plot and detection \( P \)-value plot) without changing the default thresholds (0 sample) and samples that were outliers for the first two principal components (1 sample). The procedure was repeated in the same manner for the validation cohort where none of the samples were excluded.

Evaluation of DNA methylation sites and mQTLs in brain and blood

To investigate to what extent epigenetic shifts in whole blood are of functional relevance in the brain, we correlated chromatin marks in brain and blood. On the basis of the availability of chromatin state data derived using Hidden Markov Models (HMMs), the following eight brain tissues were analyzed: brain angular gyrus, brain anterior caudate, brain cingulate gyrus, brain hippocampus, brain inferior temporal lobe, brain substantia nigra and peripheral blood mononuclear primary cells. Data were loaded from Roadmap Epigenomics Project of 37/hg19 version of human genome in the WashU Epigenome Browser. For easier visualization, the 18-state model for the production of the segmentations according to gene-regulatory role was reduced to five regions, indicating the functionality by different colors: red, for active/bivalent active/bivalent/poised transcription start site (TSS); yellow, for active/bivalent/genic enhancer; orange, for flanking bivalent TSS/ enhancer; green, for active transcription; and grey, for repressed polyComb state. Using this reliable tool, information about regions representing chromatin states overlapping significant CpG sites and mQTL was obtained. In addition, potential regulatory effects of the mQTL site and the multiplicity of genes and the specificity of the association with mQTLs were considered by examining long-range interactions. The long-range interaction mapping was derived using chromatin analysis by paired-end tag sequencing libraries from the ENCODE consortium. Four different cell lines, that is, erythrocytic leukemia cells (K562), breast cancer (MCF-7), cervical cancer (HeLaS3) and human colon carcinoma (HCT-116), targeting the transcription factors RNA polymerase II and CCCTC-binding factor (CTCF), an insulator protein with diverse functions,\(^{20}\) were used. Data were downloaded from the WashU Epigenome Browser, 37/hg19 version.

Ubiquitous, tissue-specific and cell type-specific in vivo transcribed enhancers

In order to get information about the in vivo relevance of the CpG sites for expression, we used data produced by the FANTOMS project. Ubiquitous, tissue-specific (brain, blood) and cell type-specific (T cells, neurons) in vivo enhancers as defined by CAGE tags were downloaded from the Transcribed Enhancer Atlas website.\(^{21}\)

Linkage disequilibrium of mQTLs

Linkage disequilibrium (LD) data were obtained using SNAP Proxy Web tool\(^{22}\) with Northern Europeans from Utah (CEU) as the population selection. Two SNPs having \( r^2 = 1 \) and \( D^2 = 1 \) were considered in perfect LD.

Statistical analysis

Beta values of methylation were used for graphical illustration. For statistical analysis, we transformed the beta values to \( M \)-values, which have been shown to be statistically more robust.\(^{23}\) Statistical analyses were performed using Bioconductor, R (version 3.1.3) and SPSS software (version 22; SPSS, Armonk, NY, USA). To guarantee the reliability and power of data analyses, a confirmatory approach was chosen, investigating two independent cohorts with regard to SNP–methylation interaction.

Linear models. The association between SNPs and DNA methylation was tested through linear models using the limma R package, suitable for large-scale methylation studies\(^{16,24}\) applying an empirical Bayes method based on a moderated t-statistic. We assumed a linear model where the \( M \)-values of each individual CpG site \( i \) were used as the quantitative dependent trait and categorical variables, for example, genotype (G) and sex (S), together with continuous variables, for example, age (A), body mass index, PC1 and PC2, were included as covariates. The coefficient \( b_i \) represents the strength of association between continuous traits and variable of interest and \( r \), refers to the unexplained variability.

\[ M_i = a_i + b_i G + b_i S + b_i A + b_i BMi + b_i PC1 + b_i PC2 + e_i \]

All analyses were adjusted for multiple testing using the Bonferroni correction. Adjusted two-sided \( P \)-value \( < 0.05 \) was considered significant. A known limitation of the epigenome-wide association analyses is an inflated
number of false positives.\textsuperscript{25,26} Therefore, the genomic inflation factor (λ) for all SNP-CpG analyses was calculated using the \texttt{estlambda} function of \texttt{GenABEL} R package.\textsuperscript{27} The analyses were restricted to 500 kb up- and downstream of each SNP. The targeted analyses were performed applying a likelihood ratio test, with \texttt{lrtest} function of the \texttt{lmtest} package.\textsuperscript{28} Here, to correct for false positives, two-sided \(q\)-values were calculated using the \texttt{qvalue} package\textsuperscript{29} and values < 0.05 were considered significant.

### RESULTS

#### Study data sets

The outcome of demographic and clinical variables of the discovery and replication set is illustrated in Table 1. The discovery data set was retrieved from 129 adolescents, who were in the majority female subjects. The mean age was 15.33 ± 0.60 years. There were no substantial demographic differences between the discovery and replication groups. The subjects from both cohorts were categorized into two categories according to the level band score of DAWBA, that is, in the ‘Low risk’ \((n=113, \text{respectively}, n=44)\) and ‘High risk’ group \((n=16, \text{respectively}, n=49)\). The DAWBA general band was used as the outcome variable in the analyses, whereas separate symptoms were not analyzed, given the small number of cases in the discovery cohort (Supplementary Table 1A).

#### Effect of cell heterogeneity across samples

Our mQTL analyses were adjusted for cell-type proportions detected in whole blood. To test the reliability of the correction for blood cell proportions, we calculated the first two principal
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Table 1. Discovery and replication data sets’ description

|                      | Discovery set (n = 129) |                | Replication set (n = 93) |                |
|----------------------|-------------------------|-----------------|-------------------------|-----------------|
|                      | Low risk                | High risk       | P-value*                | Low risk       | High risk       | P-value*                |
| Sex (male) n (%)     | 33 (25.6)               | 4 (3.1)         | NS                      | 14 (15.0)      | 5 (5.3)         | **0.019**               |
| Age (years) ± s.d.   | 15.34 ± 0.59            | 15.25 ± 0.68    | NS                      | 15.77 ± 0.61   | 15.70 ± 0.64    | NS                      |
| BMI (kg/m²) ± s.d.   | 21.37 ± 2.89            | 24.68 ± 6.3     | NS                      | 22.23 ± 3.16   | 22.06 ± 3.32    | NS                      |

Abbreviations: BMI, body mass index; DAWBA, Development and Well-Being Assessment. Continuous variables are shown as mean ± s.d. Individuals with a general DAWBA psychiatric risk score below 50% were defined as ‘Low risk’ and included 0 (< 0.1%), 1 (0.5%), 2 (3%) and 3 (15%) level bands of the DAWBA score. Individuals with level bands 4 (50%) and 5 (> 70%), having a risk higher than 50%, were assigned to the ‘High risk’ category. Two-tailed analysis tests the difference between the ‘Low risk’ and ‘High risk’ group using the Student’s t-test for continuous variables and the χ²-test for categorical variables. Bold value signifies P-values < 0.05.

A highly significant positive association was found for the methylation site cg01089319 (GAD1 gene) with two genetic variants of the gene GAD1 (rs2058725 (P = 0.0003) and rs2241165 (P = 2.61e−06)). In addition, cg01089249 showed a strong relationship to the variant rs2241165 (GAD1, P = 1.44e−02). The CpG site represented by cg24137543 was significantly associated with rs2530223 within HDA3 (P = 0.009). All significant identified methylation sites on the genome-wide scale were validated using targeted analyses, except in case of cg18766608, which was not located in the range of 500 kb up- or downstream of rs2058725 (data not shown).

We sought to increase the power of our analyses by confirming our findings in a replication set comprising of 93 individuals. During this step the limma models were restricted to five genetic variants significantly associated with DNA methylation levels in the discovery set. After correction for multiple testing, five SNP–CpG pairs were validated, including the SNPs rs10914453 (HCRTR1), rs2058725 (GAD1), rs2241165 (GAD1) and rs2530223 (HDA3). Four of seven CpG loci were confirmed to have associations with the genetic variants in the same direction as in the discovery data set. All associations with a Pcorr < 0.05, corresponding to P < 1.58 × 10⁻⁷, were considered significant. In line with the results obtained in the discovery data set, the CpG site cg01089319 was significantly associated with the GAD1 SNPs rs2058725 (P = 5.89e−07) and rs2241165 (P = 5.98e−06). The other two associations detected in the discovery group were also confirmed in the replication group (cg24137543 and rs2530223 (P = 7.59e−06); cg01089249 and rs2241165 (P = 1.33e−06; Table 2).

Given our hypothesis that a subset of SNPs are associated with methylation levels, DNA methylation levels were stratified according to the number of minor alleles in the discovery data set (Figure 3). Carriers of the minor allele (G allele) of GAD1 SNPs rs2058725 and rs2241165 showed higher methylation levels at cg01089319 and cg01089249. Conversely, carriers of the ancestral allele at rs2530223 showed higher methylation levels. In the discovery cohort, the inflation factor was below 1, except for one variant at rs2530223 (λ = 1.09). For the replication set all λ values were below 1.

Genomic context of the CpG sites significantly associated with GAD1 and HDA3 mQTLs

The four CpG sites significantly associated with GAD1 and HDA3 SNPs were further investigated with regard to the tissue specificity of the association and their potential functional implication in gene regulation. Therefore, we compared the functional role of these CpG sites in different brain tissues and blood cells with regard to gene regulatory relevance and/or in interaction with nearby genes, offering a broad landscape between chromatin states and potential regulatory activity. All four CpG sites show...
To our knowledge, this is the first study to investigate the association between SNPs and the genome-wide methylation pattern. By further investigating and phenotyping psychiatric-related CpG sites with DAWBA risk scores, the association between methylation levels at validated CpGs and DAWBA risk scores was tested. The analysis supported the association between methylation levels at cg01089319 and gene expression levels, indicating that the associations may not be tissue-specific, as the cg01089319 site is located within important enhancer regions of the tool chromHMM (Figure 4). The second CpG site in the hippocampus did not show significant associations with methylation levels at validated CpGs and DAWBA risk scores. The cg01089319 site was detected in the hippocampus. The CpG site cg24137543 was detected in the hippocampus (Figure 5).

Abbreviations: bp, base pair; Bonf, Bonferroni corrected; NS, nonsignificant; PC, principal component; SNP, single-nucleotide polymorphism; TSS, transcription start site; Unadj., unadjusted. The table shows the associations that remain significant after the Bonferroni correction. All 37 investigated SNPs and the top association CpG using unadjusted analyses are shown in Supplementary Table 2. Associations between SNPs and DNA methylation in the discovery cohort were performed genome-wide using linear models (limma package, R). In the replication data set, analyses were restricted to the significantly associated SNPs and CpGs. Covariates included were age, sex, BMI, PC1 and PC2. Shown are the raw and Bonferroni P-values. *Non-carriers of the coding allele. #Carriers of the coding allele. Bold values signify P-values < 0.05.

**Table 2. Genome-wide significant psychiatric-associated CpG sites**
hypothesized to have a pathogenetic role in psychiatric diseases in adolescents. We detected that the methylation state of eight CpG sites was associated with SNPs and replicated five of these pairs in whole blood, after cell-type confounding and strict Bonferroni correction. These significant variants include the following genes HCRTR1, GAD1, HDAC3 and FKBP5, earlier associated with neuropsychiatric diseases.32 Interestingly, the GAD1 SNP rs2241165 is in perfect LD with rs2270335, which has been previously linked with grey matter loss in childhood-onset schizophrenia.33 Moreover, we extended our analyses on the relationship of the methylation levels with gene expression, identifying associations with GAD1 and COMT expression. Importantly, one of our identified CpG site (cg01089319) within the GAD1 gene is significantly hypermethylated in the individuals with higher general psychiatric risk (DAWBA) score in discovery data set. These findings point that these SNPs are partly reflecting epigenetic regulatory loops, changing the expression of important psychiatric susceptibility genes.

Previous studies revealed that epigenetic shifts appear to be especially of importance for the regulation of pathways involved in neuronal development.34–38 Indeed, in our study on adolescent individuals, we found five psychiatric risk variants to be associated with DNA methylation. One of the most striking finding is the role of these methylation shifts in GAD1 gene expression. GAD1 is a critical enzyme for the synthesis of GABA, the most important inhibitory neurotransmitter in the brain. Here we show that the two GAD1 variants are significantly associated with DNA methylation of the CpG sites cg01089319 and cg01089249, detecting a difference of 3% between carriers and non-carriers of the minor allele. Importantly, chromatin states map the CpG loci in an enhancer region in the hippocampus (Figure 4), an observation that is consistent with the role of GAD1 expression in the hippocampus.39

Alterations in GABA activity have been shown to be a result of lower GAD1 expression induced by epigenetic mechanisms.40,41 Lower GABA concentrations were found in patients with mood disorder, bipolar disorder or depression compared with controls.42–44 Another gene, which might regulate GABA neuronal excitability, is COMT (ref. 45). This enzyme is involved in dopamine inactivation. The exact mechanism of how this neurotransmitter regulates GABAergic activity is, however, unclear. On the basis of our results, we support the hypothesis of a potential functional interaction of COMT and GAD1 in the GABA circuit.5,46 We
identified a significant inverse correlation between methylation levels at cg01089319 (GAD1) and COMT expression. However, we did not observe a change in GAD1 messenger RNA (mRNA) expression. This could be because of the fact that GAD1 undergoes important post-transcriptional modification.\(^57\) The lack of correlations between methylation levels and mRNA profiles can be attributed to the effect of methylation changes on alternative splicing\(^48\) or to an unspecific signal as multiple transcripts are recognized by one probe.\(^49\)

The small difference in DNA methylation at cg01089319 observed is significantly associated with the level of DAWBA general band. Our regression model accounted for 20% of the variation of the risk level measured by DAWBA in the discovery set. The methylation levels at this CpG site are associated with two variants within the GAD1 gene, suggesting an additional mechanism of how these SNPs affect the risk for psychiatric disorders. This finding allows the hypothesis that genetic variants and associated methylation changes are not disorder-specific but associated to several psychiatric disorders.\(^50\)

Importantly, the discovery cohort is a population-based cohort, whereas the replication cohort has a more homogenous composition with regard to DAWBA scores. This might be a possible reason that this association was not validated.

Another variant that was associated with differences in DNA methylation profiles was the exonic SNP rs2530223, within the gene HDAC3. This gene is strongly expressed in the hippocampus.\(^51\) The enzyme HDAC3 was shown to have a role in normal brain development\(^22\) and specifically in long-term memory.\(^53\) Moreover, this protein represses the transcription factor GATA-2 (ref. 54), which modulates GABAergic neurons in mice.\(^57\) The cg24137543 location within an in vivo enhancer region of neurons strengthens the evidence for a potential modulatory effect on γ-Pcdh transcripts in neurons.

The strength of our study is the genome-wide approach, revealing the most relevant changes of the CpG sites. Consistently, we could validate the associations of the identified CpG sites with distinction SNPs by independent local analyses. We specifically investigated 37 SNPs known to be strongly associated with psychiatric diseases. It cannot be excluded that additional SNPs may show similar association patterns with methylation. Although we accounted for different cell-type proportions in blood, the preferred tissue for biomarker analysis, additional studies in brain tissue would provide valuable information about additional tissue-specific SNP/CpG site associations and connected expression changes, which are not reflected in blood. Furthermore, it will be of value to validate the CpG/mQTL associations in larger cohorts, taking changes of the transcriptional expression of associated genes into account. Finally, to increase the power of the analysis on the association between methylation levels and the DAWBA general bands, we combined scores generated exclusively by adolescent and parent questionnaires with scores generated based on both adolescent and parent questionnaires. As the adolescent questionnaire does not cover all behavioral and hyperactivity-type disorders\(^57\) present in the parent questionnaire, this heterogeneity in our primary outcome variable could bias downstream results. However, we adjust for this potential bias by controlling for this difference in our analysis. In addition, the separate analysis including only the complete DAWBA general band score, based on both adolescent and parent questionnaires, confirmed the positive association between level of methylation and DAWBA score. We were specifically interested in analyzing SNP–methylation interactions in individuals at risk for psychiatric diseases in adolescence, encompassing multiple psychiatric diseases and neurobiological changes. Future studies of larger sample size are
Figure 5. Genomic context of the most significant CpG sites (cg08155325 and cg24137543) associated with SNP rs2530223. Genomic positions of RefSeq genes are displayed in the top part, indicated by blue arrows. The positions of the significant CpG sites are highlighted by black lines. For the investigation of the potential regulatory effect of the significant CpG sites on other genes and of the specificity of the associations, long-range interactions were derived from four cell lines targeting two transcription factors. Associations are represented by arcs. Only long-range interactions containing significant CpGs are illustrated. The intensity of the arc is proportional to the strength of the interaction between the two regions. As analyses were performed based on data obtained in blood, chromatin marks overlapping in brain and blood cells were investigated. Chromatin states of eight tissues downloaded from the 37/hg19 WashU Epigenome Browser are illustrated. Each functional role of a segment is indicated by a particular color. BrainAC, brain anterior caudate; BrainAG, brain angular gyrus; BrainCG, brain cingulate gyrus; BrainDPC, brain dorsolateral prefrontal cortex; BrainHIPPO, brain hippocampus; BrainITL, brain inferior temporal lobe; BrainSN, brain substantia nigra; PBMC, peripheral blood mononuclear primary cells; SNP, single-nucleotide polymorphism; TSS, transcription start site.

Table 3. Associations of the significant and validated CpG sites and gene expressions in 11 adult healthy individuals

| CpG site   | Affymetrix transcript IDa | Gene expression | Coefb | P-valueb | Coefc | P-valuec |
|------------|---------------------------|-----------------|--------|----------|--------|----------|
| cg01089319 | NM_000817, NM_001379      | GAD1            | −1.14  | 0.03     | —      | NS       |
|            | NM_001130823, NM_153759   | DNMT1           | —      | NS       | NS     | NS       |
|            | NM_022552, NM_144589      | COMT            | —      | NS       | NS     | NS       |
| cg01089249 | NM_000817                 | GAD1            | —      | NS       | —      | NS       |
|            | NM_001130823, NM_001379   | DNMT1           | —      | NS       | NS     | NS       |
|            | NM_022552, NM_153759      | DNMT3A          | —      | NS       | NS     | NS       |
|            | NM_144589                 | COMT            | —      | NS       | −0.61  | 0.04     |
| cg24137543 | NM_003883                 | HDAC3           | 1.49   | 1.25e−06 | NS     | 0.04     |
|            | NM_018919                 | PCDHGA6         | −1.44  | 0.01     | −0.60  | NS       |
|            | NM_001525                 | HCRTR1          | —      | NS       | NS     | NS       |

Abbreviations: Coef, coefficient; NS, nonsignificant. aAccording to GeneChip Human Gene 2.1 ST Array annotation file. bCorrelations between methylation levels at CpG sites (M-values) and gene expression levels were performed using robust linear regression models. Shown are P-values and coefficients. cCorrelations between methylation levels at CpG sites (M-values) and gene expression levels are performed using Pearson’s correlation analyses. Shown are P-values and coefficients. Bold values signify P-values < 0.05.
needed to further investigate the associations in specific diseases, such as, for example, depression, general anxiety and obsessive-compulsive disorder.

In conclusion, we succeeded to define an epigenetic landscape that associates with genetic markers related to psychiatric diseases and gene expression. Our findings show that the effect of several important psychiatric disease-related SNPs appears at least partly to be the result of associated epigenetic shifts that lead to alterations in GABAergic signaling in the human brain.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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