Genome-wide mapping of genetic determinants influencing DNA methylation and gene expression in human hippocampus

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Emerging evidence emphasizes the strong impact of regulatory genomic elements in neurodevelopmental processes and the complex pathways of brain disorders. The present genome-wide quantitative trait loci analyses explore the cis-regulatory effects of single-nucleotide polymorphisms (SNPs) on DNA methylation (meQTL) and gene expression (eQTL) in 110 human hippocampal biopsies. We identify cis-meQTLs at 14,118 CpG methylation sites and cis-eQTLs for 302 3′-mRNA transcripts of 288 genes. Hippocampal cis-meQTL-CpGs are enriched in flanking regions of active promoters, CpG island shores, binding sites of the transcription factor CTCF and brain eQTLs. Cis-acting SNPs of hippocampal meQTLs and eQTLs significantly overlap schizophrenia-associated SNPs. Correlations of CpG methylation and RNA expression are found for 34 genes. Our comprehensive maps of cis-acting hippocampal meQTLs and eQTLs provide a link between disease-associated SNPs and the regulatory genome that will improve the functional interpretation of non-coding genetic variants in the molecular genetic dissection of brain disorders.

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Understanding the functional complexity of the human brain is a major challenge for the genetic dissection of common brain disorders. Genome-wide association studies (GWAS) successfully identified a large number of susceptibility loci for these disorders\(^1\). However, the majority of associated single-nucleotide polymorphisms (SNPs) are located in non-coding genomic regions and usually their functional effects remain elusive\(^3\). Differential spatiotemporal DNA methylation and gene expression play a key role in normal neurodevelopmental processes and the complex and heterogeneous pathogenesis of brain disorders\(^4,5\). The rapidly evolving mental processes and the complex and heterogeneous pathogenesis of brain disorders 4,5. The rapidly evolving mental processes and the complex and heterogeneous pathogenesis of brain disorders 4,5. The rapidly evolving mental processes and the complex and heterogeneous pathogenesis of brain disorders 4,5. The rapidly evolving mental processes and the complex and heterogeneous pathogenesis of brain disorders 4,5. The rapidly evolving mental processes and the complex and heterogeneous pathogenesis of brain disorders 4,5. The rapidly evolving mental processes and the complex and heterogeneous (LD, pair-wise \(r^2 \leq 0.8\) within a window of 50 SNPs) 344,106 CpG probes and 15,708 \(3^{\prime}\)-RNA expression probes were included in the QTL study. We performed cis-meQTL/eQTL analyses within a cis-window of ±500 kb between SNP genotypes and quantitative methylation rate (\(\beta\)-value) of CpGs or \(3^{\prime}\)-RNA expression levels, using a linear regression model implemented in Matrix eQTL\(^28\). We corrected for gender, age at surgery as well as the proportion of neuronal cells, and adjusted by principal components for population stratification, batch effects and for hidden confounders (see Methods). For cis-QTL analyses, we chose a false discovery rate (FDR) of 1%. CpGs and \(3^{\prime}\)-transcripts with at least one significantly associated SNP (FDR of 1%) were considered as meQTL or eQTL, respectively. The study power was sufficient to detect cis-acting hippocampal QTL-SNPs that

Dysfunction of hippocampal–prefrontal interactions has been implicated in a variety of neurological and psychiatric disorders, such as temporal lobe epilepsy (TLE), Alzheimer’s disease, schizophrenia, and depression\(^26,27\). Given the substantial impact of a polygenic component in the etiology of these common brain disorders, it is of special interest to identify those genetic variants that regulate DNA methylation and gene expression in hippocampal tissue. Accordingly, the present study aims to generate a comprehensive map of cis-acting meQTLs and eQTLs in human hippocampal brain tissue by correlating genome-wide SNP genotypes with high-density CpG methylation and gene expression profiles. Therefore, we took advantage of the unique access to fresh-frozen surgically resected hippocampal biopsies from 110 European patients with pharmacoresistant TLE. By annotating meQTLs and eQTLs to the tissue-specific landscape of regulatory genomic elements characterized by the Encyclopedia of DNA Elements (ENCODE\(^7\) and the NIH Roadmap Epigenomics Consortia\(^8\), we provide deeper insights into the epigenomic regulation of gene expression in human hippocampal tissue. Specifically, the map of hippocampal meQTLs/eQTLs will improve the functional interpretation of SNPs associated with brain disorders.

**Results**

**Study design of meQTL and eQTL analyses.** To explore the cis-regulatory effects of SNPs on DNA methylation and gene expression, we performed genome-wide mapping of cis-acting meQTLs/eQTLs and correlations of CpG methylation and mRNA expression in human hippocampal tissue from 110 European TLE patients (Supplementary Table 1). After stringent array and SNP quality control, 536,041 SNPs pruned by linkage disequilibrium (LD, pair-wise \(r^2 \leq 0.8\) within a window of 50 SNPs), 344,106 CpG probes and 15,708 \(3^{\prime}\)-RNA expression probes were included in the QTL study. We performed cis-meQTL/eQTL analyses within a cis-window of ±500 kb between SNP genotypes and quantitative methylation rate (\(\beta\)-value) of CpGs or \(3^{\prime}\)-RNA expression levels, using a linear regression model implemented in Matrix eQTL\(^28\). We corrected for gender, age at surgery as well as the proportion of neuronal cells, and adjusted by principal components for population stratification, batch effects and for hidden confounders (see Methods). For cis-QTL analyses, we chose a false discovery rate (FDR) of 1%. CpGs and \(3^{\prime}\)-transcripts with at least one significantly associated SNP (FDR of 1%) were considered as meQTL or eQTL, respectively. The study power was sufficient to detect cis-acting hippocampal QTL-SNPs that

![Fig. 1 DNA methylation in context of genetic determination.](image1.png)

**Fig. 1** DNA methylation in context of genetic determination. **a** Distribution of the degree of methylation (\(\beta\)-value) of CpGs. The 14,118 meQTL-CpG sites display a rather intermediate distribution of their median \(\beta\)-values (red) compared to the bimodal distribution observed for all 344,106 CpG sites (black). **b** The relationship of explained variance \(R^2\) to the genomic distance of 14,118 meQTL-SNPs and their associated CpG site.
Cis-meQTL analyses. For an FDR of 1% (P < 9.8 x 10^-6), cis-meQTL analysis identified 66,970 significant SNP-CpG methylation associations at 14,118 CpG sites (Supplementary Data 1). The median \( \beta \)-values across all 344k CpGs displayed a bimodal distribution reflecting an excess of either completely methylated or unmethylated CpGs, whereas the hippocampal cis-meQTL-CpGs showed a rather unimodal and intermediate distribution with a prominent peak at a \( \beta \)-value of 0.86 (Fig. 1a). The median distance between the meQTL-CpGs and the most significantly associated SNP was 11.7 kb (IQR = 3.2–36.7 kb) (Fig. 1b). For explained >10% of the variance of CpG methylation, and >16% of the variance in gene expression, respectively.

We display gene symbols (Ensembl), CpG islands (UCSC), the Roadmap Hippocampus middle (E071) 15-chromatin-states: active TSS (#1 TssA, black), flanking active TSS (#2 TssAFlnk, gray), enhancer (#7 Enh, black), genic enhancers (#6 EnhG, gray) and cis-meQTL-SNPs. The plot was created using coMET69.

Fig. 2 Genomic organization of hippocampal cis-meQTLs at the ADARB2 locus. Regional plot of hippocampal cis-meQTL P-values and CpG co-methylation patterns in the chromosomal region 10p15.3 (chr10:1,130,405–1,815,625, hg19) encompassing the RNA-editing adenosine deaminase-2 gene (ADARB2). We display gene symbols (Ensembl), CpG islands (UCSC), the Roadmap Hippocampus middle (E071) 15-chromatin-states: active TSS (#1 TssA, black), flanking active TSS (#2 TssAFlnk, gray), enhancer (#7 Enh, black), genic enhancers (#6 EnhG, gray) and cis-meQTL-SNPs. The plot was created using coMET69.

Cis-meQTLs generally explained a substantial proportion of the methylation variance ranging from 10.8 to 84.9% (median = 24.0%; IQR = 19.1–34.2%). Remarkably, the proportion of variance explained by cell-type heterogeneity was modest (~5%, range: 0–54%) and relatively low for the age-at-sampling (~3%, range: 0–39%) and gender (~1%, range: 0–31%), respectively (Supplementary Data 1). Notably, 9375 (66.4%) of cis-meQTL-CpGs resided in 4,905 ENSEMBL-73 genes. Overall, 3578 (25.3%) of the hippocampal meQTL-CpGs were located within or nearby 1,140 (73.0%) out of 1561 candidate genes recently implicated for neurodevelopmental disorders.
based on literature and database queries. Several of these high-ranking candidate genes (e.g., ADARB2, GABRB3/GABRA5, HDAC4, NRXN1, RBFOX3, RIMBP2, SLC2A1, and SLC6A1) were covered by more than one cis-meQTL (Fig. 2; Supplementary Data 1). To facilitate the selection of accessible epigenic biomarkers, we performed cis-meQTL analysis of the 14,118 hippocampal meQTL-CpGs in DNA from whole blood cells of 494 German population controls. We observed a moderate correlation between the brain and blood samples (r = 0.33). However, about 66% of the hippocampal cis-meQTL-CpGs displayed significant SNP-CpG methylation associations (FDR of 1%) with similar effect sizes in whole blood cells (Supplementary Data 1).

Co-localization of cis-meQTLs with regulatory genomic motifs. To explore the effects of CpG methylation on hippocampal transcription activity, we examined whether hippocampal cis-meQTL-CpGs were co-localized with epigenic marks annotated by the ENCODE and the Roadmap Epigenomics Projects. We interrogated 189 epigenic marks comprising six hippocampal histone marks and 15 hippocampal chromatin states from Roadmap, 161 transcription factor-binding sites (TFBS), three DNase I hypersensitivity tracks, three UCSC CpG island (CGI) definitions and regions of intermediate methylation in brain. Given distinct differences of the CpG methylation states across the Roadmap 15-core chromatin states (Supplementary Figs. 1 and 2), we compared the frequency of hippocampal cis-meQTL-CpGs within epigenic marks with those obtained in 98,826 non-meQTL control-CpGs matched for median β-values (FDR > 10%, seven-fold match). We found strong relative enrichments of cis-meQTL-CpGs in the flanking genomic regions of active promoters (TssAFlink state, OR = 1.35, P = 1.44 × 10^-27), marked by an enrichment in chromatin immunoprecipitation sequencing (ChIP-seq) peaks of the promoter-associated histone mark H3K4me3 (OR = 1.13, P = 6.85 × 10^-12) and the enhancer-associated H3K4me1 mark (OR = 1.15, P = 5.51 × 10^-13) (Table 1, Supplementary Data 2). Moreover, cis-meQTL-CpGs were enriched at chromatin marks with repressed Polycromb states (ReprPCWk state, OR = 1.19, P = 3.41 × 10^-10) marked by an enrichment in H3K27me3 (OR = 1.15, P = 2.14 × 10^-14). Otherwise, hippocampal cis-meQTL-CpGs were depleted in actively transcribed regions (TX state, OR = 0.60, P = 1.81 × 10^-31) corresponding with a depletion in H3K36me3 (OR = 0.81, P = 2.96 × 10^-26). We observed a strong over-representation of cis-meQTL-CpGs in CGI shores (OR = 1.20, P = 4.04 × 10^-20) and a depletion in neighboring shelves (OR = 0.75, P = 3.21 × 10^-20). A strong enrichment (OR = 1.84, P = 1.26 × 10^-74) of cis-meQTL-CpGs was found in 6.654 regions of intermediate DNA methylation, which encodes a conserved signature of genome regulation. Notably, hippocampal cis-meQTL-CpGs were significantly enriched in the ChIP-seq peaks of the TFBS for CTCF (OR = 1.33, P = 8.3 × 10^-31) (Table 1), whereas significant depletions were observed for 35 out of 161 ENCODE transcription factors interrogated in this study (Supplementary Data 2).

Cis-eQTL analyses. The cis-eQTL analysis on quantile normalized expression values was done in a cis-window of ±500 kb using the linear regression model of Matrix eQTL. The eQTL analysis led to 1337 significant SNP-3′-RNA expression associations (FDR of 1%, P < 4.48 × 10^-6) of 302 expression probes of 288 genes (Supplementary Data 3). The median genomic distance was 35.8 kb (IQR = 13.2–76.2 kb) between the SNP and the 5′-TSS of the corresponding gene. The eQTL-SNP's explained proportions of variance ranging from 16.0–79.7% (median = 25.4%; IQR = 20.1–32.3%). Notably, the proportion of variance explained by cell-type heterogeneity was modest (~4%, range: 0–33%) and relatively low for the age-at-sampling (~2%, range: 0–16%) and gender (~1.4%, range: 0~15%), respectively (Supplementary Data 3).

Correlation of CpG methylation and mRNA expression. For an FDR of 1%, we detected 80 cis-related correlations of CpG methylation and RNA expression, comprising 73 CpGs and 38 mRNA probes, annotating to 34 genes (Supplementary Data 4). The genomic distance between the mRNA transcription start site and CpG site varied between 46 bp and 487.5 kb with a median distance of 11.2 kb (IQR = 0.6–24.6 kb). The CpG methylation levels explained proportions of variance of mRNA expression ranging from 22.3 to 64.2% (median = 27.8%; IQR = 24.7–32.5%). Overall, 70% of the methylation-correlated mRNA expressions were negatively correlated. Particularly, the methylation levels of 30 CpGs in the 5′-regulatory gene regions were predominantly negative correlated with gene expression (OR = 0.267, P = 0.025). The majority (50 out of 80) of methylation-correlated mRNA expressions were based on coincidental eQTLs and meQTLs, of
which 35 pairs of QTL-SNPs were in close LD ($r^2 > 0.4$) indicating a shared genetic driver (Fig. 3).

**LD overlap of cis-eQTL- and meQTL-SNPs in brain tissue.** We tested for a clumping-based LD relationship between hippocampal cis-meQTL-SNPs with the present set of 1223 hippocampal cis-eQTL-SNPs as well as 16,791 cis-eQTL-SNPs identified in 10 adult brain regions recently published by Ramasamy and co-workers. Applying the clumping procedure of PLINK (LD pruning of SNPs with pair-wise $r^2 < 0.25$ within 250 kb, maintaining the most significant QTL-SNP), we created a set of 10,890 quasi LD-independent hippocampal cis-meQTL-SNPs (FDR of 1%) and a set of quasi LD-independent cis-eQTL-SNPs from each eQTL data set (FDR of 1%). For the clumping-based enrichment analysis, we counted the number of 10,890 quasi LD-independent hippocampal cis-meQTL-SNPs which showed pair-wise LD ($r^2 > 0.25$ within 1 Mb) with one of the quasi LD-independent cis-eQTL-SNPs. Likewise, we randomly generated 10^6 sets of 10,890 MAF-matched control-SNPs from 92,308 quasi LD-independent non-meQTL control-SNPs (FDR > 10%). Empirical significance for enrichment was determined by counting the number of control-SNP sets that reached or exceeded the number of hippocampal cis-meQTL-SNPs showing LD (pair-wise $r^2 > 0.25$ within 1 Mb) with the sets of quasi LD-independent cis-eQTL-SNPs. We found significant (empirical $P < 10^{-6}$) relative enrichments of 6.70 of hippocampal cis-meQTL-SNPs in the set of hippocampal cis-eQTL-SNPs and a 3.62-fold enrichment in the set of brain cis-eQTL-SNPs obtained from Ramasamy et al. (Table 2).

**Epigenomic profiling of hippocampal QTL-SNPs.** To prioritize regulatory, deleterious, and disease-relevant QTL-SNPs among numerous LD-correlated QTL-SNPs, we carried out epigenomic profiling of hippocampal cis-meQTL-SNPs ($n = 229,235$ for an FDR of 0.001) and cis-eQTL-SNPs ($n = 16,635$ for an FDR of 0.01) derived from the imputed data set of 3,239,626 autosomal SNPs (MAF > 5%). Therefore, we estimated the relative pathogenicity of hippocampal QTL-SNPs using the Combined Annotation Dependency Depletion (CADD v1.3) framework, which integrates annotations across a wide range of functional categories into a single quantitative score (CADD-Phred score) for each SNP. At a threshold of a CADD-Phred score > 5, we selected 53,409 meQTL-SNPs at 6226 unique CpG sites (Supplementary Data 5) and 3,899 eQTL-SNPs for 304 unique RNA transcripts (Supplementary Data 6). To facilitate the functional interpretation of the CADD5 QTL-SNPs, we implemented genomic annotations using the Ensemble Variant Effect Predictor (VEP) and hippocampus-related epigenetic annotations for the Roadmap ChromHMM 15-states and relevant histone marks. Notably, 997 CADD5 meQTL-SNPs and 42 CADD5 eQTL-SNPs directly matched GWAS risk-SNPs from the GWASdb catalog at a significance level of $P < 5.0 \times 10^{-8}$ (Supplementary Data 5 and 6).

Considering only the QTL-SNPs with the highest CADD score per CpG (best-CADD5 meQTL-SNPs: $n = 6226$; mean CADD score: 12.73, s.d. = 5.32) or RNA transcript (best-CADD5 eQTL-SNPs: $n = 304$; mean CADD score: 14.06, s.d. = 5.19), the distribution of the functional annotations of the CADD5 QTL-SNPs displayed a predominant proportion of QTL-SNPs with genic localizations. Remarkably, a considerable proportion of the best-CADD5 meQTL-missense SNPs create/abolish CpG sites and could be a potential target for allele-specific methylation. We explored potentially regulatory effects of best-CADD5 QTL-SNPs due to their allelic alterations of TFBS affinities predicted by the SNP2TFBS database. In total, 857 out of 6226 best-CADD5 meQTL-SNPs and 44 out of 304 best-CADD5 eQTL-SNPs were predicted to alter TFBSs and the binding affinity of at least one transcription factor (Supplementary Data 5 and 6). We

![Fig. 3](image-url)
observed a four-fold enrichment of CTCF-binding sites that overlap with the best-CADD5 meQTL-SNPs (P = 1.81 × 10⁻¹¹).

### Discussion

Dysfunction of the hippocampal formation and its interaction with the prefrontal cortex plays an important role in neurobiological pathways implicated in a wide variety of human brain disorders. The primary aim of our study was to generate genome-wide catalogs of cis-regulatory meQTLs and eQTLs in human hippocampal tissue and to explore the functional impact of cis-meQTL-CpGs and cis-meQTL/eQTL-SNPs on DNA methylation and gene expression. Here we present comprehensive lists of hippocampal cis-meQTLs at 14,118 unique CpG sites (Supplementary Data 1) and hippocampal cis-eQTLs for 302 3′-RNA probes annotating to 288 genes (Supplementary Data 3). We also provide cis-meQTL/eQTL results for the complete set of 3,239,626 imputed SNPs (accessible online: https://uni-bonn.scielo.de/index.php/s/Njy2o9GKcnZl2pn). Concomitantly, we carried out epigenomic profiling of hippocampal QTL-SNPs to explore their cis-regulatory effects on gene expression and to prioritize pathogenic regulatory SNPs at genetic risk-loci of common brain disorders (Supplementary Data 5 and 6). Besides known rare deleterious coding mutations and structural genomic variations, cis-regulatory SNPs affecting hippocampal gene expression may extend the allelic mutation spectrum of brain disorders. Given that rare causal deleterious gene mutations act on a strong polygenic background and the substantial impact of eQTL-SNPs on tissue- and cell type-specific gene expression, compound heterozygosity of rare loss-of-function mutations and common regulatory cis-eQTL-SNP alleles lowering gene expression could synergistically aggravate haplosufficiency of genes causing brain disorders. The compound recessive mode of action may explain the remarkable phenotypic variability among carriers of loss-of-function gene mutations and could account for a relevant proportion of the missing heritability.

The present catalogs of cis-acting meQTLs and eQTLs were generated from fresh-frozen hippocampal biopsies, whereas the majority of previous studies used post-mortem specimens. Notably, hippocampal biopsies from TLE patients frequently show neuropathological alterations with neuronal cell loss and gliosis. Thus, it is of critical relevance for QTL analyses to correct for confounding factors such as cell-type heterogeneity and other known or unidentified confounders arising from the TLE-related pathology. To correct for cell-type heterogeneity, we included the individual neuronal proportion in our linear regression model for QTL analysis. For the cis-acting hippocampal QTLs (FDR of 1%), the linear regression model revealed a strong impact of SNPs on the variance of CpG methylation and gene expression (average explained variance: 28%), whereas the proportion of variance explained by cell-type heterogeneity was modest (~5%) and relatively low for the age-at-sampling (~2–3%) and gender (~1%), respectively (Supplementary Data 1 and 3). With regard to the wide range of effects attributable to these covariates for each probe-set, we provide the estimated proportion of explained variance of each covariate for each single probe-set (Supplementary Data 1 and 3). This information allows distinguishing probes, for which the CpG methylation or gene expression states are strongly influenced by the covariate of interest. We also applied an independent surrogate variable analysis (ISVA) based adjustment to assess whether our supervised linear model may have missed relevant confounders. The variation covered by the ISVs is mostly represented in at least one of our supervised covariates and principal components (Supplementary Fig. 3). Overall, these analyses confirm the large majority (~88%) of cis-acting meQTLs and eQTLs discovered by the supervised linear model, and emphasize that our QTL findings are not adversely affected by hidden confounding factors (Supplementary Data 1 and 3, Supplementary Fig. 3).

The epilepsy pathology underlying our hippocampal specimens may selectively change methylation and transcription levels of some CpGs and mRNAs. Evidence suggests that interactions between genetic variation and environmental factors may contribute to eQTLs and meQTLs. However, conditional allele-dependent shifts of mRNA transcription levels by gene-by-environment (GxE) interaction seem to affect only a small fraction (0.4%) of the investigated eQTL-genes and explain relatively small proportions of variance of gene expression. To explore the potential influence of the epilepsy status on the present cis-meQTLs/eQTLs, we estimated the proportion of variance of CpG methylation and gene expression attributable to TLE-related clinical factors (number of epileptic seizures, duration of epilepsy, type of antiepileptic medication, and therapy outcome after epilepsy surgery). Compared to the strong impact of SNP genotypes on cis-acting hippocampal meQTLs and eQTLs (FDR of 1%; average explained variance: 28%, range: 11–85%), the average proportion of variance of CpG methylation and gene expression explained by the investigated TLE-related factors was relatively small ranging between 0.4 and 1.1% (range per probe-set: 0.0–14.9%). Moreover, ISV-adjusted QTL analyses did not provide evidence for a substantial effect of epilepsy-related or environmental factors. The epilepsy status thus appears to exert only marginal effects on CpG methylation and gene expression in the QTLs identified in the present study. However, the epilepsy status or environmental factors may induce an upregulation of the expression levels of at least some genes, possibly even in a genotype-dependent manner, which may increase the power to detect epilepsy trait-related eQTLs. This is supported by a cis-eQTL study investigating hippocampus tissue derived from 22 TLE patients and 22 normal individuals, which demonstrated that epilepsy-associated SNPs of an epilepsy GWAS meta-analysis were significantly more enriched with hippocampal cis-eQTLs of

### Table 2 Linkage disequilibrium overlap of cis-eQTL-SNPs with cis-meQTL-SNPs

| Source | Hippocampal cis-eQTL-SNPs | Brain cis-eQTLs |
|--------|--------------------------|-----------------|
| Number of “clumped” cis-eQTL-SNPs | 362 | 7961 |
| eQTL-SNPs overlapping meQTL-SNPs | 201/10,890 | 2196/10,890 |
| Mean (eQTL-SNPs overlapping control-SNPs) | 30.0/10,890 | 606.7/10,890 |
| s.d. (eQTL-SNPs overlapping control-SNPs) | 5.09 | 22.29 |
| Error for the mean | 0.010 | 0.044 |
| Average enrichment | 6.70 | 3.62 |
| Empirical P-value | <10⁻⁶ | <10⁻⁶ |

Overlapping SNPs of the cis-eQTL-SNPs and cis-meQTL-SNPs were identified by the linkage disequilibrium (LD) clumping procedure implemented in PLINK (SNP pair-wise r² > 0.25 within 1Mb). Empirical P-values were derived on the basis of 1,000,000 simulated sets of LD-clumped control-SNPs (non-QTL-SNPs with FDR > 10%; LD clumping: SNP pair-wise r² < 0.25 within 250 kb with reference to the most significantly associated SNP matched for allele frequency.

*Brain cis-eQTL-SNPs were obtained from a recent exon-level eQTL study from 10 adult brain regions.
TLE patients than of normal individuals. In line with this interpretation, we identified a hippocampal cis-eQTL for the protocadherin $PCDH7$ gene (ILMN_1670383, nominal $P = 6.55 \times 10^{-7}$, $R^2 = 21.1\%$), which is expressed in thalamocortical circuits and the hippocampus. The eQTL-SNP rs7674790 (chr4:31149277, hg19, MAF = 34%) is located in the 3′-region of the $PCDH7$ gene and in close LD ($r^2 = 0.90$) with the epilepsy GWAS risk-SNP rs28498976 (chr4:31151357; $P = 5.44 \times 10^{-9}$). Notably, the $PCDH7$ cis-eQTL identified in hippocampal biopsies of patients with pharmacoresistant TLE has not been reported in current brain cis-eQTL catalogs.

Two recent studies reported cis-meQTL maps in post-mortem brain specimens, using the HumanMethylation450 array. For an FDR of 1%, Jaffe and co-workers identified cis-meQTLs at 138,962 unique CpG sites in tissue from prefrontal cortex of 258 adult non-psychiatric individuals. In total, 91.3% (12,894/14,118) of the present hippocampal cis-meQTLs were also found in the set of prefrontal cortex cis-meQTLs. For a conservative Bonferroni-corrected significance threshold, Hannon et al. reported cis-meQTLs at 3243 unique CpG sites in tissue from 166 human fetal brains. Using adult post-mortem tissue from three distinct brain regions (prefrontal cortex, striatum, cerebellum), the majority (83.4%) of fetal brain meQTLs were present in at least one of the investigated adult brain regions and the meQTL effect sizes were highly correlated. For a subset of 1390 fetal brain meQTL-CpGs, which were also investigated in the present study, we observed an overlap of 81.0% with adult hippocampal meQTLs. Taken together, these findings implicate that the majority of hippocampal meQTLs are conserved across adult brain regions and most of them are likely to be developmentally stable. The considerable overlap of brain meQTLs indicates a high replicability and validity of meQTLs in human brain tissue (Supplementary Data 1). In addition, we found that 66.6% of hippocampal cis-meQTL-CpGs represent also meQTLs in whole blood cells (Supplementary Data 1). Likewise, we observed a 55% overlap of hippocampal eQTL-genes with corresponding eQTLs in whole blood cells, based on the Genotype-Tissue Expression (GTEx) database (Supplementary Data 3). Obviously, our screening procedure will preferentially detect those QTLs which display strong allelic effects on CpG methylation and gene expression across the bulk of various hippocampal cell-types. Therefore, it is not surprising that many of the cis-meQTLs/eQTLs detected in hippocampal bulk tissue are not cell type- or tissue-specific. Future studies in single cells will provide deeper insights into cell-type specificity of QTLs in normal and disease-related brain tissue.

Collectively, hippocampal cis-meQTL-CpGs were annotated to 4905 ENSEMBL-73 genes and were located within or nearby 1140 out of 1561 candidate genes recently implicated in neurodevelopmental disorders. To deepen insights into basic regulatory mechanisms of the transcriptional activity in the human hippocampus, we examined whether cis-meQTL-CpGs were co-localized to 189 functional regulatory epigenetic elements annotated in the adult human hippocampus. We identified a hippocampal cis-eQTL signature of gene regulation and exon usage in hippocampal cis-meQTL-CpGs in 6,654 genomic regions characterized by an intermediate methylation state, which has been implicated to encode a conserved epigenomic signature of gene regulation and exon usage. Hip-

| **Table 3** Linkage disequilibrium overlap of schizophrenia-associated SNPs with hippocampal cis-meQTL/eQTL-SNPs |
|---|---|---|
| **Hippocampal cis-QTL** | meQTL | eQTL |
| Number of ‘clumped’ cis-QTL-SNPs | 1806 | 362 |
| GWAS Trait | Schizophrenia | Schizophrenia |
| GWAS P-value threshold | $P < 5 \times 10^{-5}$ | $P < 5 \times 10^{-8}$ |
| Number of ‘clumped’ GWAS SNPs | 1806 | 1806 |
| QTL-SNPs overlapping GWAS SNPs | 352 | 51 |
| Mean (QTL control-SNPs overlapping GWAS SNPs) | 161.43 | 18.28 |
| s.d. (QTL control-SNPs overlapping GWAS SNPs) | 11.72 | 3.96 |
| Error for the mean | 0.023 | 0.008 |
| Average enrichment | 2.18 | 2.79 |
| Empirical P-value | $< 10^{-6}$ | $< 10^{-6}$ |
| | $< 10^{-6}$ | $< 5 \times 10^{-8}$ |

Two significance thresholds were used to select risk-SNPs from a recent large-scale GWAS of schizophrenia. Overlapping SNPs of the cis-QTL-SNPs and GWAS SNPs were identified by the linkage disequilibrium (LD) clumping procedure implemented in PLINK. Empirical P-values were derived on the basis of 1,000,000 simulated sets of LD-clumped control-SNPs (non-QTL-SNPs with FDR > 10%; LD clumping: SNP pair-wise $r^2 < 0.25$ within 250 kb with reference to the most significantly associated SNP) matched for allele frequency.
eQTL-genomes when the comparison was restricted to hippocampal tissue (Supplementary Data 4). In addition, 157 (54.5%) out of 288 hippocampal eQTLs were also eQTLs in whole blood cells in the GTEx eQTL database. This substantial overlap implies that many regulatory SNPs exert more ubiquitous effects on gene expression independent of the tissue source. Correlation analysis between cis-acting hippocampal CpG methylation and 3’-RNA expression revealed 34 genes with methylation-driven gene expression (Supplementary Data 4). Correlation of CpG methylation and gene expression frequently occurred in coincidence of cis-acting hippocampal meQTL and eQTL pairs that often share the same genetic driver SNP (Supplementary Data 4; Fig. 3). Overall, the number of methylation-driven gene expressions identified in our study is remarkably low considering that we have found hippocampal cis-meQTL-CpGs at 4905 ENSEMBL-73 genes. Of note, the HumanHT-12 v3 Expression BeadChip employed in this study detects gene-level expression signals. A considerable proportion of the best-CADD5 meQTL-SNPs and 44 cis-eQTL-SNPs with CADD-scores $>$5 were predicted to alter TFBs (Supplementary Data 5 and 6). Consistent with an enrichment of cis-meQTL-CpGs in the ChiP-seq peaks of CTCF, we found a four-fold enrichment of CTCF binding sites overlapping with the best-CADD5 meQTL-SNPs ($P=1.81\times10^{-11}$). A considerable proportion of the best-CADD5 meQTL-SNPs represents deleterious coding SNPs that also create/abolish CpG sites. SNPs affecting CpG sites account for up to 20% of common SNPs in human genome19,20,53, and were found to be significantly enriched in eQTLs and in trait-associated SNPs. The potentially methylation-sensitive hippocampal QTL-SNPs might exert meaningful susceptibility effects of trait-associated SNPs. Notably, 997 CADD5 meQTL-SNPs and 42 CADD5 eQTL-SNPs directly matched GWAS trait-SNPs from the GWASdb v2 catalog38 at a significance level of $P<5.0\times10^{-8}$ (Supplementary Data 5 and 6). In summary, our catalogs of cis-acting hippocampal meQTLs and eQTLs provide a valuable resource for the scientific community to identify genetic drivers of epigenetic and transcriptional variation in the human hippocampus and will deepen our insights into neurodevelopmental processes and neurobiological pathways involved in brain disorders. The majority of these QTLs appear to be conserved across brain regions and developmentally stable. More than 50% of hippocampal cis-meQTL-SNPs and cis-eQTLs are also detected in blood cells and could be used as easily accessible epigenetic biomarkers. The regulatory influence of SNPs on hippocampal CpG methylation and gene expression will inform the interpretation of GWASs and epigenome-wide association studies of brain disorders. Epigenomic profiling of hippocampal QTL-SNPs and meQTL-CpGs and their predicted alteration of TFBs within brain- and cell type-specific promoters and enhancers will facilitate the dissection of causal regulatory SNPs/CpGs at GWAS risk-loci of brain disorders and will provide valuable functional hints for their leading molecular pathways.

The currently available catalogs of brain eQTLs and meQTLs are incomplete and emphasize the need for larger sample sizes of specimens from diverse brain regions in context of various neurodevelopmental stages and disease states.

Methods

Study participants and surgical specimens. Biopsies of hippocampal tissue from 110 European patients with chronic pharmacoresistant temporal lobe epilepsy (TLE) were collected in the Epilepsy Surgery Program at Bonn University. All epilepsy patients were medically resistant and underwent surgical removal after standardized presurgical evaluation to achieve seizure control54. The clinical parameters of the TLE patients (58 males, 52 females; range of age at seizure onset: 1 to 67 years, average age: 13.1) are summarized in Supplementary Table 1. For each TLE patient, array-based SNP genotyping, gene expression, and methylation were performed in hippocampal brain tissue specimens. Informed and written consent was obtained from all patients. Procedures were carried out in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of the University of Bonn Medical Center (No. 360/12).

Fresh-frozen human hippocampal segments had been surgically removed from identical regions of the hippocampus and were prepared as tissue-slices at cryostat-conditions. All fresh-frozen hippocampal segments were analyzed by an experienced neuropathologist using international standards and a diagnostic classification was established55,56. The majority (>65%) of the hippocampal specimens displayed Ammon’s horn sclerosis. In a smaller proportion of hippocampal specimens, lesional alterations such as cortical dysplasia or tumors were diagnosed. For the extraction of genomic DNA and RNA, we used up to five tissue sections with a thickness of 20 μm. Isolation of total DNA and RNA was conducted using the AllPrep DNA/RNA Micro Kit (Qiagen, Hilden, Germany) using the manufacturer’s recommendations. Quality control of total RNA was monitored by analysis with RNA 6000 nano lab chips on a BioAnalyzer 2100 (Agilent Technologies, Waldbronn, Germany). All used RNA samples showed intact 28S and 18S ribosomal RNA signals and a RNA integrity number of $\geq$ 8.

To dissect causal regulatory QTL-SNPs among LD-correlated QTL-SNPs, we performed epigenomic profiling of cis-acting hippocampal meQTL- and eQTL-SNPs (Supplementary Data 5 and 6; Supplementary Fig. 4). We prioritized potentially regulatory hippocampal QTL-SNPs by estimating their pathogenicity with Ensemble VEP tool57 and CADD-Phred scores36 in context of complementary hippocampus-related epigenetic annotations for the Roadmap ChromHMM 15-states and relevant histone marks. We implemented predictions of potentially regulatory effects of QTL-SNPs due to genetic variation of TFBs affinities using the SNP2TFBS database39. In total, 857 cis-meQTL-SNPs and 44 cis-eQTL-SNPs with CADD-scores $>$5 were predicted to alter TFBs (Supplementary Data 5 and 6). Consistent with an enrichment of cis-meQTL-CpGs in the ChiP-seq peaks of CTCF, we found a four-fold enrichment of CTCF binding sites overlapping with the best-CADD5 meQTL-SNPs ($P=1.81\times10^{-11}$). A considerable proportion of the best-CADD5 meQTL-SNPs represents deleterious coding SNPs that also create/abolish CpG sites. SNPs affecting CpG sites account for up to 20% of common SNPs in human genome19,20,53, and were found to be significantly enriched in eQTLs and in trait-associated SNPs. The potentially methylation-sensitive hippocampal QTL-SNPs might exert meaningful susceptibility effects of trait-associated SNPs. Notably, 997 CADD5 meQTL-SNPs and 42 CADD5 eQTL-SNPs directly matched GWAS trait-SNPs from the GWASdb v2 catalog38 at a significance level of $P<5.0\times10^{-8}$ (Supplementary Data 5 and 6). In summary, our catalogs of cis-acting hippocampal meQTLs and eQTLs provide a valuable resource for the scientific community to identify genetic drivers of epigenetic and transcriptional variation in the human hippocampus and will deepen our insights into neurodevelopmental processes and neurobiological pathways involved in brain disorders. The majority of these QTLs appear to be conserved across brain regions and developmentally stable. More than 50% of hippocampal cis-meQTL-SNPs and cis-eQTLs are also detected in blood cells and could be used as easily accessible epigenetic biomarkers. The regulatory influence of SNPs on hippocampal CpG methylation and gene expression will inform the interpretation of GWASs and epigenome-wide association studies of brain disorders. Epigenomic profiling of hippocampal QTL-SNPs and meQTL-CpGs and their predicted alteration of TFBs within brain- and cell type-specific promoters and enhancers will facilitate the dissection of causal regulatory SNPs/CpGs at GWAS risk-loci of brain disorders and will provide valuable functional hints for their leading molecular pathways. The currently available catalogs of brain eQTLs and meQTLs are incomplete and emphasize the need for larger sample sizes of specimens from diverse brain regions in context of various neurodevelopmental stages and disease states.
SNP genotyping and imputation. SNP genotyping of the 110 genomic DNA samples was performed using the Illumina Human660W SNP array (Illumina, San Diego, CA, USA). SNP were assessed according to the Illumina Human660W Assay_Hugo build 37. Using the bead position manifest version 1 (Illumina, San Diego, CA, USA). Data normalization were extracted using GenomeStudio version 2011.1 and the HumanMethylation450 manifest version 1 (Illumina, San Diego, CA, USA). In pre-phasing was performed using the SHAPEITv2 workflow. Imputation was conducted using IMPUTE2, using the provided 1000 Genomes haplotypes Phase I integrated variant release (v3, March 2012) with a MAF > 1%. The post-imputation SNP genotypes were filtered using SNPsTestv2 and PLINK 1.94. IMPUTE2 genotype probabilities were converted into best-guess genotypes according to the following inclusion criteria: info quality score >0.90, call rate >99%, MAF > 1%, and Hardy–Weinberg equilibrium p >0.001. To diminish redundancy of the imputed SNP data set, an LD-based SNP pruning was carried out (r2 > 0.8 for a window size of 50 SNPs), resulting in 356,041 SNPs.

Preparation and filtering of gene expression profiles. The Illumina HumanHT-12 v3 Expression BeadChip (Illumina, San Diego, CA, USA) was used to assess 3′-mRNA transcription in mRNA samples of 110 hippocampus biopsies. Hybridization of biotin-UTP-labeled cRNA to the expression BeadChips was followed by washing steps as described in the Illumina protocol. The BeadChips were scanned using the Illumina iScan system and RNA expression raw data were quantile normalized on probe level and without background correction using the Expression Module of the GenomeStudio software (v2011.1). The resulting signals were log-transformed and subjected to an additional quality control using the provided ambiguous library of hybridizing expression probes with more than one genomic location according to Illumina and Ensembl v73 gene annotations, and probes containing a SNP with a MAF > 1% according to 1000 Genome Phase 1 and phase 35. We excluded weakly expressed probes having an Illumina detection P > 0.05 in 95% of the samples. After quality filtering, 15,708 expression probes were included in the eQTL analysis.

Preparation and filtering of DNA methylation data. Bisulfite conversion of genomic DNA was applied using Zymo EZ DNA Methylation kit (Illumina, Irvine, CA, USA) according to the manufacturer’s protocol. A total of 500 ng of bisulfite converted DNA was analyzed using the Illumina Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA, USA) according to the manufacturer’s instruction. The images were extracted using GenomeStudio version 2011.1 and the HumanMethylation450 manifest version 1 (Illumina, San Diego, CA, USA). Data normalization and filtering was processed using the R packages “watermelon” and “minfi” according to the protocol described by Lehne et al.51. In brief, intensities of six-probe type bisulfite-converted cDNA was defined by Unique SNP, color channel and M/U type for Infinium type I probes were quantile normalized and intensities with a detection P > 0.05 were set missing. To control for technical bias across samples, a PCA over 220 array control probes was performed. According to the distribution of the PC variance proportions, the first six PCs were added as linear predictors to the regression model. Quality filtering of 47,863 autosomal CpG probes was carried out based on the following exclusion criteria: (i) detection P > 0.01 in at least 5% of samples (n = 4333), (ii) less than three beat counts per probe in at least 5% of the samples (n = 522), (iii) cross-hybridization of the CpG probe to more than one genomic location (n = 29,978%), (iv) SNP (MAF > 1%, 1000 Genome phase 1 and phase 3) and phasing according (phasing 99.7%), (v) CpGs without any SNP (LD-pruned SNP set) within the flanking region of 500 kb (n = 96). After quality filtering, 344,106 CpGs were included in the meQTL analysis.

Statistical quantitative trait loci analyses. For cis-meQTL analysis, we confirmed individual sample identity of the SNP and CpG methylation data sets based on the genotypes of 65 CpG/SNP probes on the HumanMethylation450 array comparison with those of the corresponding type II probes assessed by the Illumina Human660W SNP array. The eQTL and meQTL analyses were performed for an additive linear regression model using the R package Matrix eQTL. Given that the majority of hippocampus biopsies showed neuropathological alterations with neuronal cell loss and gliosis, the inter-sample adjustment for the neuronal proportion as well as known confounders was conducted using IMPUTE2. The neuronal and glial proportion of each hippocampus specimen was calculated using the FASTER R package. To design the model, we first build residuals of the expression and methylation matrix (lin, stats R package) over the known confounders: neuronal proportion, age, and gender. Moreover, the first three Eigenstrat PCs from SNP genotype data were included to control for ethnic differences. For the meQTL model, the six PCs from the 220 array control probes were added to control for technical bias. Subsequent principal component analyses (PCAs) on residuals were calculated using prcomp (stats R package). PCA for RNA expression resulted in a variance proportion of 17.1% and 11.9% for the first two principal components (PCs), respectively. SNP and eQTL data were filtered for expression Richmond et al.62 and mindy 63 according to the following criteria: i: correlation, r2 > 0.8 for a window size of 50 SNPs, resulting in 536,041 SNPs.
**Epigenomic profiling of hippocampal QTL-SNPs.** For the prioritization of potentially regulatory hippocampal cis-QTL-SNPs, we selected 229,235 cis-QTL-SNPs with an FDR of 0.001 and 16,635 cis-QTL-SNPs with an FDR of 0.01, which were derived from the QTL results of the imputed data set of 3,239,626 autosomal SNPs (MAF ≥ 5%) to achieve a best possible SNP coverage of the QTL-associated LD blocks. Next, we estimated the relative pathogenicity of hippocampal cis-QTL-SNPs using the Combined Annotation Dependent Depletion (CADD V13) framework. A threshold of a CADD-PHEd score > 5, we selected 33,409 cis-QTL-SNPs at 6226 unique CpG sites and 3899 cis-eQTL-SNPs for 304 unique RNA transcripts for functional epigenomic profiling, using genomic annotations from the Ensemble Variant Effect Predictor (VEP), and hippocampus-related (EHT) Roadmap Epigenomics annotations for ChromHMM 15-state and histone marks, and the ENCODE transcription factor ChiH-seq peaks of CTCF and POLR2A. Co-localization of hippocampal CADDS5 cis-QTL-SNPs with TFBs and allelic alterations of the binding affinities were predicted by the SNP2TFBS database. In addition, we matched overlapping hippocampal CADDS5 cis-QTL-SNPs with GWAS trait-SNPs from the GWASdb v2 catalog at a significance level of P < 10^{-5}.

**Data availability.** Due to data protection issues, the raw data cannot be made publically available. However, individual researchers may request to use the data for specific projects on a collaborative basis. Inquiries should be made to S.C., A.B., and T.S. The full results of the eQTLmeQTL study are available at: https://true-bonn.sciendo.de/index.php?/Nnjzog9GKcmZlZ2pm

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Author contributions

A.-K.R., A.J.F., S.S., S.M., A.H., N.F. and H.V. generated gene expression and methylation data or provided tissue and phenotype data. H.S., A.K.R., S.H., C.W., N.-M.S., O.S., D.C., R.P. and B.M-M. performed statistical analyses. H.S., A.K.R., P.H., T.S. and S.C. wrote the manuscript. A.J.B., P.H., M.M.N. and T.S. and S.C. directed the project and designed the strategies.

Additional information

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