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Investigation of *MORC1* DNA methylation as biomarker of early life stress and depressive symptoms

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Abstract

Early life stress (ELS) is associated with an increased risk of depression and this association may be mediated by epigenetic mechanisms. A previous epigenome-wide DNA methylation (DNAm) study investigating human newborns and two animal models of ELS suggested that the epigenetic regulator *MORC1* is differentially methylated following ELS. The ELS-induced DNAm alterations were long-lasting in the animal models. However, whether this finding is also transferable to humans experiencing ELS in childhood was not investigated. Further, *MORC1* may provide a link between ELS and adult depression, as *MORC1* DNAm and genetic variants were found to be associated with depressive symptoms in humans. In the present study, we investigated

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Availability of data and material

The Tuebingen dataset used in the current study is available from the corresponding author on request. The raw methylation data and all related phenotypes for the Grady Trauma Project cohort have been deposited into NCBI GEO (GSE72680).

the validity of *MORC1* DNAm as a biomarker of ELS in humans and its role in linking ELS to depression later in life by studying childhood maltreatment. We analyzed whole blood *MORC1* DNAm in an adult cohort (N=151) that was characterized for both the presence of depressive symptoms and childhood maltreatment. Further, we investigated the association between *MORC1* DNAm, depressive symptoms and childhood maltreatment in two additional cohorts (N=299, N=310). Overall, our data do not indicate an association of *MORC1* DNAm with childhood maltreatment. An association of *MORC1* DNAm with depressive symptoms was present in all cohorts, but was inconsistent in the specific CpG sites associated and the direction of effect (Tuebingen cohort: standardized β=0.16, unstandardized β=0.01, 95% CI [−0.0004, −0.0179], p=0.061, PReDICT cohort: standardized β=−0.12, unstandardized β=−0.01, 95% CI [−0.0258, −0.0003], p=0.045), Grady cohort: standardized β=0.16, unstandardized β=0.008, 95% CI [0.0019, 0.0143], p=0.01). Our study thus suggests that peripheral *MORC1* DNAm cannot serve as biomarker of childhood maltreatment in adults, but does provide further indication for the association of *MORC1* DNAm with depressive symptoms.

Keywords

Epigenetics; DNA methylation; childhood trauma; early life stress; depression

Introduction

DNA methylation (DNAm) is a chemical modification of cytosine bases in the DNA and a highly conserved epigenetic mark (Law and Jacobsen, 2010). It is present throughout the genome, but most frequently found and best studied in the context of CpG dinucleotides (Jones, 2012). DNAm affects gene transcription by changing chromatin structure and modifying the affinity to transcription factors and other DNA-binding proteins (Boyes and Bird, 1991; Eden and Cedar, 1994; Kass et al., 1993). DNAm in somatic tissues, including blood, is dynamic to some extent (Ziller et al., 2013). Factors influencing blood DNAm are cell type composition, genetic variants, age, sex, and also environmental exposures and lifestyle factors, such as smoking, nutrition and stress (Lam et al., 2012). Therefore, blood DNAm has been extensively studied as a predictor and biomarker for diseases that are characterized by a strong influence of environmental risk factors, such as stress-related psychiatric disorders (Cortessis et al., 2012; Klengel et al., 2014). For example, many studies report associations between blood DNAm and major depressive disorder (MDD) (Menke and Binder, 2014). Data from family and twin studies suggests that the heritability of MDD is approximately 40 %, while the remaining variance is attributed to environmental risk factors (Rice et al., 2002).

One important environmental risk factor for adult depression is early life stress (ELS) (Pietrek et al., 2013; Syed and Nemeroff, 2017), a broad term describing various forms of stress experienced early in life. Different types of ELS range from maternal stressors affecting the developing fetus including *in utero* exposure to harmful substances, childhood maltreatment or traumatic experiences early in life. The risk of depression later in life is particularly increased by the occurrence of childhood abuse, neglect, lack of caregiver, and parental loss (Brown et al., 1999; de Carvalho Tofoli et al., 2011). This effect is believed, in

part, to be facilitated through long-lasting influences of ELS on the endogenous stress regulation system, the hypothalamus-pituitary-adrenal axis (de Carvalho Tofoli et al., 2011), and through immunological effects (Baumeister et al., 2016; Slavich and Irwin, 2014). It has been proposed that this translation of psychological stressors to long-lasting, biological changes is partly mediated by DNAm (Hornung and Heim, 2014).

In support of this theory, recent studies have identified a number of DNAm loci associated with ELS (Bustamante et al., 2016; Kang et al., 2013; Klengel et al., 2013; Nieratschker et al., 2014; Weder et al., 2014). These were found within genes that are known to be relevant to the pathogenesis of depression (i.e. *SLC6A4* (Kang et al., 2013)) or the stress response (i.e. *NR3C1* (Bustamante et al., 2016), *FKBP5* (Klengel et al., 2013)), and also within genes that have no prior connection to stress-related psychiatric disorders (Labonte et al., 2012; Nieratschker et al., 2014; Suderman, 2014; Weder et al., 2014; Yang et al., 2013). One of these, *MORC1*, was identified in a cross-species epigenome-wide association study (EWAS), where its promoter was hypomethylated in rats, rhesus macaques, and human newborns that had experienced different forms of ELS (Nieratschker et al., 2014). The differential methylation was present in the brain and in peripheral tissue (blood cells). *MORC1* is a highly conserved nuclear protein that is increasingly recognized as an epigenetic regulator (Li et al., 2013). In plants, the *MORC1* homolog *AtMORC1* plays an important role in heterochromatin condensation and gene silencing (Dong et al., 2018) and accumulating evidence suggest that it is implicated in gene silencing in humans and animals as well (Koch et al., 2017). Although human *MORC1* was initially believed to be expressed exclusively in male testis, it is now known that *MORC1* mRNA is present in nearly all tissues (Fishilevich et al., 2016). The protein may be linked to neuropsychiatric disorders, as has been suggested for other members of the MORC protein family (Boukas et al., 2018). Providing further support for this, *MORC1* was found to be genetically associated with MDD in a European study cohort (N=1968) (Rietschel et al., 2010). Animal studies confirmed this association by showing that *MORC1* knockout mice display a depression-like phenotype (Schmidt et al., 2016). In addition, epigenetic modification of *MORC1* has been linked to depression by a study showing that *MORC1* promoter methylation in buccal tissue was associated with depressive symptoms in healthy adults (N=60) (Mundorf et al., 2018). Even though additional evidence is needed, these data suggest that *MORC1* may provide a link between ELS and depression later in life (Nieratschker et al., 2014). In support of this, assessment of DNAm across different time points in the animal models indicated that the established ELS responsive *MORC1* DNAm pattern persisted into adulthood. However, the stability of the differential *MORC1* DNAm in response to ELS was only investigated in animals and it has not yet been studied whether this pattern persists into adulthood in humans (Nieratschker et al., 2014). Therefore, we investigated *MORC1* promoter DNAm in blood in a cohort of N=151 depressive patients and healthy controls and evaluated the association of DNAm with depressive symptoms, as assessed by the Beck Depression Inventory (BDI-II) and ELS, as assessed by the Childhood Trauma Questionnaire (CTQ). In addition, we acquired *MORC1* DNAm data from a subset of the Grady Trauma Project Cohort (N=310) (Gillespie et al., 2009) and the PReDICT cohort (N=299) (Dunlop et al., 2012) to validate our findings.

Methods

In all steps of the analysis, samples were processed in balanced design in order to avoid batch effects.

MORC1 targeted bisulfite sequencing of human blood samples:

Study population: N=151 participants were included in the study. Depressive patients were recruited at the Department of Psychiatry and Psychotherapy, University of Tuebingen and had received a diagnosis of major depression by trained psychiatrists and/or psychologists according to the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 2013). Healthy controls were recruited via public announcements and screened to be free of any current and/or history of mental illness using the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). Study participants were phenotypically characterized with the self-report questionnaires Symptom Checklist 90 - Revised (SCL90R) (Franke, 2002), Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003) and Beck Depression Inventory II (BDI-II) (Beck, 1996). Further questionnaires assessed demographic information and information about smoking behavior. Complete blood counts were available from 56 % of the participants. All subjects were of Caucasian origin and gave written informed consent prior to participation in the study, which was approved by the ethics committee of the University of Tuebingen and was conducted in accordance with the Declaration of Helsinki. There was no overlap between the present sample and previous study cohorts where differential *MORC1* DNAm had been analyzed (Mundorf et al., 2018; Nieratschker et al., 2014).

Sampling and DNA extraction: Venous blood was obtained from all participants at study inclusion, collected in Ethylenediaminetetraacetic (EDTA) tubes and stored at −80 °C until further analysis. DNA was extracted using the QIAamp DNA Blood Maxi-Kit (Qiagen).

DNA methylation analysis: We selected five different regions within the promoter of the *MORC1* gene covering 56 CpG sites for methylation analysis (1.5kb upstream and 0.5kb downstream of the transcription start site, Chr3:108836490-108838489, hg19). The primer sequences, amplicons and genomic location of the analyzed CpG sites relative to the region analyzed by the previous study of Nieratschker and colleagues (Nieratschker et al., 2014) can be found in the supplementary material (Supplementary Data S4 and S5). Targeted bisulfite sequencing and processing were performed as previously described in Roeh and colleagues (Roeh et al., 2018). Briefly, sample DNAs as well as five methylated DNA standards (0, 25, 50 ,75, 100 % methylated) were bisulfite converted in triplicate using the EZ DNA Methylation Kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions and then pooled. PCRs were performed for each sample (primer sequences in Supplementary Data S2) using the PyroMark PCR Kit (Qiagen), and successful amplification was verified via agarose gel electrophoresis. The five amplicons per sample were pooled equimolarly and purified with Agencourt AMPure XP beads (Beckman Coulter GmbH, Krefeld, Germany) to remove excess primers and genomic DNA. The Illumina

TruSeq DNA PCR-Free HT Library Prep Kit (Illumina, San Diego, CA. Cat. No. 20015963) was used to prepare sequencing libraries (500 ng of bisulfite DNA per sample) according to the manufacturer's protocol. Samples were multiplexed and indexed using Illumina TruSeq DNA CD Indexes (Illumina, San Diego, CA. Cat. No. 20015949). Quality assessment and quantification of the libraries were performed using an Agilent's 4200 TapeStation (Agilent Technologies, Waldbronn, Germany) and the Kapa HIFI Library quantification kit - KK4824 (Kapa Biosystems Inc., Wilmington, MA. Cat. No. 07960140001). Libraries were then pooled equimolarly and treated with an adaptor blocking reagent before the denaturation step (Illumina Free Adapter Blocking Reagent. Cat. No. 20024144) to reduce index hopping rates. Sequencing was performed on an Illumina MiSeq using the Reagent Kit v3 – 2 x 300 bp paired-end reads (Illumina, San Diego,CA; Cat. No. MS-102-3003), with 30 % PhiX added. Read quality was verified using FastQC (Andrews, 2010), and adapter sequences were trimmed using cutadapt v.2.0 (Martin, 2012). Bismark v.0.20.0 was used to align reads to the reference sequence (Krueger and Andrews, 2011). Methylation levels were quantified using the R package methylKit (Akalin et al., 2012) with a minimum quality Phred score of 30. Two samples were excluded because they had less than 95 % bisulfite conversion efficiency (as assessed by C/T ratio in non-CpG context). A standard curve and regression line based on methylated standards was calculated for each site, and sites with $R^2 < 0.95$ were excluded from the analysis (six sites). All sites and samples had a median coverage > 1000x.

Statistical analysis: Statistical analysis was performed using R version 3.5.1. Multiple linear regression was used to predict DNAm at each CpG site, using CTQ and BDI scores as predictors and sex, age and nicotine consumption as covariates. When predicting DNAm from CTQ scores, BDI was treated as covariate and vice versa. Strength of the association was assessed by calculating partial correlation estimates using the R package RVAideMemoire (Hervé, 2019). For each CpG site, samples more than three times the interquartile range away from the 25th and 75th percentile were classified as outlier and excluded from the analysis. Correction for multiple testing for the number of CpG sites tested per dataset was performed using false discovery rate adjustment (FDR). To identify potential confounding effects of cellular blood composition, the correlations between blood cell counts and predictor variables (BDI and CTQ) were assessed in the 66 % of samples where information about blood cell composition was available, using the first principal component (PC) derived from lymphocyte, monocyte and neutrophil percentages. There was no significant correlation between blood composition and the predictors of interest. To compare the three datasets Tuebingen, PReDICT and Grady, a random-effects meta-analysis was performed on the standardized regression coefficient of interest using the R package meta (Schwarzer, 2007) and Empirical Bayes as τ^2 estimator.

MORC1 methylation analysis in Grady and PReDICT cohort

Study population: The GRADY cohort was recruited at the Grady Memorial Hospital in Atlanta, Georgia (Davis et al., 2008). All participants were from an urban population of low socioeconomic status and characterized by high prevalence and severity of trauma over the lifetime (Gillespie et al., 2009; Zannas et al., 2015). The PReDICT cohort consisted of treatment-naïve patients (18-65 years) with moderate-to-severe MDD, recruited at two sites

in Atlanta associated with the Emory University School of Medicine (Dunlop et al., 2012). All participants met criteria for current major depressive disorder (MDD) as defined in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) (American Psychiatric Association, 2000). All subjects provided written informed consent and ethical approval was given by the Institutional Review Board or Ethical Committee of each site participating in every study. Subjects were characterized for depressive symptoms by the BDI-I (PReDICT) or BDI-II (Grady) and for ELS by CTQ. Smoking scores in each cohort were calculated according to Elliott *et al.* (Elliott et al., 2014). From both cohorts, we selected all participants of which depression questionnaire, CTQ and DNAm was present. This resulted in 310 samples from Grady and 299 samples from PReDICT.

DNA methylation data: Whole blood DNA was extracted using standard techniques. DNA methylation was measured using Illumina Infinium HumanMethylation450K BeadChips. Beta values were normalized using functional normalization (Aryee et al., 2014; Fortin et al., 2014). Batch effects were removed using ComBat (Johnson et al., 2007). Subsequently, all CpGs on sex chromosomes and CpGs with single nucleotide polymorphisms (SNPs) in the probe sequence were removed. Additionally, probes were removed if the detection p-value was > 0.01 in at least 25% of the samples, the probe contained SNPs in the single base pair (bp) extension or CpG position, the probe had missing beta values, or was a cross-reactive probe (Chen et al., 2013). We used the Houseman method to estimate cell type composition (Houseman et al., 2012). Methylation data were adjusted for cell type composition using probe-wise multiple linear regression normalization, wherein all principal components generated from the estimated cell type compositions were used as predictors. Modelling and regression were performed using the R package limma (Ritchie et al., 2015). Only CpG sites within the *MORC1* promoter were selected for analysis (12 sites).

Genotype analysis: In all cohorts, genome-wide SNP genotyping was performed using Illumina HumanOmniExpress BeadChips and subsequently exported via Illumina's GenomeStudio. Quality control in all cohorts was independently performed in PLINK. Samples with low genotyping rate (< 98%) were removed. SNPs with high rate of missing data (> 2%), significant deviation from the Hardy-Weinberg equilibrium (HWE, $p < 10^{-5}$), or a low minor allele frequency (MAF < 5%) were excluded from further analyses. Afterwards, we performed MDS-analysis on the qc-ed genotypes.

Statistical analysis: Statistical analysis was performed equivalently to the analysis for the Tuebingen cohort. To assess for effects of genotype, we performed PCA on the genome-wide SNP data and included the first two axes of variation (PCs) as covariates. Additional covariates were sex, age and smoking behavior.

Results

I. Cohort statistics

The three cohorts were similar with regards to CTQ total score and percentage of study participants with moderate to severe abuse/neglect in one or more subcategories. The

average BDI total score was in the range of mild depression for the Tuebingen (full cohort: 17.5 ± 15.8; diagnosed depression: 27.7 ± 13.2, healthy controls: 4.3 ± 6.4) and Grady cohort (15.6 ± 12.6), and slightly higher (moderate depression) in the PReDICT cohort (22.7 ± 7.2). Further, there were differences in sex distribution and percentage of cases with diagnosed depression, with PReDICT containing only cases with diagnosed depression as compared to 56.3 % in the Tuebingen cohort (data not available in Grady). The Grady cohort contained the lowest amount of female participants (25.5 % vs. 57.5 % and 66.9 % in PReDICT and Tuebingen) (Table 1). Figure 1 shows that the distribution of study participants with regards to CTQ and BDI total score was most balanced in the Tuebingen cohort. The PReDICT cohort had a similar spread of CTQ scores, but a very narrow window of BDI values with low variance and a mean above the threshold for moderate to severe depression. Study participants in the Grady cohort were more balanced. In addition, this cohort contained more extreme cases (CTQ > 75 or BDI > 40).

II. No association of *MORC1* DNAm with ELS

CTQ total score was not a significant predictor of DNAm at any CpG site tested within the *MORC1* promoter, neither in the Tuebingen cohort, nor in PReDICT or Grady. The same was found for mean DNAm across all analyzed *MORC1* CpG sites (12 sites in PReDICT and Grady, 50 in Tuebingen cohort) (Fig. 2). The interaction between lifetime diagnosis of depression and ELS on *MORC1* DNAm could only be tested in the Tuebingen cohort and did not show any significant interaction (data not shown).

III. Association of *MORC1* DNAm with depressive symptoms

Depression score was a nominally significant predictor of DNAm at one CpG site within the *MORC1* promoter in the Tuebingen cohort and five sites within Grady (Table 2) (see Supplementary Data S4 and S5 for an overview of genomic location of these sites and those analyzed in previous studies (Mundorf et al., 2018; Nieratschker et al., 2014)). Two sites in the Grady cohort withstood FDR-correction. The mean DNAm across all analyzed sites was significantly associated with the depression score in PReDICT and Grady. However, this association was negative for PReDICT (standardized β=−0.12, unstandardized β=−0.01, 95% CI [−0.0258, −0.0003], p=0.045), and positive for Grady (standardized β=0.16, unstandardized β=0.008, 95% CI [0.0019, 0.0143], p=0.01). In the Tuebingen cohort, the mean DNAm was not significantly associated with depression score, though there was a trend towards a positive association (standardized β=0.16, unstandardized β=0.01, 95% CI [−0.0004, −0.0179], p=0.061) (Fig. 3). The absolute differences in DNAm between the group of individuals without depressive symptoms and those with severe depressive symptoms were small (< 2 %) (Supplementary Data S6). A random-effects meta-analysis for the effect of depressive symptoms on mean DNAm in the three datasets was not significant (standardized β estimate = 0.06, p=0.58).

The detailed results of all analyses are in the supplementary materials (Supplementary Data S1). Age, sex, smoking and genotype (if available) were included as covariates in all models. Of the identified hits, only one CpG site (Chr.108836796) was also significantly associated with one of the covariates (age) and this effect was only present in the Tuebingen cohort (standardized β=−0.21, unstandardized β=−0.02, 95% CI [−0.0343, −0.0182], p=0.048).

Further, age was a significant predictor of mean DNAm in the Grady cohort (standardized β=--0.15, unstandardized β=−0.01, 95% CI [−0.0135, −0.0022], p=0.048).

Discussion

Our study is the first to investigate the effect of childhood trauma on blood *MORC1* DNAm in adults and its potential link to depression. Although we did not find evidence for an association of *MORC1* DNAm with childhood trauma, our data indicates that *MORC1* DNAm status is associated with current depressive symptoms. It was previously reported that *MORC1* was differentially methylated in cord blood cells of newborns whose mothers had experienced stress during pregnancy, in the T cells of rhesus macaques subjected to maternal separation, and in prefrontal cortex tissue of adult rats whose mothers had experienced chronic restraint stress during pregnancy (Nieratschker et al., 2014). These combined findings provided reason to hypothesize that *MORC1* might be a general, system-wide indicator of ELS that is valid for different types of stressors in multiple species and persists into adulthood. However, our results do not support this hypothesis among human adults that experienced ELS. Important differences between the current and previous studies are the method of DNAm analysis, the type and timing of stressor in the human cohort (prenatal vs. postnatal), and the type of tissue investigated. While previous results had been generated using methylated DNA immunoprecipitation (MeDIP) (Mohn et al., 2009), the results presented here were generated using single CpG site resolution methods, such as high-accuracy targeted bisulfite sequencing (Roeh et al., 2018) and 450k Illumina Bead Chip analysis (Bibikova et al., 2011). Despite their strong positive overall correlation (Clark et al., 2012), results obtained from immunoprecipitation vs. bisulfite conversion based methods are not necessarily comparable (Jeong et al., 2016). Most importantly, these methods also differ in specificity. While signals in bisulfite sequencing and 450k Illumina array derive from 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC), MeDIP is selective for 5-mC. Therefore, it is possible that the differential *MORC1* DNAm signal was masked by 5-hmC, an epigenetic mark whose significance is increasingly acknowledged (Branco et al., 2011).

It is also plausible, however, that the previously observed association of *MORC1* DNAm (Nieratschker et al., 2014) with ELS is not transferable to childhood trauma in humans. We assessed childhood trauma using the CTQ, a self-report questionnaire that is well-established and frequently used for this purpose (Martins et al., 2011). Nevertheless, the type of stress induced by abuse or neglect in childhood is different from prenatal stress and maternal separation, which were investigated in the previous study. In addition, there is a long time interval (several years) between stress exposure and *MORC1* DNAm assessment in our study. It is therefore possible that other environmental factors, such as nutrition, lifestyle factors, stressful events later in life, as well as pharmacotherapy or psychotherapy have overridden potential ELS-responsive DNAm patterns within the *MORC1* promoter. These confounding factors were not present in the previous study, where animals were kept in a controlled environment after ELS exposure (Nieratschker et al., 2014). Lastly, while the previous results were obtained in hematopoietic stem cells and T cells, here, we analyzed DNAm in whole blood. Hematopoietic stem cells are present through the entire lifespan and may, therefore, carry methylation patterns that have been established early in life. Similarly,

T cells are likely to encode epigenetic ELS marks, as their lifespan is among the longest of all blood cells and they inherit patterns from hematopoietic progenitor cells. Further, T cells may be of high relevance to ELS since it is believed that ELS exposure induces long-lasting immune alterations which contribute to vulnerability to stress-related psychiatric disorders later in life (Fagundes et al., 2013). Such immune alterations can be encoded by DNAm patterns in T cells (Morales-Nebreda et al., 2019), as has already been shown for rhesus macaques that experienced maternal deprivation (Provencal et al., 2012), humans with low early life socioeconomic status (Borghol et al., 2012) and PTSD patients (Smith et al., 2011). In whole blood, *MORC1* ELS signature in T cells might be masked by methylation patterns of neutrophils, monocytes and other short-lived cell types that are unlikely to encode long-term responses to ELS. We validated our findings in two additional whole blood cohorts, PReDICT and Grady, and found that all three datasets did not show an association of ELS and *MORC1* methylation. Most importantly, if *MORC1* links ELS and vulnerability to depression later in life, we would expect to see an interaction between the two in predicting *MORC1* DNAm. This could only be tested in the Tuebingen cohort, where no such effect was evident. In conclusion, our data suggest that there is no association between childhood trauma and *MORC1* DNAm in whole blood of adults. However, further studies are needed in order to elucidate whether ELS responsive *MORC1* DNAm patterns are present in specific immune cell types, such as T cells.

In contrast to the ELS data, we found that the average methylation calculated over all analyzed CpG sites was significantly associated with depressive symptoms in the Grady and PReDICT cohort. Further, we identified single CpG sites associated with depression score in the PReDICT and Tuebingen cohorts. These results are consistent with previous findings indicating a role of *MORC1* in depression (Mundorf et al., 2018; Nieratschker et al., 2014; Schmidt et al., 2016). Previously, positive correlations of *MORC1* DNAm and depressive symptoms were reported for buccal cells (Mundorf et al., 2018) and our results indicate the same for blood. It is not known how *MORC1* DNAm in these peripheral tissues is related to DNAm in the brain. However, both tissues contain immune cells and are therefore relevant for the study of depression independently due to the important role of stress and inflammation in the pathogenesis of depression (Slavich and Irwin, 2014; Strawbridge et al., 2017). The mechanisms underlying the association of *MORC1* and depression are not yet understood, but possibly involve the action of *MORC1* as epigenetic regulator of neuropsychiatric genes. In support of this hypothesis, it was shown that *MORC1* knockout mice displayed altered expression of hippocampal brain-derived neurotrophic factor (BDNF), a protein that is widely implicated in psychiatric disorders (Autry and Monteggia, 2012). Further, *MORC1* itself may be directly relevant for neuropsychiatric disease, as has been suggested for other members of the MORC protein family (Boukas et al., 2018). Lastly, *MORC1* plays an important role in plant immunity and may also be involved in the human immune response (Koch et al., 2017). At the same time, disturbance of the immune system is often reported in depressive patients (Strawbridge et al., 2017) and may be part of the pathogenesis of depression (Slavich and Irwin, 2014). Therefore, it is also plausible, that *MORC1* is linked to depression through its role in the immune system.

However, a meta-analysis of the three studies did not reveal a significant effect of depression score on DNAm due to the differences in sites associated with depressive symptoms in

different cohorts and the inconsistent direction of the effect. Positive associations, i.e. higher DNAm in individuals with higher depression scores, were found in the Tuebingen and Grady cohort. These results are consistent with a previous study that reported a positive association of BDI scores with buccal *MORC1* DNAm in a cohort of N=60 healthy adults (Mundorf et al., 2018). Since higher promoter methylation is generally associated with less gene transcription (Chodavarapu et al., 2010), these results suggest that individuals with depressive symptoms express lower levels of *MORC1* protein. This is consistent with animal experiments showing that *MORC1* knockout leads to a depressive-like phenotype in mice (Schmidt et al., 2016), though it remains to be proven that the observed effects indeed affect *MORC1* gene expression. In contrast, DNAm was negatively associated with depressive symptoms in PReDICT. As PReDICT contains almost no participants without symptoms of clinical depression (BDI-I ≥ 10 for 97 % of the subjects), this could indicate that the association of depressive symptoms with *MORC1* DNAm is not linear. A nonlinear, adaptive relationship between environmental exposures and DNAm has been suggested previously (Vaiserman, 2010) and this pattern was supported by a study reporting an inverted U-shape association of DNAm with exposure to organic pollutants (Na et al., 2014). In addition, PReDICT is the only treatment-naive cohort and information on medication intake in Grady and Tuebingen was not available. It is therefore possible, that intake of antidepressant medication in the Grady and Tuebingen cohort have affected *MORC1* DNAm (Menke and Binder, 2014). Lastly, the least evidence for an association of depressive symptoms with *MORC1* DNAm was found in the Tuebingen cohort, where only one site was significantly associated with depressive symptoms; however this result did not withstand correction for multiple testing. There was a trend towards an association of depressive symptoms with the mean DNAm across all 50 analyzed sites, but the effect was not significant (p=0.06). This may be because of lack of statistical power, as this cohort is the smallest one in size. Further, cell type composition was available only for a subset of the cohort and therefore not considered in the analysis. Even though we did not find evidence for an association of cell type composition with BDI score, it is still possible that the results in the full cohort are confounded by differences in cell type composition. In addition, genotype data were not available in this cohort. However, genotype was not a significant predictor of *MORC1* DNAm in Grady and PReDICT and removing it as covariate did not change the results obtained in these cohorts (data not shown). In line with this, we found that the database of methylation quantitative trait loci (mQTLdb) (Gaunt et al., 2016) lists only small effects of SNPs on DNAm within the *MORC1* promoter (difference in median DNAm between homozygote groups <1 %). Therefore, we assume that the lack of genotype data in the Tuebingen cohort did not confound the analysis. Altogether, strongest evidence for an association of *MORC1* DNAm with depressive symptoms was found in the Grady cohort, where multiple sites were significantly associated with depressive symptoms and two sites withstood correction for multiple testing. While, the absolute differences in DNAm between study participants with low and high levels of depressive symptoms were small (< 2 %), such effect sizes are very common in observational studies of human cohorts and therefore not unexpected. Therefore, the overall results in the three independent cohorts indicate that there might be an association of *MORC1* with depression and the inconsistent findings may be attributed to differences in the cohorts. In conclusion, there is previous evidence for an

association of *MORC1* DNAm and depression and our study supports these findings, but further validation in additional datasets is necessary.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of Interest Statement

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All other authors declare that they have nothing to disclose.

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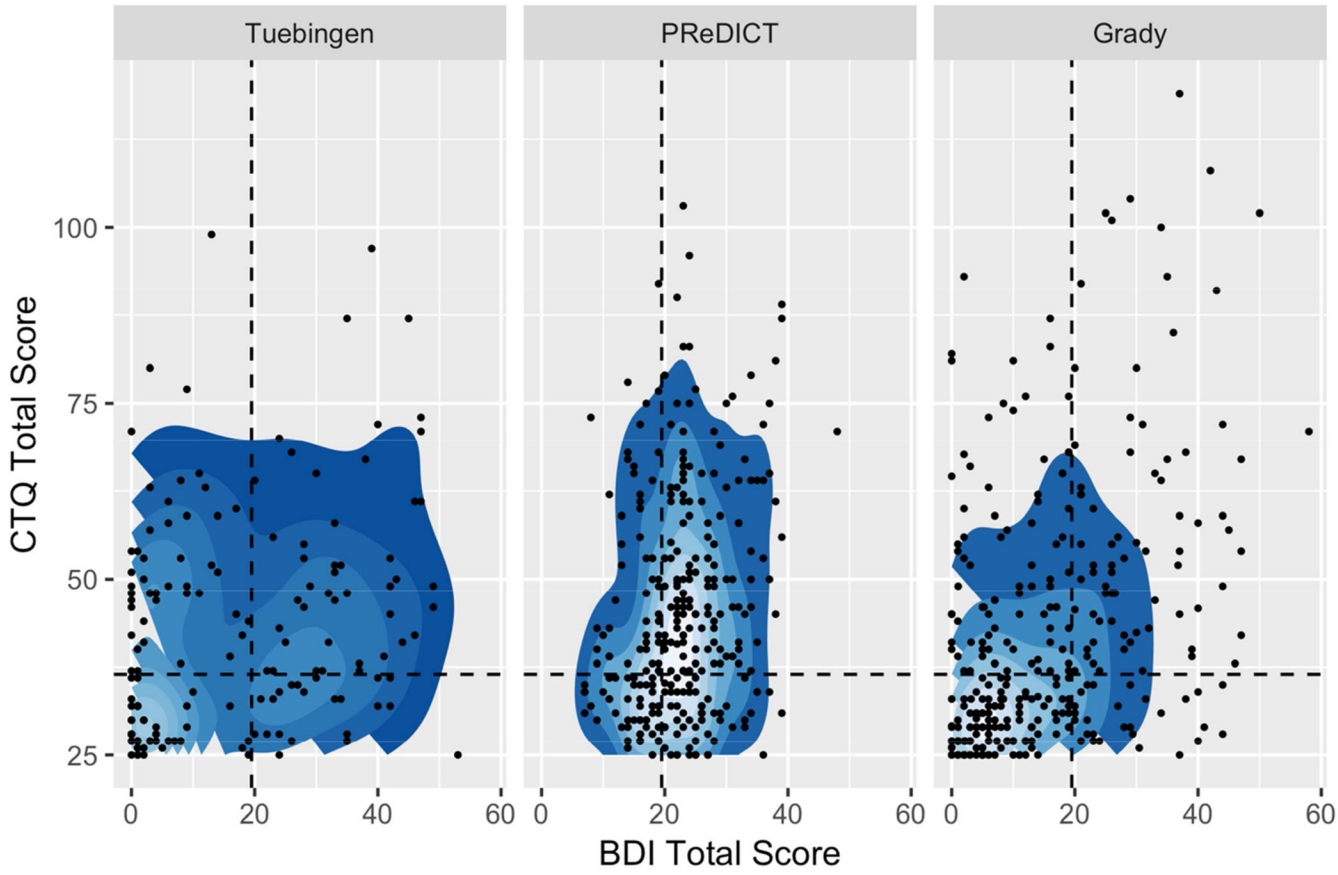

Fig. 1:

Overview of distribution of BDI and CTQ total scores in the three cohorts. Each datapoint represents one study participant. Blue shapes are 2D kernel density estimations performed using R package MASS (Venables W.N., 2002). Dotted lines are thresholds for moderate to severe abuse/neglect in at least one subcategory of CTQ (CTQ total > 36) and moderate to severe depressive symptoms (BDI total > 19)(Beck, 1996)).

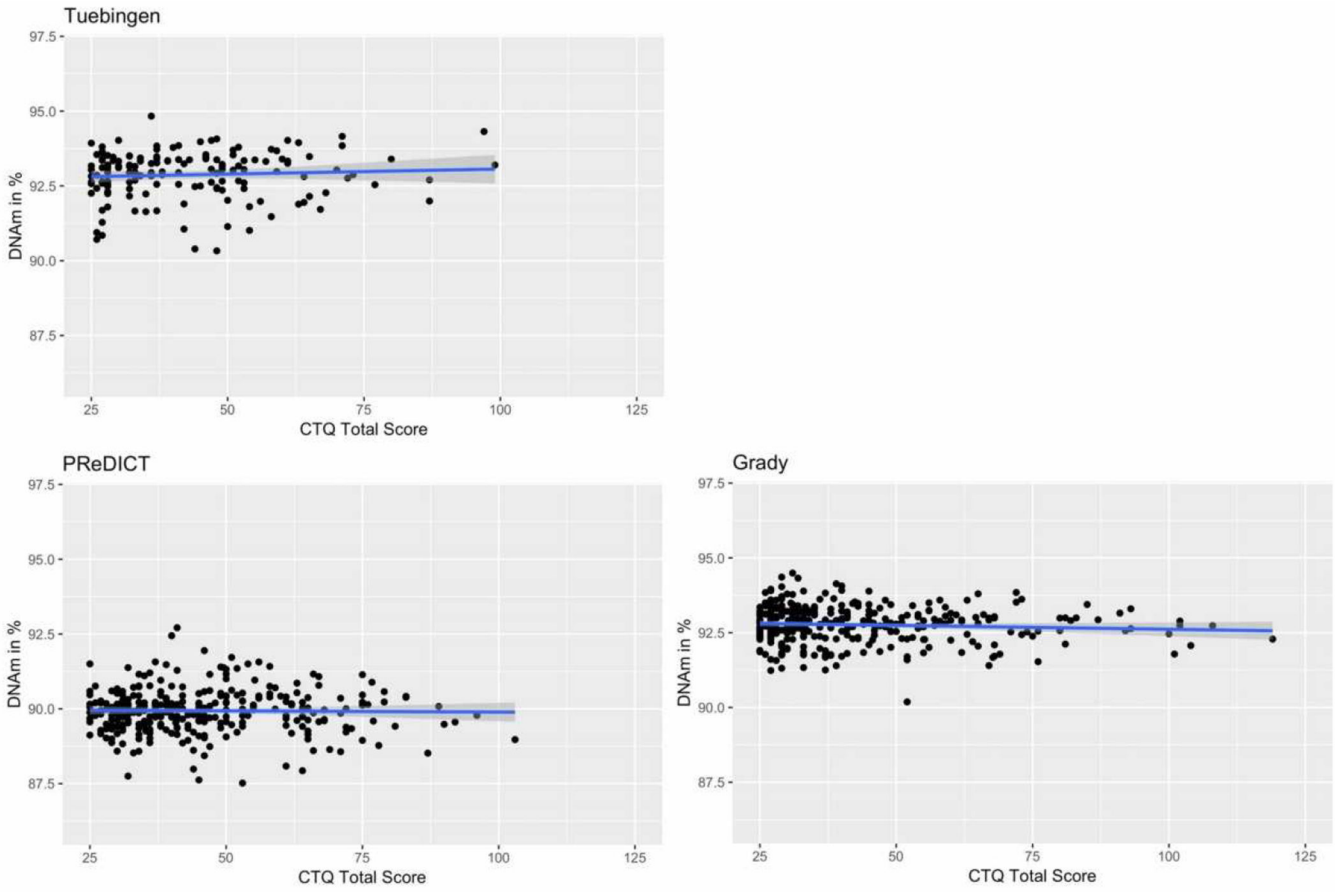

Fig. 2:

Mean MORC1 promoter DNAm in % plotted against the CTQ total score in Tuebingen, PReDICT and Grady cohort. In PReDICT and Grady, mean DNAm derives from 12 CpG sites within MORC1 promoter, whereas in the Tuebingen cohort, mean DNAm derives from 50 CpG sites. DNAm values are corrected for covariates.

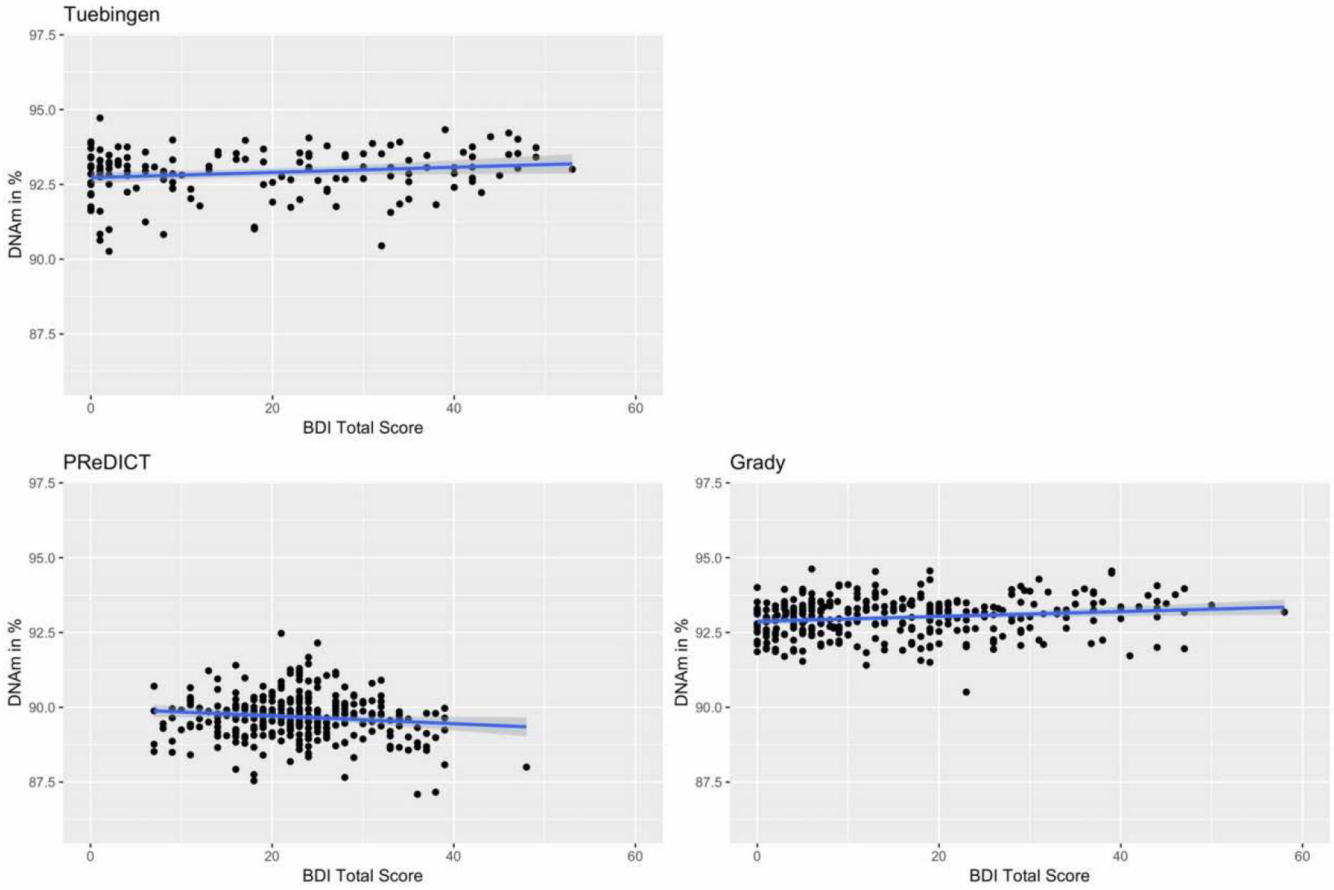

Fig. 3:

Mean MORC1 promoter DNAm in % plotted against the depression score in Tuebingen, PReDICT and Grady cohort. In PReDICT and Grady, mean DNAm derives from 12 CpG sites within the MORC1 promoter, whereas in the Tuebingen cohort, mean DNAm derives from 50 CpG sites. DNAm values are corrected for covariates.

Table 1:

Overview of cohort statistics in Tuebingen, PReDICT and Grady. Data given in percent with patient number in brackets or as mean ± standard deviation for average scores. For CTQ subscales, number of patients with moderate or severe abuse/neglect in this category were counted as cases (Bernstein et al., 2003).Data on neglect subscales was not available for the Grady cohort.

		Tuebingen (N=151)	PReDICT (N=299)	Grady (N=310)
Age		32.7 ± 11.7	40.2 ± 11.8	42.0 ± 13.1
% Females		66.9 % (101)	57.5 % (172)	25.5 % (79)
% Diagnosed depression		56.3 % (85)	100 % (299)	n.a.
Average BDI total score		17.5 ± 15.8	22.7 ± 7.2	15.6 ± 12.6
Average CTQ total score		43.4 ± 15.8	45.9 ± 15.8	43.0 ± 18.4
%	Emotional abuse	34.4 % (52)	46.2 % (138)	39.7 % (123)
Cases	Physical abuse	9.27 % (14)	12.0 % (36)	15.2 % (47)
	Sexual abuse	18.5 % (28)	22.4 % (67)	32.9 % (102)
	Emotional neglect	35.1 % (53)	32.3 % (97)	n.a.
	Physical neglect	21.9 % (33)	27.1 % (81)	n.a.
	One or more of the above	58.3 % (88)	65.6 % (196)	51.0 % (158)

Table 2:

Overview of partial correlation estimates between the depression score and the DNAm at single CpG sites, using PC1+2 generated from genotype data (for Grady and PReDICT only), sex, age, smoking behavior and CTQ total score as covariates. Results shown for Tuebingen (N=151), PReDICT (N=299) and Grady (N=310) datasets. Only sites that were nominally significant in at least one of the three datasets are listed. Total number of analyzed sites was 50 for Tuebingen and 12 for Grady and PReDICT cohort. Unstandardized betas for depression score are shown in brackets and in italic letters. Both estimates are rounded to two decimal places, $_{abs}$<x denotes that the absolute value is smaller than x. Statistical significance of depression score as predictor of DNAm was assessed using multiple regression analysis. Nominally significant associations are marked in bold and designated with * =p-value<=0.05, **=p-value<=0.01, ***=p-value<=0.001. Sites are described by genomic position (hg19). Results that withstand FDR-correction are underlined. Mean DNAm was calculated from all analyzed sites (50, 12, 12).

	Tuebingen	PReDICT	Grady
Ch3:108.836.796	**0.17 * (*0.03*)**	NA	NA
Chr3:108.836.878	0.06 (*0.01*)	−0.1 (*−0.01*)	**0.17 * (*0.01*)**
Chr3:108.837.069	0.01 ($_{abs}$*<0.01*)	−0.05 (*−0.01*)	**0.1 * (*0.01*)**
Chr3:108.837.086	$_{abs}$*<0.01* ($_{abs}$ *< 0.01*)	$_{abs}$*<0.01* (*−0.01*)	**0.16 ** (*0.02*)**
Chr3:108.837.088	0.03 ($_{abs}$*<0.01*)	−0.07 (*−0.02*)	**0.19 *** (*0.02*)**
Chr3:108.837.620	NA	$_{abs}$*<0.01*($_{abs}$*<0.01*)	**0.13 * (*0.01*)**
Mean of all analyzed sites	0.15 (*0.01*)	**−0.06 * (*−0.01*)**	**0.15 * (*0.01*)**