MORC1 methylation and BDI are associated with microstructural features of the hippocampus and medial prefrontal cortex

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\textbf{ABSTRACT}

\textbf{Background:} Alterations in the hippocampus and prefrontal cortex (PFC) have frequently been reported in depressed patients. These parameters might prove to be a consistent finding in depression. In addition, peripheral DNA methylation of the \textit{MORC1} gene promoter showed stable associations with depression across independent samples. However, the question arises whether \textit{MORC1}, supposedly acting as transcription factor, might also be involved in neurobiological alterations accompanying depression. This study further analyses the role of \textit{MORC1} in depression by investigating a potential correlation between peripheral \textit{MORC1} DNA methylation and neuronal structural properties previously associated with depression in humans.

\textbf{Methods:} Beck Depression Inventory (BDI) was assessed in 52 healthy participants. DNA was extracted from buccal cells and \textit{MORC1} methylation correlated with micro- and macrostructural properties derived from magnetic resonance imaging (MRI) and neurite orientation dispersion and density imaging (NODDI) in the hippocampus and medial prefrontal cortex (mPFC).

\textbf{Results:} \textit{MORC1} methylation was associated with volume reduction and neurite orientation dispersion and density markers in the hippocampus and mPFC. BDI was positively associated with neurite orientation dispersion and density markers in the hippocampus.

\textbf{Limitations:} The study was conducted in a small sample of healthy participants with subclinical depressive symptoms. Peripheral tissue was analyzed.

\textbf{Conclusion:} We found significant negative associations between peripheral \textit{MORC1} methylation and macro- and microstructural markers in the hippocampus and mPFC. Thus, \textit{MORC1} might be involved in neurobiological properties. Studies investigating neuronal methylation patterns of \textit{MORC1} are needed to support this hypothesis.

1. Introduction

More than 300 million people are suffering from Major Depressive Disorder (MDD) worldwide (\textit{World Health Organization, 2018}). In the global burden of disease study across 188 countries, MDD was within the top ten leading causes for years lived with disability in every country (\textit{Global Burden of Disease Study 2013 Collaborators, 2015}). So far, multifactorial origins have been determined for MDD, including environmental factors as well as genetic predispositions (\textit{World Health Organization, 2018}). Researchers have been trying to enable early detection and understand the pathological alterations accompanying MDD patients on all possible levels. Besides genetic variation investigated in genome-wide association studies (GWAS) (\textit{Aragam et al., 2011; Sullivan et al., 2009}) and epigenetic changes of DNA packing (Farrell...
and O’Keane, 2016) structural as well as functional changes in the brain are also associated with MDD (Armone, 2019; Belleau et al., 2019).

In MDD patients, volume reductions have been most profound in the hippocampus and prefrontal cortex (PFC) and could be linked to symptom severity (Belleau et al., 2019). Besides volume reduction, decreased functional activity or reduced neuron density are also described for some regions in MDD patients (Grimm et al., 2008; Santos et al., 2018; Uranova et al., 2004). Hippocampal volume, for example, is found to be significantly reduced in MDD patients compared to healthy controls in multiple studies (Malykhin et al., 2010; Videbech and Ravnikilde, 2004; Zhao et al., 2017). A meta-analysis of 29 magnetic resonance imaging (MRI) studies including over 1300 patients and 100 controls found significant hippocampal volume atrophy even though the studies were highly heterogeneous regarding age, sex, age of onset and illness duration (Santos et al., 2018) indicating a strong link between reduced hippocampal volume and depression. This association was already found in a previous meta-analysis of MRI studies in depressive and bipolar patients (Videbech and Ravnikilde, 2004). Another study investigating early-onset depression and hippocampus volume with MRI in a small sample found that hippocampal volume correlated negatively with age of onset of depression, with more reduction in the left hemisphere (MacMaster and Kusumakar, 2004). Brain activity measuring blood flow or energy metabolism can also be observed using functional MRI (fMRI). A study analyzing fMRI while viewing emotional pictures found hypoxia in regions of PFC, temporal and parietal cortex, insula, hippocampus, and basal ganglia in MDD patients compared to controls. Interestingly, this hypoxia could be aligned after antidepressant treatment (Schaefe et al., 2006).

In a post mortem electron microscopy study in the PFC, a significant reduction of oligodendrocyte density was found in MDD, bipolar disorder, and schizophrenia patients compared to controls (Uranova et al., 2004). Regarding the involvement of the PFC, a study using fMRI with medication-free MDD patients revealed less activity in the left dorsolateral (dl) PFC during an emotional judgment task but hyperactivity in the right dlPFC compared to healthy controls indicating a left-right dlPFC imbalance (Grimm et al., 2008). Microstructural alterations can also be investigated using diffusion-weighted imaging (DWI). A new DWI technique to investigate the in vivo microstructural brain architecture is neurite orientation dispersion and density imaging (NODDI) (Zhang et al., 2012). This method allows the quantitative investigation of neurite morphology based on a multi-shell high-angular-resolution diffusion imaging protocol thus enabling the analysis of diffusion-weighted data regarding the microstructure in the gray and white matter (Jespersen et al., 2010, 2007). Using NODDI, current studies have investigated, for example, interindividual differences in cognitive ability (Genç et al., 2018), language processing (Ocklenburg et al., 2018), hemispheric asymmetries (Schmitz et al., 2019a) as well as alterations in MDD (Ota et al., 2018). Analyzing DWI changes in MDD patients revealed multiple asymmetrical alterations such as anisotropy reduction and neurite density reduction in cortical regions as well as in thalamic and parahippocampal regions, so far (Ota et al., 2018). Moreover, another DWI study in MDD patients and matched healthy controls revealed reduced subfield connectivity in the left hippocampus in MDD patients as well as a positive correlation between subfield connectivity and age of onset of depression (Rutland et al., 2019). However, studies using DWI to investigate microstructural changes in MDD are still rare and have rather small samples sizes. Given all previous findings in different brain regions in MDD, it is still unclear how exactly these alterations arise. A possible explanation would be altered neuronal gene expression leading to reduced neurite density and thus resulting in reduced volume and connectivity.

Recently, the MORC family CW-type zinc finger 1 (MORC1) gene has attracted increasing attention for being a possible biomarker for depression (Mundorf et al., 2018; Nieratschker et al., 2014; Thomas et al., 2020). Nieratschker and colleagues found altered MORC1 methylation being associated with early life stress (ELS) in a cross-species study including human cord blood, primate blood and rat brain tissue (Nieratschker et al., 2014). Moreover, they were the first to report an association between certain genetic variations of MORC1 and MDD (Nieratschker et al., 2014). In a study investigating a Morc1 knockout, mice lacking the Morc1 gene showed depressive-like behavior in the forced swim test (Schmidt et al., 2016). This link between reduced Morc1 expression and depressive symptoms could be validated in healthy humans. There, we found an association between increased MORC1 methylation in buccal cells and increased depressive symptoms (measured with the Beck Depression Inventory; BDI) (Mundorf et al., 2018). Lastly, in a multicentric study investigating MORC1 methylation in whole blood cells, childhood trauma and depression in depressive patients and healthy controls, no association between methylation and childhood trauma were found but again, the association between depressive symptoms and MORC1 methylation was significant in all three cohorts investigated (Thomas et al., 2020).

Besides the validity of MORC1 methylation as a biomarker for MDD the question arises whether MORC1, potentially acting as a transcription factor (Perry and Zhao, 2003), might also be associated with macro- and microstructural brain properties. The present study therefore expands our previous study investigating MORC1 methylation and subclinical depressive symptoms by correlating MORC1 methylation and macro- and microstructural properties in the medial (m) PFC and hippocampus from 52 healthy participants. We therewith investigated whether MORC1 might also be involved in neurobiological alterations accompanying depression.

2. Methods

2.2. Sample

We tested a cohort of 52 healthy adults of German descent (25 females, $M_{age} = 24.27, SD_{age} = 2.95$). Participants were genetically unrelated and free from psychiatric or neurological disorders as determined by self-report. Depressive symptoms were assessed using the Beck Depression Inventory (BDI) (Beck et al., 1996). The BDI ranged from 0-15 ($M_{BDI} = 4.38, SD_{BDI} = 3.86$) and years of education ranged from 11-23 years ($M_{education} = 16.96, SD_{education} = 2.87$). All participants gave written informed consent and were treated in accordance with the Declaration of Helsinki. The study was approved by the ethics committee of the Faculty of Psychology, Ruhr University Bochum.

2.3. DNA methylation

DNA was collected from buccal swabs and isolated using the black-PREP Swab DNA Kit (Analytik Jena, Germany). Bisulfite conversion was performed using the EpiTect Kit (Qiagen, Germany). Bisulfite-converted DNA was processed on the Illumina MethylationEPIC array (Illumina, United States). Quality control, preprocessing and processing were performed using RStudio version 0.99.903 (RStudio, Inc., United States) and RnBeads (Max Planck Institute for Informatics, Germany) (Assenov et al., 2014) as described previously (Schmitz et al., 2018). The Illumina MethylationEPIC array covered 13 CGsites in the MORC1 promoter region, defined as 1500 bp upstream and 500 bp downstream of the TSS (chr3: 108836490-108838489) (Mundorf et al., 2018).

2.4. Neuroimaging

Acquisition: All neuroimaging data were acquired at the Bergmannsheil hospital in Bochum, Germany, using a Philips 3T Achieva scanner (Best, The Netherlands) with a 32-channel head coil.

Anatomical imaging: We acquired T1-weighted high-resolution anatomical images (MP-RAGE, TR (repetition time) = 8.2 ms, TE (echo time) = 3.7 ms, flip angle = 8°, 220 slices, matrix size = $240 \times 240$, voxel size = $1 \times 1 \times 1 \text{ mm}$).

Diffusion-weighted imaging: Diffusion-weighted images were acquired
using echo planar imaging (TR = 7652 ms, TE = 87 ms, flip angle = 90°, 60 slices, matrix size = 112 × 112, voxel size = 2 × 2 × 2 mm). We employed a multi-shell, high-angular-resolution scheme using diffusion-weighted images with b-values of 1000, 1800, and 2500 s/mm², applied along 20, 40, and 60 uniformly distributed directions. Diffusion directions within and between shells were generated orthogonal to each other using the MASSIVE toolbox (Froeling et al., 2017). Eight volumes were acquired with no diffusion weighting (b = 0 s/mm²) for anatomical reference for motion correction and computation of NODDI coefficients.

**Analysis of anatomical data:** We used FreeSurfer version 5.3.0 (http://surfer.nmr.mgh.harvard.edu) to reconstruct the cortical surfaces of T1-weighted images (Dale et al., 1999; Fischl et al., 1999). The automatic reconstruction steps included skull stripping, gray and white matter segmentation and cortical surface reconstruction and inflation, individually performed for each participant. Each segmentation slice was checked for inaccuracies and manually edited if necessary. Hippocampus and mPFC were parcellated per hemisphere from the T1-weighted images using an automated segmentation procedure based on the Desikan atlas (Desikan et al., 2006) implemented in FreeSurfer. Hippocampus and mPFC were linearly transformed into the native space of the diffusion-weighted images to serve as anatomical landmarks for the extraction of NODDI coefficients.

**Analysis of diffusion data:** Preprocessing of diffusion images was performed using FMRIB’s Diffusion Toolbox (FDT) in FMRIB Software Library (FSL) version 5.0.7. Preprocessing was performed as described previously (Genç et al., 2018; Schmitz et al., 2019b). NODDI coefficients were computed using the AMICO toolbox (Daducci et al., 2015). NODDI features a three-compartment model distinguishing intra-neurite, extra-neurite, and cerebrospinal environments. First, the proportion of free moving water within each voxel is estimated (isotropic volume fraction, ISO), reflecting isotropic diffusion with Gaussian properties likely to be found in the cerebrospinal fluid. The remaining portion of the signal is divided into the intra-neurite volume fraction (INVF) and neurite orientation dispersion (ODI) (Zhang et al., 2012). For a detailed description of NODDI coefficients and their histological validation see (Grussu et al., 2017; Jespersen et al., 2010, 2007; Zhang et al., 2012). As described above, hippocampus and mPFC were transformed into the native space of the diffusion-weighted images to compute voxel-wise NODDI coefficients (INVF, ODI, ISO).

### 2.5. Statistical analysis

As previous studies suggest a differential effect of the left and right hippocampus (MacMaster and Kusumakar, 2004), macro- and micro-structural properties of the hippocampus were analysed for the left and right hemisphere separately, resulting in eight variables (INVF, ODI, ISO, and volume; for the left and right hemisphere).

For the mPFC, there was no indication for an effect of hemispheric asymmetry and average values over both hemispheres were analysed. This resulted in six variables (INVF, ODI, ISO, volume, surface, thickness).

Bivariate Pearson correlations were performed to investigate the relationship between BDI score, DNA methylation levels at the 13 CG sites as well as averaged promoter region (15 variables) with macro- and microstructural properties of the hippocampus (8 variables) and mPFC (6 variables). To adjust for multiple comparisons, FDR correction was applied for hippocampus and mPFC, respectively. To control for sex and age effects, we repeated the analysis using partial correlations adjusting for sex and age.

As we previously reported a sex effect on DNA methylation in cg07090057 (Mundorf et al., 2018), we tested for a moderating effect of sex on the left and right hippocampus INVF in a linear regression model.

**3. Results**

**3.1. MORC1 DNA methylation and hippocampus micro- and macrostructure**

**Microstructure:** The overall MORC1 promoter region was not significantly correlated with either microstructural feature (INVF, ODI, ISO) (all p > .05). Among the individual CG sites, cg19748686 showed nominally significant positive correlation with ISO in the left hippocampus (r = 0.315, p = .023) (see Fig. 1A). To analyze whether there are any associations between MORC1 methylation and NODDI indices in males or females, respectively, both genders were analyzed separately. However, even though some associations differ between males and females, no CpG site reached FDR-corrected significance, see supplementary figure 1.

The strongest effect was a significant negative correlation between cg07090057 and left- (r = -0.361, p = .009) as well as right-hemispheric (r = -0.472, p = .0004) hippocampal INVF (see Fig. 2).

**Macrostructure:** The overall MORC1 promoter region was not significantly correlated with left- or right-hemispheric hippocampal volume (both p > .05). Cg16259931 showed a nominally significant positive correlation whereas cg27175191 achieved a significant negative correlation with right hippocampal volume (cg16259931: r = 0.307, p = .027; cg27175191: r = -0.300, p = .031). Cg07090057 and cg19748686 showed a nominally significant negative correlation with left hippocampal volume (cg07090057: r = -0.299, p = .031; cg19748686: r = -0.287, p = .039).

**3.2. MORC1 DNA methylation and mPFC micro- and macrostructure**

**Microstructure:** The overall MORC1 promoter region showed a nominally significant negative correlation with mPFC ODI (r = -0.280, p = .044) and ISO (r = -0.316, p = .023). For ODI, this effect was based on nominally significant correlations of cg05148217 (r = -0.310, p = .026) and cg07090057 (r = -0.282, p = .043) with mPFC ODI. For ISO, the overall effect of the promoter region was based on a significant negative correlation of cg18733433 (r = -0.385, p = .005) with mPFC ISO, while cg16259931 showed the opposite trend (r = 0.331, p = .016). Cg07090057 was also negatively correlated with mPFC INVF (r = -0.285, p = .041) (see Fig. 1B). To analyze whether there are any associations between MORC1 methylation and NODDI indices in male or female, respectively, both genders were analyzed separately. However, even though some associations differ between males and females, no CpG site reached FDR-corrected significance, see supplementary figure 2.

**Macrostructure:** The overall MORC1 promoter region was not significantly correlated with mPFC volume, surface or thickness (all p > .05). Among the individual CG sites, cg16259931 was positively correlated with mPFC volume (r = 0.303, p = .029) and surface (r = 0.291, p = .036), while cg05148217 (r = 0.298, p = .032) and cg25456186 (r = 0.286, p = .040) were positively correlated with mPFC thickness. Cg07090057 was negatively correlated with mPFC volume (r = -0.342, p = .013) and surface (r = -0.320, p = .021).

**3.3. BDI scores and hippocampus and mPFC micro- and macrostructure**

BDI scores were not significantly correlated with INVF in the left or right hippocampus (both p > .05). However, BDI scores showed a nominally significant positive correlation with ODI in the left (r = 0.353, p = .010) and right hippocampus (r = 0.288, p = .039) as well as ISO in the right hippocampus (r = 0.333, p = .010) (see Fig. 1A). BDI scores were not significantly correlated with hippocampal volume (both p > .05, see Fig. 1A) or mPFC micro- or macrostructure (all p > .05) (see Fig. 1B). For all correlation coefficients and p values, see supplementary tables S1 and S2.

**Partial correlations:** We repeated the analysis by running partial
correlations adjusting for the effects of sex and age. While the pattern of correlations remained similar (see Fig. 3), no CpG site reached FDR-corrected significance.

3.4. Moderator analysis

Based on the sex effect on DNA methylation in cg07090057, we tested whether sex moderated the association between cg07090057 and...
INVF in the left and right hippocampus by running a linear regression including the interaction term. There was no evidence for a moderating effect of sex for INVF in the left (βinteraction = -0.377, p = .336) nor in the right hippocampus (βinteraction = -0.102, p = .733).

4. Discussion

We reported nominally significant associations of MORC1 methylation and macrostructural features as well as different neurite density, neurite orientation dispersion, and cerebrospinal properties in the hippocampus and mPFC of healthy participants. Interestingly, self-rated BDI scores correlated positively with left and right hippocampus ODI and right hippocampus ISO. Regarding the MORC1 promotor region methylation, increased methylation of cg07090057 was associated with multiple macro- and microstructural features of the hippocampus and mPFC. Other CGsites of the promotor region were nominally associated with different features. Still, the prominent and repeatedly negative association of cg07090057 marks this site as potentially most important with different features. However, whether the CGsites have different biological functions regarding MORC1 expression is still unknown. The found nominal positive correlation between self-rated BDI scores and hippocampus ODI and ISO might be indicative of a structural more distinct hippocampus in participants that reported to be more depressive. Unfortunately, this study does not render enough information to further interpret this result at this point. Of interest, we reported a significant positive association between MORC1 methylation and BDI in our previous study (Mundorf et al., 2018). The current study is conducted with a subset of the previous sample (52 instead of 60 participants) and the previously reported association is still significant in this subset.

In the hippocampus, increased cg07090057 methylation was associated with decreased INVF in the left and right hemisphere as well as with a reduction in volume in the left hemisphere. Reduced hippocampus volume has previously been described in depressed patients (MacMaster and Kusumakar, 2004). More precisely, in an MRI study with early-onset depression patients (13-18 years of age) a reduced volume in the left hippocampus was found (MacMaster and Kusumakar, 2004). Moreover, hippocampus volume correlated negatively with the age of onset meaning a smaller hippocampus volume with increasing years after the first diagnosis (MacMaster and Kusumakar, 2004). Thus, the effect might be less severe in young and subclinical people. Reduced subfield connectivity in the left hippocampus was also found in MDD patients (Rutland et al., 2019). Again, subfield connectivity and age of onset of depression correlated positively (Rutland et al., 2019). In the mPFC, increased cg07090057 methylation was associated with decreased INVF and ODI as well as reduced volume and thickness. Decreased ODI and INVF might be early signs of the previously identified reduction of oligodendrocytes PFC density found in MDD patients (Uranova et al., 2004). As oligodendrocytes myelinate neurons, reduces neuronal density would also lead to a reduced number of oligodendrocytes.

Generally, INVF gives an estimate of gray matter dendrite and axonal density proving to be negatively related to cell body density (Jepsen et al., 2007). By using stereological analysis, a strong correlation between INVF and density of myelinated axons (r = 0.97) was found indicating that INVF is a marker of myelinated axonal density (Jepsen et al., 2010). With INVF as a marker of neurite density it can be assumed that the here found reduced INVF indicates reduced neurite density which might reflect less neurons. Given the here found association between a higher degree of MORC1 methylation and reduced neurite density it can be hypothesized that MORC1 might be involved in pathways leading to less neurons. Of note, a higher degree of methylation is frequently associated with less gene activity resulting in less expression (Razin and Cedar, 1991; Razin and Riggs, 1980). Thus, increased MORC1 methylation might result in reduced gene expression which might lead to reduced NODD signals. The MORC1 protein is described to have a CW-zing finger protein domain which can be related to chromatin methylation status (Perry and Zhao, 2003) and thus, holds the potential to influence transcription. But studies investigating brain DNA methylation are needed to further investigate this association.
Generally, the found volume reductions might be mediated by stress. It is hypothesized that every new stress exposure or depressive episode activates neurotoxic pathways leading to a decline of brain structures with the progress of the disorder (Belleau et al., 2019). As we investigated healthy participants, with some of them reporting subclinical depressive symptoms, the here found decrease in volume and NODDI markers might be an early detection of induced alterations. Possibly, the associations between MORC1 methylation and macro- and microstructural features will be stronger in clinically depressed patients.

Also, the found asymmetrical hemispheric alterations are in line with previous studies regarding general hemispheric functioning. Multiple studies already have reported hemispherical lateralization of specific functions such as emotion processing (Davidson, 1992; Rutherford and Lindell, 2011; Silberman and Weinhardt, 1986). Lesion studies in humans revealed that the left frontal hemisphere controls positive and the right frontal hemisphere negative emotions (Rutherford and Lindell, 2011). Damage to one hemisphere leads to reduced respective emotionality and an asymmetrical control of emotions (Rutherford and Lindell, 2011). This hypothesis of hemispheric lateralization of emotion is called valence hypothesis (Silberman and Weinhardt, 1986).

Regarding this principle, the here found negative correlation of cg07090057 methylation and INVF in the right hippocampus might be responsible for lower activity thus less positive memory processing.

So far, MORC1 methylation has been linked to depressive symptoms in subclinical and clinical participants (Mundorf et al., 2018; Thomas et al., 2020). Single nucleotide polymorphisms in the DNA sequence of MORC1 have been associated with MDD (Nieratschker et al., 2014) as well as a gene knockout with depressive behavior in mice (Schmidt et al., 2016). Finding associations between peripheral MORC1 methylation and reduced volume and neurite morphology in key regions in the brain reinforces the potential role of MORC1 in depression. More studies analyzing different peripheral and neuronal markers of depression would be helpful in advancing disentangling possible etiologies of depression. As MORC1 potentially acts as a transcription factor it holds the potential to be involved in stress-regulated pathways leading to alterations found in depression.

4.1. Limitations

As we performed multiple statistical analyses and significant results are not corrected for FDR, the results should be interpreted carefully. Moreover, the sample size is rather low (52 participants) and does not include clinically diagnosed depressed patients. However, we believe that this data set does render important insights into the function and involvement of MORC1 in the development of depression. Further studies conducted with clinically depressed patients and larger sample sizes are needed to strengthen this finding.

Authors Contribution

A.M. and J.S. designed the study and wrote the manuscript. All authors revised the manuscript. C.S., C.F. and J.S. collected data and were supported by K.H. E.G., C.F., and J.S. analyzed the data. All authors approved the manuscript. This manuscript is our original work and it is submitted for first publication.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials


References


