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Methylation of *MORC1*: A possible biomarker for depression?

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A B S T R A C T

New findings identified the *MORC1* gene as a link between early life stress and major depression. In this study, *MORC1* methylation was investigated in 60 healthy human adults (30 women, 30 men) between 19 and 33 years of age. For analysis, DNA was isolated from buccal cells. The results show that DNA methylation in the *MORC1* promoter region significantly correlates with the Beck Depression Inventory score in the examined non-clinical population. Sum score of birth complications, however, seems to correlate negatively with methylation. These findings further confirm that *MORC1* is a stress sensitive gene and a possible biomarker for depression.

1. Introduction

Major depressive disorder (MDD) is one of the leading causes of disability worldwide as 6–18% of the population will develop MDD during their life span (Drevets and Furey, 2009; World Health Organization, 2016). It has been widely shown that early life stress (ELS) contributes to the risk of developing MDD by inducing structural and functional changes in the brain (Carr et al., 2013; Kendler et al., 2004; Teicher et al., 2003). Recent data point to an involvement of the epigenome in explaining the link between ELS and MDD (Essex et al., 2013). Epigenetic changes after ELS could not only predispose for MDD, they might also have the potential to be used as early detection markers. Nieratschker and colleagues (Nieratschker et al., 2014) identified a relationship between ELS and an altered methylation state of the *MORC family CW-type zinc finger 1* (*MORC1*) gene (Nieratschker et al., 2014). In a cross-species, multi-tissue approach, the researchers investigated the effects of early life stress on DNA methylation. They compared DNA methylation from human cord blood following prenatal stress, the prefrontal cortex tissue of adult rats that had been exposed to prenatal stress, and blood cells of adolescent nonhuman primates after maternal separation with matched non-stressed control groups. Significantly reduced methylation of the *MORC1* gene was found in all tissues of all species (Nieratschker et al., 2014). To further investigate these results, the authors performed a gene-based case-control analysis utilizing data from a previous GWAS MDD study. Specific gene variants of the *MORC1* gene were associated with MDD. The link between *MORC1* and MDD was supported by a study by Schmidt et al., in 2016. In female MORC1 knockout mice, depressive-like behavior was observed, as well as differences in *BDNF* mRNA levels in the hippocampus (Schmidt et al., 2016). Of interest, differential *BDNF* expression has also been previously reported in MDD patients (Jiang and Salton, 2013).

The *MORC* gene was first discovered in 1998 by Watson and colleagues. It was attributed a major role in spermatogenesis based on findings that male *MORC−/−* mice were infertile while female *MORC−/−* mice did not show any symptoms (Watson et al., 1998). The *MORC* gene family exists only in meiotic and mitotic germ cells, further suggesting importance in male mouse spermatogenesis. However, given that other closely related genes of human *MORC*, such as human *KIAA0852* and *KIAA0136,* are found in several somatic tissues, it seemed likely that *MORC* also exists in somatic tissues (Inoue et al., 1999). In recent years, the *MORC* family has been characterized more precisely, with seven defined members; *MORC1, MORC2, MORC3, MORC4, MORC5, MORC6* and *MORC7* (Li et al., 2013; Moissiard et al., 2014). Moreover, the role of *MORC*s was specialized to epigenetic regulation in diverse nuclear processes due to the special domain architecture of this highly conserved nuclear protein superfamily (Li et al., 2013). This protein domain also suggests a connection with either chromatin methylation status or early embryonic development, reinforcing its reported association with ELS (Perry and Zhao, 2003).

Some members of the *MORC* family have already been characterized more precisely in plants and mammals, with implied roles in transcription repression, e.g. *MORC2* in human cancer cells, and gene

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silencing (Brabbs et al., 2013; Liggins et al., 2007; Quinlan et al., 2006; Wang et al., 2010). Given its potential role in inferring susceptibility for MDD after ELS (Nieratschker et al., 2014; Schmidt et al., 2016) we confirmed the expression of MORC1 protein in the brain and its presence during embryonic development (shown in the rodent brain, unpublished data).

In the present study we analyzed *MORC1* gene methylation in buccal cell samples from a non-clinical population. Methylation status was correlated with the scores in the Beck Depression Inventory to investigate whether *MORC1* methylation is associated with a subclinical depressive phenotype. We also examined whether *MORC1* methylation is correlated with complications during birth as one factor of ELS. This research has the potential to deepen our understanding regarding the association between ELS and the risk to develop MDD, and the potential of *MORC1* to act as a marker for predicting this risk.

2. Material and methods

We investigated *MORC1* methylation in 60 healthy human adults (30 women, 30 men) between 19 and 33 years of age (mean = 24.40, SD = 3.08). All participants gave written informed consent. The local ethics committee of the Psychological Faculty at Ruhr University Bochum approved the study procedure. Birth complications were assessed via questionnaires completed by participants and their parents. Reported items included preterm birth (before completion of the 37th week of gestation), long labour, multiple birth, caesarian birth, forceps used, breech presentation, Rhesus incompatibility, and others according to Bailey and McKeever (2004). A sum score was calculated for each participant (mean 0.52, SD = 0.89). Participants also completed the Beck Depression Inventory (BDI) (Beck et al., 1996). DNA was isolated from buccal cells using the blackPREP Swab DNA Kit (Analytik Jena, Germany), bisulfite converted with the EpiTect Kit (Qiagen, Germany) and analyzed using 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). The promoter region of the *MORC1* gene was defined as 1.5 kb upstream and 0.5 kb downstream of the transcription start site. The array covered 13 individual CGsites within this region (chr3: 108836490–108838489). Statistical analysis was conducted with the whole dataset and after elimination of all data points smaller than three SD below or larger than 3 SD above the mean DNA methylation for each CGsite. In order to control for possible sex differences, we conducted t-tests for independent samples between the sexes regarding the sum of birth complications, BDI scores, DNA methylation levels at the 13 CGsites and mean *MORC1* promoter region methylation. To control for age effects, we correlated age with DNA methylation in the CGsites and the whole promoter. We used Pearson correlation to investigate the relationship between birth complication sum score and DNA methylation levels at the 13 CGsites using Bonferroni correction for multiple comparisons ($\alpha = 0.0038$) and between birth complication sum score and mean *MORC1* promoter region methylation. Furthermore, we conducted a step-wise linear regression analysis with DNA methylation levels in all 13 CGsites as predictors and BDI score as the dependent variable. Pearson correlation was also used to investigate the relationship between birth complication sum score and BDI score.

3. Results

We did not find sex differences in birth complication sum score, BDI scores, or mean *MORC1* promoter region methylation (all $p > .05$). Only CGsite cg07090057 showed a statistically significant sex difference (males: mean = 0.926, SD = 0.016; females: mean = 0.937, SD = 0.012; $t(58) = 3.22$, $p < .01$), while the remaining 12 CGsites did not (all $t(58) < 1.4$, all $p > .05$). These results did not change after the elimination of outliers. CGsite cg04167867 showed a nominally significant correlation with age (r = -.280, $p < .05$), but no CGsite reached significance after controlling for multiple comparisons. After elimination of outliers, no CGsite significantly correlated with age.

Birth complication sum scores were correlated with DNA methylation levels in 13 individual CGsites within the *MORC1* promoter region (chr3: 108836490–108838489) using Bonferroni correction for multiple comparisons ($\alpha = 0.0038$). We also correlated birth complication scores with the mean value of DNA methylation for the whole promoter region. We found a significant negative correlation between birth complication sum score and *MORC1* promoter region total methylation (r = -.276, $p = .033$) as well as *MORC1* CGsite cg18733433 methylation (r = -.373, $p = .003$). Regression analysis with DNA methylation levels from all 13 CGsites as predictors and BDI score as the dependent variable reached significance ($F(1,58) = 4.63$, $p = .036$) with R = 0.27 and $R^2 = 0.07$. The CGsite cg27175191 was significantly positively associated with BDI score individually ($\beta = 0.27$, $t = 2.15$, $p = .036$).

After elimination of outliers, the correlation between birth complication sum score and *MORC1* promoter region methylation (r = -.101, $p = .443$) and *MORC1* CGsite cg18733433 methylation (r = -.083, $p = .531$) did not remain significant. However, the association between DNA methylation and BDI score remained significant ($F(1,51) = 4.69$, $p = .035$) with R = 0.29 and $R^2 = 0.08$. Again, CGsite cg27175191 reached individual significance ($\beta = 0.29$, $t = 2.17$, $p = .035$; see Fig. 1). Birth complication sum scores were not correlated with BDI score (r = −0.37, $p = .780$).

4. Discussion

The present data show that DNA methylation in the *MORC1* promoter region is significantly associated with BDI scores in a non-clinical population (Fig. 1). The BDI scores in our sample ranged between 0 and 15, which was expected with regard to the sample composition. Scores below 10 are considered as revealing no or minimal depression, while scores between 10 and 18 are considered as indicating mild to moderate depression (Beck et al., 1988). While our findings do not allow us to draw conclusions regarding the pathological mechanisms of *MORC1*, they might be indicative of an epigenetic regulation of *MORC1* by subtle environmental influences that induce sensitivity for subclinical depressive symptoms, possibly in line with a predisposition for MDD. This data set extends the known involvement of the *MORC1* gene in MDD patients (Nieratschker et al., 2014) to subclinical symptoms in

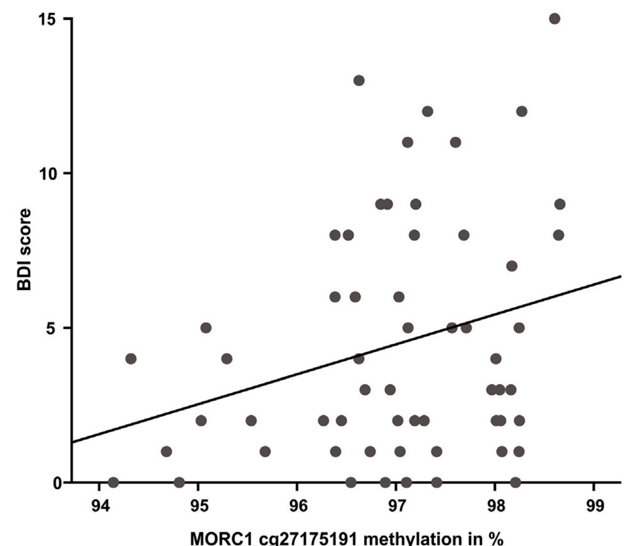

Fig. 1. Relationship between *MORC1* cg27175191 methylation in % and BDI score after elimination of outliers. The β value reached significance ($\beta = 0.29$, $p = .035$).

non-clinical subjects.

Higher methylation is traditionally associated with lower protein expression (Razin and Riggs, 1980) and therefore we can speculate that lower amounts of *MORC1* expression may infer higher levels of depressive symptoms (e.g. BDI scores in our sample). This is in line with the fact that mice lacking the MORC1 protein show a depressive-like phenotype (Schmidt et al., 2016).

In contrast, previously reported hypomethylation following ELS (Nieratschker et al., 2014), would suggest that decreased methylation of *MORC1* is associated with depression as ELS is a strong predictor of MDD (Carr et al., 2013; Leussis et al., 2012; Putnam, 2003). Interestingly, our data set shows that a sum score of birth complications is negatively correlated with DNA methylation of *MORC1* in buccal cells of healthy human adults. This finding applies to the overall *MORC1* promoter methylation as well as to CG site cg18733433 and is in line with hypomethylation after ELS (Nieratschker et al., 2014) when we consider birth complications as ELS. However, the effect in our data did not remain significant after the elimination of outliers, which makes it likely that it was partly driven by extreme values. Nevertheless, it is still an interesting result in line with the literature that requires replication in larger samples.

In general, reduced DNA methylation in *MORC1* following ELS contrasts the correlation we report between *MORC1* hypermethylation and BDI, leaving the role of *MORC1* in connecting ELS and MDD unclear. Our data set did not show a correlation between ELS (defined by birth complications) and BDI scores. Therefore, different mechanisms might be responsible for variation in methylation following ELS and methylation correlated with BDI. We also did not find a significant moderation effect for each CGsite with DNA methylation. This lack of significance may be due to different CGsites within the *MORC1* promoter region being associated with birth complication sum score and BDI scores. A further limitation which may explain the lack of correlation between BDI scores and birth complications is the self-reported measurement of birth complications and BDI by the participants.

Birth complications are just one specific adverse early life event, whilst many have been reported, particularly in the context of mental health outcomes. Furthermore, we did not control for factors which may confer a predisposition to birth complications, or any other risk exposures during pregnancy, such as maternal depression, smoking, and maternal behavior after birth. This is important, as it is known that maternal stress and prenatal stress, as well as MDD of the mother, is associated with differential DNA methylation of the offspring (Abbott et al., 2018; Bodnar et al., 2009; Vidal et al., 2014). Therefore, the correlation between the birth complications and *MORC1* methylation might not be the result of ELS but can potentially be explained by parental behavior or genetic factors that correlate with birth complications. However, in the study of Nieratschker et al. (2014), ELS was well defined in the animal studies and the hypomethylation after ELS was found in three different cell types and species, indicating strong and well-preserved mechanisms.

As DNA methylation is tissue-specific, the use of buccal cells for the investigation of birth complications and BDI may not be representative of DNA methylation signatures in our tissue of interest (the brain). However, a recent study showed that DNA methylation in peripheral tissue can reveal information on CNS-related phenotypes but should rather be interpreted as epigenetic signatures (Freytag et al., 2017). Furthermore, despite the limitations for our interpretations of our results regarding birth complications, we could extend the known connection between ELS and *MORC1* methylation that was found in $CD34^+$ cells, CD3-T cells and brain tissue (Nieratschker et al., 2014) to buccal cells.

We report a significant correlation between *MORC1* hypermethylation and BDI, giving hope for a reliable biomarker for MDD. As our results were found in buccal cells, *MORC1* hypermethylation could be an easy to apply, noninvasive biomarker for detecting subclinical depressive phenotype and therefore help to predict the risk of developing MDD.

Longitudinal studies spanning a range of developmental ages, and studies with clinical samples might improve our understanding of the connection between *MORC1* methylation status and the risk to develop MDD. Moreover, they will be essential in confirming *MORC1* methylation as a predictor of MDD risk.

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Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2018.05.026.

References

Abbott, P.W., Gumusoglu, S.B., Bittle, J., Beversdorf, D.Q., Stevens, H.E., 2018. Prenatal stress and genetic risk: how prenatal stress interacts with genetics to alter risk for psychiatric illness. Psychoneuroendocrinology 90, 9–21. https://doi.org/10.1016/j.psyneuen.2018.01.019.

Assenov, Y., Müller, F., Lutsik, P., Walter, J., Lengauer, T., Bock, C., 2014. Comprehensive analysis of DNA methylation data with RnBeads. Nat. Methods 11, 1138–1140. https://doi.org/10.1038/nmeth.3115.

Bailey, L.M., McKeever, W.F., 2004. A large-scale study of handedness and pregnancy/birth risk events: implications for genetic theories of handedness. Laterality 9, 175–188. http://dx.doi.org/10.1080/13576500342000013.

Beck, A., Steer, R.A., Brown, G.K., 1996. Beck Depression Inventory, second ed. Manual. Psychol. Corp, San Antonio TX.

Beck, A.T., Steer, R.A., Carbin, M.G., 1988. Psychometric properties of the Beck depression inventory: twenty-five years of evaluation. Clin. Psychol. Rev. 8, 77–100. https://doi.org/10.1016/0272-7358(88)90050-5.

Bodnar, L.M., Wisner, K.L., Moses-Kolko, E., Sit, D.K.Y., Hanusa, B.H., 2009. Prepregnancy body mass index, gestational weight gain, and the likelihood of major depressive disorder during pregnancy. J. Clin. Psychiatr. 70, 1290–1296. https://doi.org/10.4088/JCP.08m04651.

Brabbs, T.R., He, Z., Hogg, K., Kamenski, A., Li, Y., Paszkiewicz, K.H., Moore, K.A., O'Toole, P., Graham, I.A., Jones, L., 2013. The stochastic silencing phenotype of Arabidopsis morc6 mutants reveals a role in efficient RNA-directed DNA methylation. Plant J. Cell Mol. Biol. 75, 836–846. https://doi.org/10.1111/tpj.12246.

Carr, C.P., Martins, C.M.S., Stingel, A.M., Lemgruber, V.B., Juruena, M.F., 2013. The role of early life stress in adult psychiatric disorders: a systematic review according to childhood trauma subtypes. J. Nerv. Ment. Dis. 201, 1007–1020. https://doi.org/10.1097/NMD.0000000000000049.

Drevets, W.C., Furey, M.L., 2009. Depression and the brain. In: Squire, L.R. (Ed.), Encyclopedia of Neuroscience. Academic Press, Oxford, pp. 459–470. https://doi.org/10.1016/B978-008045046-9.00846-9.

Essex, M.J., Boyce, W.T., Hertzman, C., Lam, L.L., Armstrong, J.M., Neumann, S.M.A., Kobor, M.S., 2013. Epigenetic vestiges of early developmental adversity: childhood stress exposure and DNA methylation in adolescence. Child Dev. 84, 58–75. https://doi.org/10.1111/j.1467-8624.2011.01641.x.

Freytag, V., Carrillo-Roa, T., Milnik, A., Sämann, P.G., Vukojevic, V., Coynel, D., Demougin, P., Egli, T., Gschwind, L., Jessen, F., Loos, E., Maier, W., Riedel-Heller, S.G., Scherer, M., Vogler, C., Wagner, M., Binder, E.B., de Quervain, D.J.-F., Papassotiropoulos, A., 2017. A peripheral epigenetic signature of immune system genes is linked to neocortical thickness and memory. Nat. Commun. 8 (15193). https://doi.org/10.1038/ncomms15193.

Inoue, N., Hess, K.D., Moreadith, R.W., Richardson, L.L., Handel, M.A., Watson, M., Zinn, A.R., 1999. New gene family defined by MORC, a nuclear protein required for mouse spermatogenesis. Hum. Mol. Genet. 8, 1201–1207. https://doi.org/10.1093/hmg/8.7.1201.

Jiang, C., Salton, S.R., 2013. The role of neurotrophins in major depressive disorder. Transl. Neurosci. 4, 46–58. https://doi.org/10.2478/s13380-013-0103-8.

Kendler, K.S., Kuhn, J.W., Prescott, C.A., 2004. Childhood sexual abuse, stressful life events and risk for major depression in women. Psychol. Med. null 1475–1482. https://doi.org/10.1017/S003329170400265X.

Leussis, M.P., Freund, N., Brenhouse, H.C., Thompson, B.S., Andersen, S.L., 2012. Depressive-like behavior in adolescents after maternal separation: sex differences, controllability, and GABA. Dev. Neurosci. 34, 210–217. https://doi.org/10.1159/000339162.

Li, D.-Q., Nair, S.S., Kumar, R., 2013. The MORC family: new epigenetic regulators of transcription and DNA damage response. Epigenetics 8, 685–693. https://doi.org/10.4161/epi.24976.

Liggins, A.P., Cooper, C.D.O., Lawrie, C.H., Brown, P.J., Collins, G.P., Hatton, C.S., Pulford, K., Banham, A.H., 2007. MORC4, a novel member of the MORC family, is highly expressed in a subset of diffuse large B-cell lymphomas. Br. J. Haematol. 138, 479–486. https://doi.org/10.1111/j.1365-2141.2007.06680.x.

Moissiard, G., Bischof, S., Husmann, D., Pastor, W.A., Hale, C.J., Yen, L., Stroud, H., Papikian, A., Vashisht, A.A., Wohlschlegel, J.A., Jacobsen, S.E., 2014. Transcriptional gene silencing by Arabidopsis microrchidia homologues involves the formation of heteromers. Proc. Natl. Acad. Sci. U.S.A. 111, 7474–7479. https://doi.org/10.1073/pnas.1406611111.

Nieratschker, V., Massart, R., Gilles, M., Luoni, A., Suderman, M.J., Krumm, B., Meier, S., Witt, S.H., Nöthen, M.M., Suomi, S.J., Peus, V., Scharnholz, B., Dukal, H., Hohmeyer, C., Wolf, I. a.-C., Cirulli, F., Gass, P., Sütterlin, M.W., Filsinger, B., Laucht, M., Riva, M.A., Rietschel, M., Deuschle, M., Szyf, M., 2014. MORC1 exhibits cross-species differential methylation in association with early life stress as well as genome-wide association with MDD. Transl. Psychiatry 4, e429. https://doi.org/10.1038/tp.2014.75.

Perry, J., Zhao, Y., 2003. The CW domain, a structural module shared amongst vertebrates, vertebrate-infecting parasites and higher plants. Trends Biochem. Sci. 28, 576–580. https://doi.org/10.1016/j.tibs.2003.09.007.

Putnam, F.W., 2003. Ten-year research update review: child sexual abuse. J. Am. Acad. Child Adolesc. Psychiatry 42, 269–278. https://doi.org/10.1097/00004583-200303000-00006.

Quinlan, K.G.R., Verger, A., Kwok, A., Lee, S.H.Y., Perdomo, J., Nardini, M., Bolognesi, M., Crossley, M., 2006. Role of the C-terminal binding protein PXDLS motif binding cleft in protein interactions and transcriptional repression. Mol. Cell Biol. 26, 8202–8213. https://doi.org/10.1128/MCB.00445-06.

Razin, A., Riggs, A.D., 1980. DNA methylation and gene function. Science 210, 604–610.

Schmidt, M., Brandwein, C., Luoni, A., Sandrini, P., Calzoni, T., Deuschle, M., Cirulli, F., Riva, M.A., Gass, P., 2016. Morc1 knockout evokes a depression-like phenotype in mice. Behav. Brain Res. 296, 7–14. https://doi.org/10.1016/j.bbr.2015.08.005.

Teicher, M.H., Andersen, S.L., Polcari, A., Anderson, C.M., Navalta, C.P., Kim, D.M., 2003. The neurobiological consequences of early stress and childhood maltreatment. Neurosci. Biobehav. Rev. 27, 33–44.

Vidal, A.C., Benjamin Neelon, S.E., Liu, Y., Tuli, A.M., Fuemmeler, B.F., Hoyo, C., Murtha, A.P., Huang, Z., Schildkraut, J., Overcash, F., Kurtzberg, J., Jirtle, R.L., Iversen, E.S., Murphy, S.K., 2014. Maternal stress, preterm birth, and DNA methylation at imprint regulatory sequences in humans. Genet. Epigenet. 6, 37–44. https://doi.org/10.4137/GEG.S18067.

Wang, G.-L., Wang, C.-Y., Cai, X.-Z., Chen, W., Wang, X.-H., Li, F., 2010. Identification and expression analysis of a novel CW-type zinc finger protein MORC2 in cancer cells. Anat. Rec. Hoboken NJ 2007 (293), 1002–1009. https://doi.org/10.1002/ar.21119.

Watson, M.L., Zinn, A.R., Inoue, N., Hess, K.D., Cobb, J., Handel, M.A., Halaban, R., Duchene, C.C., Albright, G.M., Moreadith, R.W., 1998. Identification of morc (microrchidia), a mutation that results in arrest of spermatogenesis at an early meiotic stage in the mouse. Proc. Natl. Acad. Sci. U.S.A. 95, 14361–14366.

World Health Organization, 2016. WHO | Depression [WWW Document]. WHO. http://www.who.int/mediacentre/factsheets/fs369/en/, Accessed date: 29 August 2016.