Brief report

Plasticity of Functional MAOA Gene Methylation in Acrophobia

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Abstract

Epigenetic mechanisms have been proposed to mediate fear extinction in animal models. Here, MAOA methylation was analyzed via direct sequencing of sodium bisulfite-treated DNA extracted from blood cells before and after a 2-week exposure therapy in a sample of n = 28 female patients with acrophobia as well as in n = 28 matched healthy female controls. Clinical response was measured using the Acrophobia Questionnaire and the Attitude Towards Heights Questionnaire. The functional relevance of altered MAOA methylation was investigated by luciferase-based reporter gene assays. MAOA methylation was found to be significantly decreased in patients with acrophobia compared with healthy controls. Furthermore, MAOA methylation levels were shown to significantly increase after treatment and correlate with treatment response as reflected by decreasing Acrophobia Questionnaire/Attitude Towards Heights Questionnaire scores. Functional analyses revealed decreased reporter gene activity in presence of methylated compared with unmethylated pCpGfree_MAOA reporter gene vector constructs. The present proof-of-concept psychotherapy-epigenetic study for the first time suggests functional MAOA methylation changes as a potential epigenetic correlate of treatment response in acrophobia and fosters further investigation into the notion of epigenetic mechanisms underlying fear extinction.

Keywords: monoamine oxidase A, anxiety, extinction, epigenetics, DNA methylation
Introduction

An “epigenetic switch is the key to persistent extinction” (Stafford et al., 2011), a notion supported by experimental evidence suggesting that pharmacological modulation of epigenetic mechanisms such as DNA methylation or histone acetylation can produce lasting suppression of memories (for review, see Lattal et al., 2013). This seems to be particularly relevant for the psychotherapeutic treatment of anxiety disorders essentially entailing fear extinction (Whittle et al., 2014).

In female patients with panic disorder, we recently identified hypomethylation of a promoter/exon I/intron I region of the monoamine oxidase A (MAOA) gene on chromosome Xp11.4-p11.3 as a disease risk factor (Domschke et al., 2012), which was found to be reversible with a successful cognitive behavioral psychotherapeutic intervention (Ziegler et al., 2016). The MAOA gene is a prime candidate gene also for specific phobias based on evidence from animal studies (Dubrovina et al., 2006) and genetic association studies in patients with specific phobias (Samochowiec et al., 2004).

Thus, in the present proof-of-concept “psychotherapy-epigenetic” study, we for the first time investigated DNA methylation in the above-mentioned promoter/exon I/intron I region of the MAOA gene in female patients with acrophobia during a standardized 2-week cognitive behavioral psychotherapeutic intervention including exposure exercises using virtual reality technology, partly enhanced by repetitive transcranial magnetic stimulation (rTMS). In analogy to a previous study in panic disorder we predicted MAOA methylation to increase and thus to “normalize” along with treatment response. Additionally, the functional relevance of altered MAOA promoter/exon I/intron I methylation was investigated by means of luciferase-based reporter gene assays.

Methods

Sample

Twenty-eight female Caucasian patients (44.86 ± 13.67 years) with acrophobia (SCID-I) were recruited at the Department of Psychiatry, University of Würzburg, Germany. Exclusion criteria were any psychiatric comorbidities or previous treatment of acrophobia within the last 6 months, metal parts in the head, medical implants, increased intracranial pressure, pregnancy, current involvement in psycho- or pharmacotherapy, cardiovascular or neurological diseases, a history of tinnitus, or family history of epilepsy (for details see Herrmann et al., 2016). Additionally, all patients and controls were genotyped for the VNTR according to published protocols (Reif et al., 2012). The sample was matched to the sample of patients with acrophobia by age and the Attitude Towards Heights Questionnaire (ATHQ; Abelson and Curtis, 1989). Patients were instructed not to change any lifestyle patterns including smoking behavior from T0 to T1.

MAOA Methylation Analysis

In the patient sample, DNA was isolated from EDTA blood taken at T0 and T1 using the FlexiGene DNA Kit (Qiagen). After sodium bisulfite conversion (EpiTect 96 Bisulfite Kit, Qiagen), an amplicon comprising MAOA exon I as well as the adjacent promoter and intron I regions (chromosome X, GRCh38.p2 Primary Assembly, NCBI Reference Sequence: NC_000023.11, 43656260–43656613) was PCR-amplified following a published protocol (Domschke et al., 2012, 2015; Ziegler et al., 2016, 2017) and sequenced (ABI 3730XL, LGC Genomics). The obtained sequences were quantitatively analyzed using the Epigenetic Sequencing Methylation software (ESME; Lewin et al., 2004; cf. Domschke et al., 2012, 2013, 2014, 2015; Tadić et al., 2014; Ziegler et al., 2015, 2016, 2018). Electropherograms were robustly readable for 13 CpG sites (CpG1 = 43665316; CpG2 = 43656327; CpG3 = 43656362; CpG4 = 43656368; CpG5 = 43656370; CpG6 = 43656383; CpG7 = 43656386; CpG8 = 43656392; CpG9 = 43656398; CpG10 = 43656427; CpG11 = 43656432; CpG12 = 43656514; CpG13 = 43656553). Healthy controls had been assessed for MAOA methylation using the same methods as applied to the present patient sample (see Domschke et al., 2012). Quality control was performed as described previously (cf. Domschke et al., 2012; Ziegler et al., 2016). No participant had to be excluded from the reported analyses when applying quality control (data missing for CpG1 in 3 patients).

Additionally, all patients and controls were genotyped for the MAOA VNTR according to published protocols (Reif et al., 2012) and stratified into low (33/34/3a4/35) and high expression (44/45) genotype groups (cf. Reif et al., 2012; Domschke et al., 2015).

Functional Analysis

Functional analysis was accomplished using the pCpGfree-promoter-Lucia vector (InvivoGen) expressing a Luciferase under a human elongation factor-1 (hEF1) promoter. The insert comprising CpGs 1–13 was PCR-amplified and ligated into the linearized vector using T4 DNA Ligase (Invitrogen). The vectors were methylated using the CpG methyltransferase M.SsI (NEB) and transfected to HEK293 cells (ECACC) using the TransFast reagent (Promega). Luciferase assays were performed using the Stop & Glo® Reagent (Promega) on a GloMax+ LuminaMeter (Promega), and Luciferase activity was normalized using a co-transfected pGL4.74 Renilla luciferase control vector. All analyses were conducted in technical triplicates.

Statistical Analysis

Differences in MAOA methylation levels between patients and controls as well as between genotype groups were tested by means of independent samples t-tests. The association between baseline methylation and treatment response as defined as the intra-individual relative change (in percent) in clinical symptom scores (AQ Anxiety) was assessed by means of linear regression analysis and controlled for genotype and TMS status. Changes
Results

Mean scores on both questionnaires decreased significantly after the therapeutic intervention: (AQ Anxiety T0: 57.05 ± 18.11, T1: 43.47 ± 18.91, t = 4.61, P < .001; ATHQ Total T0: 37.86 ± 16.56, T1: 26.89 ± 9.53, t = 4.59, P < .001; ATHQ Danger T0: 18.32 ± 8.19, T1: 12.61 ± 5.34, t = 5.19, P < .001). For this female patient sub-sample, no difference was discerned between the CBT(-rTMS) and CBT(+rTMS) groups regarding treatment response (data not shown), allowing for a joint epigenetic analysis of the 2 groups in the present context.

At T0, average MAOA methylation (0.432 ± 0.021) did not correlate with age or disease severity according to AQ Anxiety or ATHQ Total/Danger scores (all P > .05). No influence of grouped genotype (low expression [33/34/34a/35]: n = 17 patients and n = 19 controls; high expression [44/45]: n = 11 patients and n = 9 controls) was discerned on average MAOA methylation or questionnaire scores at T0 (all P > .05). Furthermore, patients and controls did not differ in genotype distribution (χ2 = .311, P = .577).

Average MAOA methylation as well as methylation at CpGs 1-8 and 10-13 differed significantly between patients with acrophobia at baseline (T0) and healthy controls. Patients were characterized by significantly lower average methylation levels, specifically driven by single CpGs 1-8, 10, and 11 (for details see Table 1).

A positive change in AQ Anxiety scores indicating treatment response was seen in 20 patients (71.4%). While no significant association between baseline average MAOA methylation and treatment response could be discerned, differences were observed regarding CpG1 (β = .567, P = .003) and, approaching trend levels of significance, CpG5 (β = .340, P = .086) and CpG6 (β = .331, P = .091), with lower baseline methylation predicting impaired treatment response.

Following therapy, MAOA methylation increased significantly in the patient group for average methylation (T1: 0.441 ± 0.029, F = 4.703, P = .040) and specifically at CpG4 (T1: 0.420 ± 0.040, F = 4.708, P = .040), CpG6 (T1: 0.375 ± 0.051, F = 4.954, P = .036), CpG8 (T1: 0.334 ± 0.053, F = 10.204, P = .004) and CpG9 (T1: 0.484 ± 0.035, F = 10.249, P = .004) (Figure 1A). Methylation at all other CpG sites did not change significantly from T0 to T1 (all P > .05).

In the main analyses regarding methylation change dependent on treatment response as measured by changes in AQ Anxiety or ATHQ Total/Danger scores, respectively, negative correlations indicated clinical symptom improvement to go along with an increase in average MAOA methylation (AQ Anxiety: r = -.465, P = .019, Figure 1B; ATHQ Danger: r = -.496, P = .012). This pattern held true for several individual CpG sites (AQ Anxiety: CpG2: r = -0.455, P = .022; CpG3: r = -0.398, P = .049; CpG7: r = -0.459, P = .021; CpG9: r = -0.399, P = .048; CpG10: r = -0.527, P = .007; ATHQ Total: CpG9: r = -0.402, P = .047; ATHQ Danger: CpG2: r = -.412, P = .041; CpG3: r = -0.401, P = .047; CpG7: r = -0.473, P = .017; CpG9: r = -0.541, P = .005; CpG11: r = -0.424, P = .035).

Applying in vitro luciferase assays, nonmethylated pCpGfree-promoter Lucia_MAOA vectors showed a significant increase in normalized Lucia luciferase activity compared with pCpGfree-promoter Lucia_MAOA vectors methylated with M.SssI prior to transfection (t = 4.157, p < .001; Figure 2). pCpGfree-promoter Lucia vectors without insert showed no significant difference between the methylated and the nonmethylated state (t = 0.394, p = .70; Figure 2).

Discussion

The present finding of (I) decreased MAOA methylation in patients with acrophobia compared with healthy controls, (II) decreased methylation at a single MAOA CpG site to predict treatment response, and (III) increasing MAOA methylation along
with treatment response further strengthens and extends the emerging body of evidence for a potential role of MAOA with treatment response. Further support for this hypothesis is observed in recent findings that MAOA methylation is associated with treatment response in a number of different psychiatric disorders, including anxiety disorders. For example, increased methylation of the MAOA promoter/exon I/intron I region has been observed in patients with acrophobia (an anxiety disorder characterized by fear of heights) compared to healthy controls (Schiele et al., 2018). This finding is consistent with previous research showing that reduced MAOA activity is associated with fear-related disorders (Reif et al., 2012; Ziegler et al., 2016).

In anxiety disorders, besides the above-mentioned study (Ziegler et al., 2016) only 2 other studies have investigated methylation dynamics with respect to treatment effects. Here, methylation changes in the serotonin transporter (5-HTT) gene as well as in the FK506-binding protein 5 (FKBPS) gene were found to be associated with response to psychotherapy in children with mixed primary anxiety disorder diagnoses and obsessive-compulsive disorder (Roberts et al., 2014, 2015).

A major limitation of the present study entails the risk of false positive results. This is particularly true for small sample sizes, which may limit the statistical power of the study. To overcome this limitation, replication studies with larger sample sizes are needed to confirm the findings. Additionally, it is important to consider the potential role of environmental factors in the occurrence and treatment of anxiety disorders. For example, smoking patterns can influence the behavior of leukocytes and affect DNA methylation levels (Robertson et al., 2015). Therefore, smoking patterns should be taken into account in future studies to ensure the validity of the findings.

In conclusion, the present study provides preliminary evidence for a potential role of MAOA methylation with treatment response in acrophobia. Further research is needed to confirm these findings and to explore the role of other environmental factors in the treatment of anxiety disorders.
Along these lines, despite some evidence for their potential as viable markers of central processes (e.g., Shumay et al., 2012), peripheral methylation patterns do not necessarily allow for deductions regarding MAOA methylation status in brain tissue. Finally, localized changes such as the presently identified MAOA methylation patterns might reflect global changes in genomic DNA methylation either by CpG hyper- and hypomethylation necessitating investigation by means of epigenome-wide association studies in larger, sufficiently powered samples.

In sum, while warranting replication and considering all caveats mentioned above, the present psychotherapy-epigenetic study for the first time suggests functionally relevant MAOA methylation changes as a potential epigenetic correlate of response to cognitive behavioral psychotherapy in acrophobia and corroborates a previous independent finding in panic disorder (Ziegler et al., 2016). The emerging notion of epigenetic signatures as a core mechanism of action of fear extinction might aid in probing pharmacological treatment enhancers such as MAO-inhibitors or, in a more general sense, drugs inducing epigenetic changes as augmentation strategies for lasting extinction effects and is thereby hoped to contribute to a more effective treatment based on individual epigenetic information.

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Statement of Interest

None.

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