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Brief Report

BRIEF REPORT

Monoamine Oxidase A Hypomethylation in Obsessive-Compulsive Disorder: Reversibility By Successful Psychotherapy?

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ABSTRACT

Background: Epigenetic markers such as DNA methylation of the monoamine oxidase A (*MAOA*) gene have previously been shown to be altered in anxiety- and stress-related disorders and to constitute a potential mechanism of action of psychotherapeutic interventions such as cognitive behavioral therapy in these disorders. The present study for the first time, to our knowledge, investigated *MAOA* methylation in patients with obsessive-compulsive disorder applying a longitudinal psychotherapy-epigenetic approach.
Methods: The present sample comprised 14 unmedicated female patients with primary obsessive-compulsive disorder and 14 age- and sex-matched healthy controls. *MAOA* promoter methylation was analyzed via direct sequencing of sodium bisulfite-treated DNA extracted from whole blood before and after an 8- to 10-week semi-standardized, obsessive-compulsive disorder–specific cognitive behavioral therapy. Clinical response was assessed by means of the Yale-Brown Obsessive Compulsive Scale.
Results: Significantly lower *MAOA* promoter methylation was discerned in obsessive-compulsive disorder patients relative to healthy controls. Data were available for 12 patients with obsessive-compulsive disorder and 14 controls. Furthermore, following cognitive behavioral therapy, clinical improvement, i.e., decreases in obsessive-compulsive disorder symptoms as indicated by lower scores on the Yale-Brown Obsessive Compulsive Scale was found to be significantly correlated with increases in *MAOA* methylation levels in patients (data available for n = 7).
Conclusions: The present pilot data suggest *MAOA* hypomethylation as a potential risk marker of obsessive-compulsive disorder and an increase in *MAOA* methylation levels as a possible mechanistic correlate of response to cognitive behavioral therapy in obsessive-compulsive disorder.

Key Words: Epigenetics, MAOA, OCD, cognitive-behavioral therapy, CBT

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Introduction

The serotonin system is considered one of the key players in the pathogenesis and treatment of obsessive-compulsive disorder (OCD). Increased platelet monoamine oxidase (MAO) activity—crucially involved in the metabolism of serotonin—has been observed to be associated with increased symptom severity as indicated by elevated scores on the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) (Arrojo et al., 2007). Accordingly, several early case reports and 1 placebo-controlled cross-over study suggest efficacy of MAO(A) inhibitors such as phenelzine, tranylcypromine, and clorgylin in the treatment of OCD (cf. Erfurth and Schmauss, 1993). MAO(A) activity is governed by genetic variation such as the *MAOA* variable number tandem repeat (VNTR) polymorphism—with some, but not unequivocal evidence for a role in OCD pathogenesis (cf. Taylor et al., 2013)—and epigenetic mechanisms such as DNA methylation (cf. Schiele and Domschke, 2018; Ziegler and Domschke, 2018). In the anxiety-related disorders spectrum, functionally relevant hypomethylation of the *MAOA* promoter region—previously shown to result in increased gene transcription (Schiele et al., 2018) and thus presumably in a serotonergic deficit due to increased *MAOA* activity—has been reported to be associated with panic disorder (Domschke et al., 2012; Ziegler et al., 2016) and acrophobia (Schiele et al., 2018) in females. In both phenotypes, *MAOA* methylation increased significantly along with response to cognitive behavioral psychotherapy (CBT) (Ziegler et al., 2016; Schiele et al., 2018). In OCD, however, *MAOA* methylation in OCD or its dynamic course along with treatment response has not yet been evaluated. Thus, in the present proof-of-concept study, we investigated for the first time, to our knowledge, the role of *MAOA* promoter methylation in an unmedicated sample of patients with OCD applying a case-control design and a longitudinal approach allowing for evaluating potential changes in *MAOA* methylation during the course of a standardized cognitive-behavioral psychotherapeutic intervention. Given the known serotonin deficit in OCD, we predicted relative *MAOA* hypomethylation in patients compared with controls, which was expected to increase and thus to "normalize" along with response to CBT. Given previous female-specific associations of *MAOA* methylation (see above) and the X-chromosomal location of the *MAOA* gene, analyses were conducted in an all-female sample.

METHODS

Samples and Treatment

Fourteen female, unmedicated Caucasian patients with OCD (age [mean± SD]: 33.71±12.60 years) were recruited at the Psychosomatic Hospital Windach, Windach, Germany, between 2014 and 2017. OCD diagnosis was ascertained by experienced psychiatrists and/or clinical psychologists on the basis of a structured clinical interview according to DSM-IV criteria (SCID-I). The mean age of onset was 20.86±5.50 years, the mean illness duration was 14.00±11.04 years. Somatic disorders, pregnancy, psychiatric medication, and comorbid tics, trichotillomania, skin picking disorder or other current axis I diagnoses except for depression (n=6; Beck Depression Inventory score: 14.96±8.32), specific phobias (n=4), or agoraphobia (n=1) led to exclusion. In total, n=5 patients (36%) were free of any comorbid diagnoses. Six patients (43%) had 1 comorbid diagnosis, n=3 (21%) had 2 comorbid diagnoses. A comparison of OCD patients with or without comorbidities did not reveal any a priori differences in demographics, Y-BOCS scores, or average *MAOA* methylation (all $P \geq .150$). Smoking status was ascertained in detail with the total number of smoked cigarettes per day (n=3 smokers [mean no. of cigarettes/d±SD: 4.67±4.62], n=11 nonsmokers). Patients underwent an 8- to 10-week semi-standardized CBT comprising psychoeducation, exposure, and response prevention/management and intensive in vivo exposure exercises ("flooding"). Severity of OCD was assessed using the Y-BOCS before (T0; available for n=14) and after treatment (T1; available for n=11) (T0: 22.68±6.78, T1: 8.82±6.19, t(10)=6.79, $P<.001$). None of the patients received any psychiatric medication throughout the course of treatment.

A sample of 14 healthy female Caucasian controls recruited at the Department of Psychiatry, University of Wuerzburg, Germany, within the framework of the Collaborative Research Centre TRR58 were 1:1 matched to the patient sample by age (33.0±11.2 years; t(26)=–0.16, $P=.875$) and smoking status (n=3 smokers [mean no. of cigarettes/d±SD: 3.0±2.0; t(4)=0.57, $P=.597$], n=11 nonsmokers). Current and/or lifetime mental axis I disorders were excluded by experienced psychologists applying the MINI International Neuropsychiatric Interview. Further exclusion criteria as listed above also applied for the control sample.

This study was approved by the ethics committee of the University of Würzburg, Germany (ethical votes no. 128/14 and 7/08) and was conducted according to the ethical principles of the Helsinki Declaration. All patients and controls gave written informed consent prior to participation.

MAOA Methylation Analysis

DNA was isolated from EDTA blood taken at T0 and T1 using the FlexiGene DNA Kit (Qiagen, Hilden, Germany). DNA was available for controls (n=14) as well as for patients at T0 (n=14) and T1 (n=11). Following sodium bisulfite conversion (EpiTect 96 Bisulfite Kit, Qiagen), an amplicon comprising part of the *MAOA* promoter, exon 1, and part of intron 1 (chromosome X, GRCh38.p2 Primary Assembly, NCBI Reference Sequence: NC_000023.11, 43656260–43 656 613) was analyzed in analogy to previous studies on *MAOA* methylation via direct sequencing according to published protocols (Domschke et al., 2012; Ziegler et al., 2016; Schiele et al., 2018) in controls and patients at T0 (data missing for n=2 patients due to technical failure) as well as T1 (data missing for n=3 patients); methylation data at both time points were available for 9 patients. The obtained sequences were quantitatively analyzed using the Epigenetic Sequencing Methylation software (ESME, Lewin et al., 2004; cf. Domschke et al., 2012; Ziegler et al., 2016; Schiele et al., 2018). CpGs were numbered in analogy to previous studies on *MAOA* methylation (cf. Domschke et al., 2012; Ziegler et al., 2016, 2018; Schiele et al., 2018; see Table 1). Electropherograms were robustly readable for 12 CpG sites (CpGs 2–13; CpG2=43 656 327; CpG3=43 656 362; CpG4=43 656 368; CpG5=43 656 370; CpG6=43 656 383; CpG7=43 656 386; CpG8=43 656 392; CpG9=43 656 398; CpG10=43 656 427; CpG11=43 656 432; CpG12=43 656 514; CpG13=43 656 553). Quality control was performed in 2 steps, with methylation values of each duplicate with SD>0.1 defined as missing values and outliers (≥3 SD from mean methylation of the respective CpG site) set as missing data. A cutoff of >20% of missing data was defined as an exclusion criterion (cf. Ziegler et al., 2016). No participant had to be excluded from the reported analyses when applying these quality control criteria. Genotyping for the *MAOA* VNTR was performed according to published protocols (Domschke et al., 2012).

Table 1. *MAOA* Methylation in Patients With Obsessive-Compulsive Disorder and Healthy Controls at Baseline (T0)

	Patients (n = 12) M (SD)	Controls (n = 14) M (SD)	t/U	*P* value
Average methylation	0.490 (0.060)	0.580 (0.033)	4.748	<.001***
CpG2[a]	0.424 (0.094)	0.472 (0.070)	45.50	.047*
CpG3	0.401 (0.041)[b]	0.533 (0.077)	5.495	<.001***
CpG4	0.479 (0.102)	0.614 (0.053)	4.135	.001***
CpG5	0.265 (0.071)	0.414 (0.064)	5.626	<.001***
CpG6	0.413 (0.110)	0.518 (0.054)	3.188	.008**
CpG7	0.562 (0.069)	0.635 (0.069)	2.693	.013*
CpG8	0.370 (0.100)	0.503 (0.082)	3.736	.001***
CpG9	0.569 (0.066)	0.665 (0.093)	2.978	.007**
CpG10	0.579(0.064)	0.618 (0.069)	1.467	.155
CpG11	0.240 (0.045)	0.295 (0.085)	2.069	.052
CpG12[a]	0.999 (0.002)	0.997 (0.012)	83.50	.956
CpG13	0.560 (0.111)	0.587 (0.121)	0.583	.583

Abbreviation: M, mean.
Bold, significant after Benjamini-Hochberg correction for multiple testing.
*Significant at $P < .05$; **significant at $P \leq .01$; ***significant at $P \leq .001$.
[a] Data not normally distributed.
[b] Data available for n = 11 patients only.

Patients and controls were stratified into low- (33/34/3a4/35) and high-expression (44/45) groups. Grouped *MAOA* VNTR genotype distribution did not differ between patients and controls (33/34/3a4/35 vs 44; $X^2(1) = 2.286$, $P = .131$).

Statistical Analysis

Normal distribution was tested by means of Shapiro-Wilk test and visual inspection of QQ plots. Y-BOCS scores and *MAOA* methylation data were normally distributed with the exception of CpGs 2 and 12. At T0, average *MAOA* methylation did not correlate with potential confounders such as age, age of onset, disease severity, disease duration, Beck Depression Inventory-II score, number of smoked cigarettes, or grouped *MAOA* VNTR genotype (all $Ps > .05$), which were thus not considered as covariates. For case-control comparisons, normally distributed data were compared by means of independent samples *t* tests with *MAOA* methylation as dependent variable and group (OCD patients vs healthy controls) as the between factor variable; nonnormally distributed data were tested nonparametrically using Mann Whitney U test. In the patient group, *MAOA* methylation levels pre- to posttreatment were compared by means of independent samples *t* tests or Wilcoxon signed-rank test (CpGs 2 and 12). For analyzing changes in *MAOA* methylation in relation to changes in clinical symptoms (i.e., Y-BOCS scores), percentage differences in *MAOA* methylation were correlated with percentage chance in Y-BOCS scores during treatment (T1-T0 in percent of T0: [T1-T0]/T0*100) using Pearson correlation or, for nonparametric testing, Spearman correlation. Benjamini-Hochberg correction for multiple testing was applied.

RESULTS

In OCD patients, *MAOA* methylation was significantly lower compared with controls (for statistics, see Table 1). After Benjamini-Hochberg correction for multiple testing, case-control differences in average *MAOA* methylation as well as methylation at CpGs 3–9 remained significant.

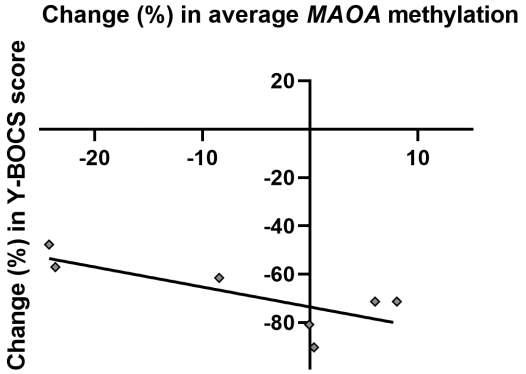

Figure 1. Changes in average *MAOA* methylation and response to cognitive behavioral therapy in obsessive-compulsive disorder (OCD). Correlation between reduction in OCD symptoms (difference in percent in Yale-Brown Obsessive Compulsive Scale scores from T0 to T1) and percentage change in *MAOA* average methylation from T0 to T1.

In OCD patients, there were no significant overall changes in *MAOA* methylation from T0 to T1 when treatment response was not taken into account (all $P \geq .061$) with the exception of CpG2 ($Z = -2.08$, $P = .038$), notwithstanding correction for multiple testing.

An exploratory analysis of Y-BOCS score change and percentage methylation change during treatment revealed a negative correlation between changes in Y-BOCS scores and changes in average methylation after treatment (n = 7; $r = -0.76$, $P = 0.046$; see Figure 1), which held particularly true for CpG6 ($r = -0.80$, $P = 0.031$) and CpG9 ($r = -0.83$, $P = .021$), indicating treatment response to go along with an increase in *MAOA* methylation. For CpGs 2–5, 7, 8, and 10–12, no significant correlations emerged (all $P \geq .137$).

Discussion

The present pilot data point to *MAOA* hypomethylation as a potential risk marker of OCD and to an increase in *MAOA* methylation as a possible mechanistic correlate of response to CBT in a sample of unmedicated patients with OCD. The present results add further support to the notion of the *MAOA* gene being a key player in the spectrum of anxiety and stress-related disorders with shared or overlapping biological risk factors (see Bandelow et al., 2016) and are in line with previous reports of decreased *MAOA* methylation of a region spanning the promoter/exon I/intron I of the *MAOA* gene in patients with panic disorder (Domschke et al., 2012; Ziegler et al., 2016), acrophobia (Schiele et al., 2018), and depression (Melas et al., 2013; Melas and Forsell, 2015). Furthermore, aside from an association with disorder risk per se, increasing *MAOA* methylation along with response to CBT—particularly CBT with exposure elements as first-line treatment for both anxiety disorders and OCD—has been observed in panic disorder (Ziegler et al., 2016) and acrophobia (Schiele et al., 2018), further supporting the notion of epigenetically driven neuroplasticity to potentially underlie response to extinction-related psychotherapeutic interventions in mental, particularly anxiety-related disorders (Stafford and Lattal, 2011). Methylation of the investigated region has, on a functional level, been associated with decreased *MAOA* gene expression in functional in vitro assays (Checknita et al., 2015; Schiele et al., 2018), decreased *MAOA* enzymatic activity in the brain (Shumay et al., 2012), and increased blood serotonin levels (Checknita et al., 2015). Therefore, *MAOA*

hypomethylation—constituting a pathogenetic marker of OCD as shown in the present study and of related phenotypes (e.g. Domschke et al., 2012; Ziegler et al., 2016; Schiele et al., 2018) – conferring a decreased availability of monoamines in clinical phenotypes may be increased, and potentially even reversed by psychotherapeutic treatment. Indeed, *MAOA* methylation has been shown to increase, that is, "normalize" to methylation levels observed in healthy controls after successful CBT in panic disorder (Ziegler et al., 2016).

This proof-of-concept case-control and psychotherapy-epigenetic study is based on a well-characterized sample (all-female sample given the X-chromosomal location of the gene under study, strictly unmedicated patients, strict exclusion criteria to minimize confounders, standardized CBT), with, however, limited sample size and consequently low statistical power owed to the above-mentioned criteria. Sixty-four percent of patients additionally had 1 or 2 secondary diagnoses of depression and/or an anxiety disorder. While OCD constituted the primary diagnosis and no differences in demographics, clinical variables or methylation status were observed between patients with or without comorbidities, a confounding effect of comorbidity status on the present results cannot be fully excluded. Also, the observed changes in *MAOA* methylation along with treatment response cannot be conclusively attributed to psychotherapy effects given that the control group was only investigated at baseline, but not longitudinally in parallel to the course of treatment. Furthermore, *MAOA* methylation was measured in peripheral blood samples, which may be subject to potentially confounding cell-composition effects and does not allow for direct conclusions about brain methylation status. However, using [^{11}C]clorgyline positron emission tomography, peripheral *MAOA* methylation obtained from leukocytes was shown to correlate inversely with MAOA enzymatic activity in the brain (Shumay et al., 2012), pointing to *MAOA* methylation in peripheral tissues to function as a viable proxy for central processes. Finally, while the focus of the present study was on extending previous findings of *MAOA* methylation to constitute a malleable disease marker targetable and potentially reversible by psychotherapeutic interventions to the clinical phenotype of OCD among the group of anxiety and stress-related spectrum disorders (see above), future efforts may want to also elucidate epigenetic markers clearly distinguishing OCD from other diagnostic entities. Delineating epigenetic signatures specifically underlying a given disorder would greatly benefit differential diagnostic decision making (cf. Schiele et al., 2019).

Thus, the present pilot findings ought to be considered preliminary and to be interpreted with caution, but might inspire future studies to further explore the role of *MAOA* methylation in the pathogenesis and treatment mechanisms of OCD. If corroborated in independent larger samples, the present findings could contribute to an individualized treatment augmentation approach, with OCD patients displaying *MAOA* hypomethylation potentially benefitting from MAO inhibitors as a catalytic adjunct to CBT.

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Interest Statement

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