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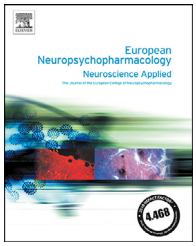

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DNA-methylation of the dopamin receptor 2 gene is altered during alcohol withdrawal

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Abstract
The dopaminergic neurotransmission is known to be of crucial importance in addictive behavior. Epigenetic regulation like methylation of DNA influences the function of dopaminergic transmission. The present study investigated alterations of DNA methylation in the dopamine D2 receptor (DRD2)-gene in patients suffering from alcohol dependence. The study sample consists of 99 alcohol dependent males admitted for alcohol withdrawal treatment and a control group of 33 healthy participants. Blood samples underwent bisulfite sequencing to determine levels of DNA-methylation of the promoter region of the DRD2 gene. Mixed linear modeling was used to test differences between patients and controls, course of methylation during detoxification. While DRD2-gene methylation did not differ significantly between patients and controls, we found a significant increase of DRD2-gene methylation during alcohol withdrawal/early abstinence. Craving, measured with the Obsessive Compulsive Drinking Scale (OCDS), was significantly associated with DRD2-gene methylation. Furthermore, smoking significantly

Abbreviations: AD, alcohol dependence; DAT, dopamine transporter; DRD2, dopamine receptor D2; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; CpG, cytosine-phosphate-guanine; DSM-4, diagnostic and statistical manual of mental disorders, 4th edition; PCR, polymerase chain reaction; EMM, estimated marginal means; SE, standard error; OCDS, obsessive compulsive drinking scale.

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influenced DRD2-gene methylation in both, patients and controls. As in other types of addictive disorders, DRD2-gene methylation is altered during alcohol withdrawal/early abstinence. The findings regarding an association with alcohol craving and tobacco consumption point towards a crucial role of DRD2-gene methylation in the neurobiology of addictive behavior.

1. Introduction

Dopaminergic circuits, particularly in the mesolimbic-striatal-prefrontal pathways, are known to play a crucial role in the genesis and in the maintenance of addictive behavior. This applies for different addictive disorders, including alcohol dependence and tobacco dependence as well as non-substance use disorders like pathologic gambling (Balfour, 2015; Boileau et al., 2013; Charlet et al., 2013). However, the mechanisms that influence the alterations in the central dopaminergic neurotransmission are still subject to intensive research. One important factor influencing these pathways may be the altered availability of dopamine receptors due to epigenetic changes in gene expression. The role of epigenetic changes has been studied not only in the neurobiology of addictive disorders but in many other psychiatric diseases, many of them occurring as frequent comorbidities in alcohol dependence. This includes affective disorders, anxiety disorders, non-substance dependent addictive behaviors, psychotic diseases as well as personality disorders (Domschke et al., 2013; Hannon et al., 2016; Hillemacher et al., 2015; Tadic et al., 2014; Teschler et al., 2016). Possible epigenetic alterations include changed DNA-methylation patterns in specific gene regions like particularly the promoter region but also histone modification processes, which are able to influence chromatine structure and subsequent transcription processes in specific genes (Robison and Nestler, 2011; Rodenhiser and Mann, 2006). In alcohol dependence, different studies have focused on the role of changes in DNA-methylation in dopaminergic genes. Thus, DNA-methylation of the dopamine transporter (DAT) gene was shown to be significantly increased at the beginning of alcohol withdrawal, compared to healthy controls (Hillemacher et al., 2009). Also, this study found a negative association between methylation of the DAT-gene and alcohol craving, measured with the Obsessive Compulsive Drinking Scale (OCDS). A recent study of Jasiewicz and colleagues described methylation changes in a particular Cystosine-phosphate-Guanine-(CpG)-position in the DAT promoter in alcohol dependent patients versus healthy controls (Jasiewicz et al., 2015). Another study found no differences in DAT promoter methylation in alcohol dependent patients compared to healthy controls but a trend towards an association between lower DAT methylation levels and elevated craving (Nieratschker et al., 2014). However, the authors state that regarding their results, this finding may be influenced by age, as older patients showed higher methylation levels. Most recently, we were able to replicate the association found between DAT methylation and alcohol craving and furthermore showed that DAT methylation was associated with amygdala activation (BOLD response) during a cue reactivity task in the fMRI (Wiers et al., 2015).

A recent investigation from our group described alterations in DNA methylation of the dopamine-2 receptor gene (DRD2) in subjects with pathologic gambling continuing with gambling behavior compared to subjects able to abstain, which points towards an involvement of DRD2 methylation changes in addictive behavior (Hillemacher et al., 2015). Also, we have found differences in DRD2 methylation between women with eating disorders and healthy controls and in Gilles-de-la-Tourette-syndrome (Frieling et al., 2010; Muller-Vahl et al., 2017).

Recent studies describe an association of DRD2 methylation and alcohol problem severity in a community sample (Hagerty et al., 2018). Also DRD2 methylation was associated with striatal activation in response to alcohol-associated reward cues (Bidwell et al., 2019). Recent studies also show that epigenetic aging and specifically DNA methylation is accelerated in subjects suffering from alcohol dependence (Rosen et al., 2018). Also, a recent investigation showed that genome-wide methylation changes in alcohol dependence (compared to healthy controls) at least partially reverse during short-term abstinence (Philibert et al., 2014). In a further recent approach, Philibert and co-workers were able to show the discriminative ability of two small methylation based marker sets to quantify alcohol use status in patients (Philibert et al., 2018).

Thus, changes in DNA methylation of specific genes in alcohol dependence are an interesting target to identify specific mechanisms in the neurobiology of addictive behavior and deserve further attention. Aim of the present investigation was to analyze changes in DNA-methylation in the DRD2 the promoter region compared to healthy controls.

2. Experimental procedures

2.1. Alcohol-dependent patients and control group

The present study is part of a large prospective research project on neurobiological mechanisms in alcohol dependence (NENA: Studies in Neuroendocrinology and Neurogenetics in Alcoholism), which was approved by the local Ethics Committee of the University of Erlangen-Nuremberg and complies with the declaration of Helsinki. Written, informed consent was obtained from every participant. All patients suffered from alcohol dependence according to the International Classification of Diseases (ICD-10) and the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and were included in the study after admission for detoxification treatment to the Hospital for Psychiatry, Psychotherapy and Psychosomatics, Obermain, Germany. A detailed physical examination as well as a routine laboratory testing and urine drug screening were assessed in all patients. Patients with concomitant psychiatric illnesses, other substance abuse apart from alcohol or nicotine, cerebral ischemia, cerebral hemorrhage, epilepsy, cardiovascular and renal diseases were not included in the study. The extend of alcohol craving was

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