Journal of Psychiatric Research 86 (2017) 1–8

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Gilles de la Tourette syndrome is associated with hypermethylation of the dopamine D2 receptor gene

Kirsten R. Müller-Vahl [a, *], Gesa Loeber [a], Alexandra Kotsiari [a, b], Linda Müller-Engling [a, b], Helge Frieling [a, b]

[a] Clinic of Psychiatry, Socialpsychiatry and Psychotherapy, Hannover Medical School, Germany
[b] Molecular Neuroscience Laboratory, Hannover Medical School, Germany

ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form
7 November 2016
Accepted 10 November 2016

Keywords:
Tourette syndrome
Tic
Methylation
DAT
DRD2
Dopamine

ABSTRACT

Several lines of evidence support a "dopaminergic hypothesis" in the pathophysiology of Gilles de la Tourette syndrome (TS). The aim of this study was to investigate for the first time epigenetic changes in DNA methylation in different dopamine genes in adult patients with TS. We included 51 well characterized adult patients with TS (41 males, 10 females, mean age = 35 ± 12.6 years, range, 18–71 years) and compared results with data from a group of 51 sex- and age-matched healthy controls. Bisulfite sequencing was used to measure peripheral DNA methylation of the dopamine transporter (DAT), the dopamine D2 receptor (DRD2), and the catechol-O-methyltransferase (COMT) genes. Compared to healthy controls, patients with TS showed significantly elevated methylation level of the DRD2 gene that positively correlated with tic severity. In contrast, DAT methylation was lower in more severely affected patients. Our results provide evidence for a role of altered epigenetic regulation of dopaminergic genes in the pathophysiology of TS. While DRD2 hypermethylation seems to be directly related to the neurobiology of TS that may lead to dopaminergic dysfunction resulting in enhanced thalamo-cortical movement-stimulating activity, DAT hypomethylation might reflect a secondary mechanism in order to compensate for increased dopaminergic signal transduction due to DRD2 hypermethylation. In addition, it can be speculated that spontaneous fluctuations of tics may be caused by short-term alterations of methylation levels of dopaminergic genes resulting in dynamic changes of tonic/phasic dopaminergic signaling in the striatum and thalamo-cortical output pathways.

1. Introduction

Gilles de la Tourette syndrome (TS) is a childhood-onset chronic neuro-psychiatric disorder characterized by the combination of multiple motor and at least one vocal tic. The majority of patients, in addition, suffers from psychiatric comorbidities such as attention deficit hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), self-injurious behavior, depression, and anxiety disorder. The underlying cause of the disease is still unknown. Several studies provided evidence for an involvement of cortico-striato-thalamo-cortical (CSTC) circuitry. Mink (2001) suggested an aberrant activity in a particular set of neurons in the striatum (caudate nucleus and putamen) leading consequentially – via increased inhibition of the globus pallidus internus and the substantia nigra pars reticulata and reduced inhibition of the thalamus - to an excitation of cortical neurons involved in movements. In line with this hypothesis, most structural neuroimaging studies have shown abnormalities of the basal ganglia nuclei, most consistently reduced volume of the caudate nucleus (Makki et al., 2008; Müller-Vahl et al., 2009; Peterson et al., 1993). In addition, a negative correlation between caudate volume in childhood and tic severity later in life could be demonstrated (Bloch et al., 2005). It is thought that atypical neurochemical transmission within the basal ganglia leads to the aberrant integrative interplay of CSTC circuitry (Mink, 2001; Singer, 2013). Different neurotransmitter systems have been suggested to be involved in the pathogenesis of TS including the dopaminergic, serotonergic, glutamatergic, gamma-amino butyric acid-(GABA)ergic, histaminergic, and endocannabinoid systems (Martino and Leckman, 2013).

The strongest evidence, however, supports a "dopaminergic

* Corresponding author. Clinic of Psychiatry, Socialpsychiatry and Psychotherapy, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625, Hannover, Germany.
E-mail address: mueller-vahl.kirsten@mh-hannover.de (K.R. Müller-Vahl).

http://dx.doi.org/10.1016/j.jpsychires.2016.11.004

hypothesis" in TS with an increased signal transduction. This assumption is not only supported by the well-known beneficial effects of dopamine receptor blocking drugs (antipsychotics) on tics (Roessner et al., 2011), but also several other findings including alterations in both presynaptic dopamine transporters (DAT) and postsynaptic dopamine D2 receptors (DRD2) in striatal and extrastriatal regions (Gilbert et al., 2006; Minzer et al., 2004; Müller-Vahl et al., 2000; Serra-Mestres et al., 2004; Steeves et al., 2010; Turjanski et al., 1994; Wong et al., 1997; Yoon et al., 2007a). Accordingly, different "dopaminergic hypotheses" have been proposed in TS due to either supersensitive postsynaptic striatal receptors, dopamine hyperinnervation within the striatum, presynaptic abnormality in dopa carboxylase, or elevated intrasynaptic dopamine release as a result of an imbalance between tonic and phasic levels. Of these various proposed changes within the dopaminergic system, most evidence supports an alteration of the tonic-phasic dopamine release system (Singer, 2013). Based on the detection of increased presynaptic dopamine uptake sites in both the striatum (Singer et al., 1991) and the frontal cortex (Minzer et al., 2004) and increased striatal DAT binding (Malison et al., 1995; Müller-Vahl et al., 2000; Serra-Mestres et al., 2004), a reduction in extrasynaptic tonic (homeostatic) dopamine levels has been suggested. It is believed that tonic dopamine is regulated by presynaptic D2 and D3 autoreceptors (Rice et al., 2011). In addition, the observation of an increased release of dopamine in the striatum following amphetamine stimulation (Singer et al., 2002; Wong et al., 2008) - as a measure of synaptically focused, phasic (spike-dependent) dopamine - suggests abnormalities in the transient phasic release of dopamine into striatal regions. In summary, it is believed that in TS an abnormal interaction between tonic and phasic dopaminergic signaling results in altered modulation of CSTC circuitry (Singer, 2013).

Based on twin and family studies it is generally believed that there is a genetic etiology in TS. It can be assumed that multiple variations in multiple genes are likely to be carrying the risk for TS. However, the only available genome-wide association study (GWAS) of TS failed to demonstrate genome-wide significant loci (Scharf et al., 2013). A recently performed large multicenter study failed to replicate previously implicated individual single nucleotide polymorphisms (SNPs) and candidate genes in TS (unpublished data). During the last years, a large number of neurotransmitter related candidate gene association studies has been performed that included - besides several other genes involved in neurodevelopmental, neuroendocrine, and immunological function - various dopamine receptor genes including the DRD2, dopamine D3 receptor (DRD3), dopamine D4 receptor (DRD4) gene as well as the DAT gene and the dopamine catabolizing enzyme catechol-O-methyltransferase (COMT) gene. However, all these studies failed to demonstrate significant reproducible findings (for review see Paschou, 2013).

Most of the available evidence points to the hypothesis that TS is related to an interplay of both genetic and epigenetic factors. Until today, several different environmental factors have been suggested to play a crucial role in the onset and natural course of the disorder including perinatal risk factors, hormonal factors, psychosocial stress, immune mechanisms, and infections (in particular group A streptococcal (GAS) infections). For example, there is evidence that older paternal age, maternal smoking during pregnancy, severe maternal psychosocial stress during pregnancy, delivery complications, low Apgar score, and low birth weight contribute to the development of TS and are associated with increased tic severity, respectively (Burd et al., 1999; Hyde et al., 1992; Leckman et al., 1987; Mathews et al., 2006). Although there is little doubt that environmental factors are involved in the etiology of TS, no single factor could yet be identified (for review see Hoekstra et al., 2013).

During recent years, it became clear that gene expression can be affected not only by DNA sequences, but also epigenetic modifications. Since these epigenetic changes can be induced by environmental factors, a major role of epigenetic variations has been suggested in the etiology of neurodevelopmental disorders via alteration of neural gene function (Kubota et al., 2014). There are several lines of evidence suggesting that DNA methylations at cytosine-phosphate-guanine (CpG) dinucleotides are involved in several complex neuro-psychiatric disorders. Only recently, the first epigenome-wide association study has been published including DNA methylation data consisted of 411,169 autosomal methylation sites in 1678 individuals (188 cases and 1490 controls) (Zilhão et al., 2015). Although no probes reached genome-wide significance, some of the top ranking probes mapped to genes that have been previously described in association with neuropsychiatric disorders (including GABBRI, BLM, and ADAM10).

The aim of this study was to investigate for the first time epigenetic differences in DNA methylation in different dopaminergic genes including DRD2, DAT, and COMT in a group of adult patients with TS.

2. Material and methods

2.1. Patients

In this study, 51 adult patients with TS (≥18 years) were included between 5/2012 and 3/2013. Patients were recruited from the Tourette outpatient department at the Hannover Medical School and the German Tourette advocacy group (Tourette Gesellschaft Deutschland e.V.). In all patients, the diagnoses of TS and psychiatric comorbidities according to DSM-IV-TR were confirmed by one of the authors (KMV), who is a neurologist and adult psychiatrist and a well experienced TS expert. The following exclusion criteria were defined: (1) age < 18 years, (2) secondary tic disorders, and (3) additional diagnosis of psychosis, epilepsy, drug and alcohol dependency, clear mental retardation, regular cannabis use (>once a week for at least 6 months) at present time and/or during the last 5 years. Common psychiatric comorbidities of TS (such as ADHD, OCD, depression, anxiety), other behavioral problems as well as medication for tics and comorbidities were no exclusion criteria.

For clinical characterization of the patients' group we used several different well established assessments: (1) Yale Global Tic Severity Scale (YGTSS) (Leckman et al., 1989) including YGTSS − total tic score (TTS) and YGTSS − global score (GS) (=YGTSS-TTS + impairment score), (2) Premonitory Urge for Tics Scale (PUTS) (Woods et al., 2005), (3) Yale-Brown Obsessive Compulsive Scale (Y-BOCS) (Goodman et al., 1989), (4) Conners' Adult ADHD Rating Scale (CAARS) (Conners et al., 1999), (5) DSM-IV symptom list for ADHD (APA − American Psychiatric Association, 2000), (6) Wender Utah Rating Scale short version (WURS-K) (Retz-Junginger et al., 2002), (7) Beck Depressions Inventory (BDI) (Beck et al., 1961), (8) State-Trait-Anxiety Inventory (STAI) (Laux et al., 1981), (9) Brief Symptom Inventory (BSI) (global severity index (GSI)) (Franke, 2000), (10) Gilles de la Tourette Syndrome − Quality of Life Scale (GTS-QOL) (Cavanna et al., 2008).

For further analyses (see below), we formed three groups of patients according to tic severity (as assessed by YGTSS-TTS): "mild" TS (YGTSS-TTS≤14), "moderate" TS (YGTSS-TTS = 15–34), and "severe" TS (YGTSS-TTS≥35). Used YGTSS-TTS cut-off values follow general recommendations for treatment: a score ≥15 is suggested to indicate clinically significant tics, while a score ≥35 seems to be adequate to display tics as "severe" (Müller-Vahl et al., 2011; Wilhelm et al., 2012). To investigate the influence of comorbidities, in addition, we made not only the diagnoses of comorbid OCD, ADHD, and depression (based on clinical interview and