Psychiatry Research 246 (2016) 275–283

Contents lists available at ScienceDirect

Psychiatry Research

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

Glucocorticoid receptor variants in childhood attention-deficit/hyperactivity disorder and comorbid psychiatric disorders

Andrea B. Schote[a,*], Martina Bonenberger[a], Haukur Pálmason[a], Christiane Seitz[b], Jobst Meyer[a], Christine M. Freitag[c]

[a] Department of Neurobehavioral Genetics, Institute of Psychobiology, University of Trier, Trier, Germany
[b] Department of Child and Adolescent Psychiatry and Psychotherapy, Saarland University Hospital, Homburg, Germany
[c] Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, JW Goethe University, Frankfurt am Main, Germany

ARTICLE INFO

Keywords:
NR3C1
SNPs
Association
ADHD
Cortisol
Conduct disorder

ABSTRACT

Stress results in a variety of neuroendocrine, immune and behavioral responses and represents a risk factor for many disorders. Following exposure to stress, glucocorticoids are secreted from the adrenal cortex and act via the ligand-activated glucocorticoid receptor (GR). Several polymorphisms of the GR-encoding gene *NR3C1* have been described and functionally investigated. However, the impact of these variants on complex diseases such as Attention-Deficit/Hyperactivity Disorder (ADHD) is still unclear. In this study, 251 children with ADHD, 19 affected and 35 unaffected siblings, and their parents were included in a family-based association study assessing seven common variants of *NR3C1* (*TthIII*I_rs10052957; NR3C1-I_rs10482605; ER22/23EK_rs6189/rs6190; N363S_rs56149945; *Bcl*I_rs41423247; GR-9beta_rs6198). A four-marker haplotype (*TthIII*I-NR3C1-I-ER22/23EK) was nominally associated with ADHD. In addition, in index children with ADHD, associations with comorbid disorders, inattentive and hyperactive-impulsive symptoms were explored. N363S minor allele carriers were more likely to show comorbid conduct disorder (CD). In our study, *NR3C1* variants moderately affected ADHD and had a significant effect on comorbid CD. Therefore, *NR3C1* as an important gene of the hypothalamic–pituitary–adrenal axis seems to be particularly relevant for the pathophysiology of ADHD combined with comorbid CD. For a deeper understanding, investigations in larger samples of healthy, ADHD and CD individuals are warranted.

1. Introduction

With a prevalence of 3–5%, attention deficit/hyperactivity disorder (ADHD) is one of the most common psychiatric disorders in children (Polanczyk and Jensen, 2008). ADHD is characterized by age inappropriate impulsiveness and hyperactivity as well as attention problems, and - according to DSM-IV TR - shows three subtypes: hyperactive/impulsive (HI), inattentive (I) and combined (C) ADHD (American Psychiatric Association, 1999). Previous twin and adoption studies showed a strong heritability (70–80%) of ADHD (Freitag et al., 2010). In children with ADHD, psychiatric comorbidity is high, especially anxiety disorder (AnxD, around 30%), oppositional defiant disorder (ODD, around 50%), and conduct disorder (CD, around 30%) are frequently present (Willcutt et al., 2005).

The hypothalamic-pituitary-adrenal (HPA) axis is the major physiological stress response- and regulation system. Its activation results in the secretion of cortisol from the adrenal glands. Cortisol as the major stress hormone acts on hypothalamus, pituitary and hippocampus, and inhibits its own production by a negative feedback loop. The HPA axis shows a diurnal rhythm as well as an increase in cortisol response to awakening (cortisol awakening response, CAR) (Clow et al., 2010; Pruessner et al., 1997). The CAR represents a sensitive and stable measurement for the physiological reactivity of the HPA axis (Clow et al., 2010; Wust et al., 2000), although in younger children, it is less pronounced or even absent (O'Connor et al., 2005; Freitag et al., 2009). In children with ADHD, a reduced CAR was found in comorbid ODD (Freitag et al., 2009), in hyperactive/impulsive (Ma et al., 2011) and combined ADHD (Blomqvist et al., 2007). Studies implementing cortisol measures that represent other stages of the HPA axis activities revealed conflicting results (Gispen-de Wied et al., 1998; Hirvikoski et al., 2009; Pesonen et al., 2011; Sondeijker et al., 2007). In response to an experimental, standardized psychosocial stress test such as the Trier Social Stress Test for Children (TSST-C) (Dickerson and Kemney, 2004; Foley et al., 2004), a blunted cortisol response was reported for

* Corresponding author.
E-mail address: schotefrese@uni-trier.de (A.B. Schote).

http://dx.doi.org/10.1016/j.psychres.2016.10.001
Received 26 November 2015; Received in revised form 10 August 2016; Accepted 2 October 2016
Available online 02 October 2016

children with combined ADHD (van West et al., 2010), hyperactive/impulsive ADHD (Maldonado et al., 2009), and inattentive ADHD (Randazzo et al., 2008; Pesonen et al., 2011). However, other studies did not find these differences in children with ADHD symptoms (Snoek et al., 2004; Hastings et al., 2009). Hypo-reactivity of the HPA axis during psychosocial stress was shown for comorbid disruptive behavior disorders (DBD) comprising ODD and CD (Fairchild et al., 2008; Popma et al., 2006; van Goozen et al., 1998, 2000). In ADHD with comorbid DBD, the cortisol response was blunted (Snoek et al., 2004; Hastings et al., 2009; Stadler et al., 2011), whereas an increased cortisol response to stress was observed for ADHD and comorbid anxiety disorder (Hastings et al., 2009). The HPA axis clearly plays a role in ADHD, and several underlying mechanisms are discussed. One theory that might underpin the reported lower activity of the HPA axis in more severe ADHD is Zuckerman's sensation-seeking theory (Zuckerman and Neeb, 1979). It postulates that people tend to normalize the unpleasant status of under-arousal through an increase of their physical tension and sensation seeking behavior to temporarily obtain a higher sympathetic arousal level. In line with this, individuals with low sympathetic arousal levels as seen in e. g. hyperactive/impulsive ADHD may display low HPA axis activity (Sondeijker et al., 2007).

The genomic effects of cortisol are mediated via two receptors, the mineralocorticoid receptor (MR; encoding gene *NR3C2*) and the glucocorticoid receptor (GR, encoding gene *NR3C1*). Both receptors orchestrate the neuroendocrine and behavioral response to a stressor via trans-activation or trans-repression of their target genes. While MR, with a 10-fold higher affinity for cortisol, maintains corticosteroid homeostasis by controlling basal HPA axis activity; GR prevents the initial stress reaction from overshooting and facilitates the recovery. Although both receptors are abundantly expressed, co-localized in neurons from e.g. the hippocampus, prefrontal cortex (PFC) and amygdala (Kloet et al., 1998), and exert complementary effects, GR rather than MR modulates the action of other transcription factors involved in the initial stress response (Oitzl et al., 2010). Recently, it was shown that genetic variants of *GR* have direct effects on the functioning of the PFC (El-Hage et al., 2013), and thus possibly also on top-down regulation of attention and executive control in ADHD (Arnsten, 2006). Furthermore, GR might be directly involved in the dysregulation of the HPA axis reported for hyperactive/impulsive ADHD (Ma et al., 2011) via negative feedback regulation in the hypothalamic neurons and pituitary corticotropes (Kloet et al., 1998). Finally, GR interacts with other neurotransmitter systems in the brain such as the dopaminergic system (Inoue et al., 2004; McEwen, 2007), which has been shown to be involved in the ethiology of ADHD (Faraone and Biederman, 1998).

The GR-encoding gene, *NR3C1* (OMIM+138040, located at 5q31), is genetically highly complex, which leads to different tissue-specific receptor isoforms (Kloet et al., 1998; Zhou and Cidlowski, 2005) showing different GC sensitivities (Derijk et al., 2002). Seven functional genetic polymorphisms have been associated with alterations of HPA axis feedback regulation as well as changes in stress responsiveness (Derijk et al., 2002; Kumsta et al., 2008; Manenschijn et al., 2009; van Rossum and Lamberts, 2004). The single nucleotide polymorphisms (SNPs) *Bcl*I (rs41423247) and N363S (rs56149945) have been associated with increased GC sensitivity, enhanced salivary cortisol response after psychosocial stress as well as with unchanged or lower baseline cortisol levels, respectively (Derijk et al., 2002; Huizenga et al., 1998; Manenschijn et al., 2009; van Rossum and Lamberts, 2004; Wust et al., 2004). Minor alleles of the SNPs *TthIII*I (rs10052957), NR3C1-I (rs10482605), ER22/23EK (rs6189/rs6190) and 9beta (rs6198) were associated with relative GC resistance and higher baseline cortisol levels (Derijk et al., 2002; Kumsta et al., 2008; Manenschijn et al., 2009; van Rossum and Lamberts, 2004), probable due to the destabilization of the active receptor isoform (Russcher et al., 2005; Kumsta et al., 2008). Despite constantly evolving research, the underlying biological mechanisms of the actions of *NR3C1* SNPs and the role of these variants and haplotypes in complex disorders are still not fully understood. Interestingly, *NR3C1* variants have been associated with complex psychiatric disorders such as schizophrenia, bipolar and major depressive disorder (Derijk et al., 2002; Manenschijn et al., 2009; Spijker and van Rossum, 2009; Zobel et al., 2008) and were targeted by the recent microarrays used for genome wide association studies from the Psychiatric Genomics Consortium for ADHD, however without any significant association (Hawi et al., 2015). In addition, four association studies investigated stress relevant candidate genes in ADHD (Kortman et al., 2013; Isaksson et al., 2015; Fortier et al., 2012; van der Meer et al., 2016). It was shown that carriers of the *NR3C1* 9beta haplotype showed a positive correlation between long-term stress exposure and ADHD severity compared to non-carriers (van der Meer et al., 2016). Fortier and colleagues reported an association of a specific *NR3C1* haplotype G-A-G-G (E22E-N363S-*Bcl*I-9beta) with ADHD and comorbid ODD symptoms (Fortier et al., 2012). In our study, we also included two SNPs of *NR3C1* (NR3C1-I and *TthIII*I), which are located in the promoter region of the gene. They are known for their action on *NR3C1* expression regulation and showed association with other stress-related disorders.

Due to the important role of *NR3C1* variants on HPA axis feedback regulation, brain functioning and neurotransmitter mediated processes, the aim of the study was to test the association of seven *NR3C1* polymorphisms with childhood ADHD. Furthermore, we explored an association with the three most frequent comorbid disorders, ODD, CD, and AnxD as well as with dimensional ADHD symptoms in the index children with ADHD.

2. Materials and methods

2.1. Sample and assessment instruments

Families were recruited at the Department of Child and Adolescent Psychiatry and Psychotherapy, Saarland University Hospital, Germany, and at the Department of Neurobehavioral Genetics, University of Trier, Germany. All patients and participants gave their informed consent for participation in the study. Ethical approval of the study design was obtained from the local ethics committee (Ethikkommission der Ärztekammer des Saarlandes) in accordance with the declaration of Helsinki. Attention deficit/hyperactivity disorder (ADHD) shows three subtypes: hyperactive/impulsive (HI), inattentive (I) and combined (C) ADHD (DSM-IV TR, American Psychiatric Association, 1999). The number of index children included in this study was N=251 children, N=60 (23.9%) with ADHD-I, N=22 (8.8%) with ADHD-HI and N=169 (67.3%) with ADHD-C. Genetic data were available for 245 mothers, 170 fathers, 35 unaffected sibs and 19 affected sibs. An overview about demographic data of the index children is given in Table 1. All clinical details have been published previously (Freitag et al., 2012).

Briefly, in index children and affected siblings, ADHD and comorbid psychiatric disorders were diagnosed according to the fourth and text revision of the diagnostic and statistical manual of mental disorders (DSM-IV TR) by a standardized, structured interview with the parent or primary caregiver (Kinder-DIPS) and a clinician rated German Hyperkinetic Syndrome diagnosis checklist (DCL-HKS) for each DSM-IV TR-derived symptom. Symptoms were rated on a Likert-scale of 0–3, with scores of 2 and 3 indicating the presence of the respective symptom. Global, inattentive and hyperactive-impulsive ADHD symptom scores were calculated from the DCL-HKS by summarizing the scores of the respective items (9 inattentive, 11 hyperactive-impulsive symptoms, coded from 0= no symptoms to 3= severe symptoms). Additionally, parents and teachers filled in a questionnaire on DSM-IV TR-derived ADHD symptoms to ensure pervasiveness of ADHD symptoms across different settings in affected children or to exclude ADHD in unaffected siblings. Intelligence testing