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The Effect of Nicotine on HPA Axis Activity in Females is Modulated by the *FKBP5* Genotype

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Summary

Tobacco smoking modulates activity in the hypothalamic-pituitary-adrenal (HPA) axis and is used to cope with stress, especially by females. The single nucleotide polymorphism (SNP) rs1360780, linked to FK506-binding protein 51 (FKBP5), has been shown to affect HPA axis functioning, and has thus been suggested as a promising candidate for indicating vulnerability to stress-related disorders. The aim of this study was to investigate the interaction between nicotine consumption and rs1360780 on cortisol plasma levels in females. A total of 296 female smokers (assessed by the Fagerström Test for Nicotine Dependence; FTND) were genotyped for the SNP rs1360780. We measured participants' cortisol plasma concentration in blood plasma collected 3 h after standardized tobacco smoking exposure. In the 36 *TT-homozygotes*, we found a significant negative correlation between the FTND sum score and cortisol plasma concentrations. Using linear regression analysis, we found that the FTND sum score accounted for 12.4% of the variance of cortisol plasma levels. This association was not detected in *C-allele carriers*. Our results suggest that nicotine is an important confounder in the modulation of HPA axis activity by FKBP5. In light of these findings, future studies on FKBP5 should seek to include data on nicotine consumption as a covariate.

Keywords: Nicotine, FKBP5, HPA axis

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is the backbone of the human stress response system. Acute stress stimulates the release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus of the hypothalamus, which in turn activates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. AVP and CRH serve to regulate cortisol release from the adrenal gland, both directly and indirectly by means of their effect on ACTH. Cortisol initiates the body's physiological stress response, but also triggers negative feedback on the HPA axis by activating the glucocorticoid receptors (GRs) and by modulating CRH and AVP expression (Kovács et al., 2000). The negative feedback loop is a critical element in healthy stress response, as it prevents overactivation of the stress response system. A lack of sensitivity in the GRs results in an impairment of this regulation system.

Activity in the GRs is in turn influenced by cochaperones (Pratt & Toft, 1997; Pratt et al., 2006). FK506-binding protein 51 (FKBP5) is a cochaperone of heat shock protein 90 (hsp90), which regulates GR sensitivity. When cortisol is absent, FKBP5 binds to hsp90 via a tetratricopeptide repeat domain, resulting in a low affinity of the GR complex to cortisol (Wochnik et al., 2005). On the other hand, when cortisol is present, FKBP5 is replaced by a different cochaperone,

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FK506-binding protein 52 (FKBP4). In this conformation, the GR complex is capable of binding itself to dynein, which allows for its translocation to the nucleus and a further activation of gene transcription (Wochnik et al., 2005). FKBP5 also stimulates the translocation of the biologically inactive isoform of GR (the GR beta) decreasing the GR signaling pathway (Zhang et al., 2008). This is in line with the research of Wochnik et al. (2005), who found that increased expression of FKBP5 leads to a decrease in receptor affinity, as well as reduced translocation of the GR complex to the nucleus, thereby impairing steroid sensitivity.

The *FKBP5* gene is located on chromosome 6p21 and consists of 10 exons (Binder, 2009). High rates of *FKBP5* expression have been observed in the brain as well as in a broad range of human cells, including in muscle, liver and thymus tissue (Gawlik et al., 2006). Furthermore, activation of the glucocorticoid response elements by cortisol triggers *FKBP5* expression (Vermeer et al., 2003).

The functioning of *FKBP5* is modulated by some common single nucleotide polymorphisms (SNPs) within the *FKBP5* gene (Binder et al., 2004). The SNP rs1360780, which is located closest to a functional glucocorticoid response element, is said to have a functional role in the regulation of *FKBP5* expression in addition to helping regulate GR sensitivity (Klengel et al., 2013). The minor *T-allele* is associated with higher *FKBP5* expression (Binder et al., 2004) and with a relative reduction in GR sensitivity (Binder et al., 2008). Additionally, Klengel et al. (2013) were able to show that the functional polymorphism in SNP rs1360780, which alters the interaction between the chromatin start site and long-range enhancers in the *FKBP5* gene, increases the risk of developing stress-related psychiatric disorders in adulthood. This occurs by means of allele-specific, childhood-trauma-dependent DNA demethylation in the functional glucocorticoid response elements of FKBP5. This demethylation is linked to increased stress-dependent gene transcription, followed by long-term dysregulation of the stress hormone system.

An impairment of the HPA axis is common in psychiatric patients. FKBP5 is a promising candidate for indicating vulnerability to stress-related disorders, such as depression, anxiety and posttraumatic stress disorder (Koenen et al., 2005; Binder et al., 2008; Koenen & Uddin, 2010; Xie et al., 2010; Zimmermann et al., 2011; Hauger et al., 2012; Klengel et al., 2013; Pérez-Ortiz et al., 2012). However, it has also been observed in healthy individuals that the polymorphism in SNP rs1360780 is linked to changes in the stress response of the HPA axis, with *T-allele carriers* showing an impaired recovery of cortisol levels in response to stress (Ising et al., 2008; Buchmann et al., 2014).

Although the majority of previous studies on this subject have identified the minor *T-allele* as a risk factor for psychiatric disorders such as depression, posttraumatic stress disorder, and substance use disorders (e.g., Binder et al., 2004; Lekman et al., 2008; Brent et al., 2010; Shinozaki et al., 2011; Minelli et al., 2013; Levran et al., 2014; Szczepankiewicz et al., 2014), a handful of other studies have been unable to confirm these findings, or have even identified the minor *T-allele* as a protective factor against psychiatric disease (e.g., Gawlik et al., 2006; Sarginson et al., 2010; Huang et al., 2014). These conflicting results suggest that there may be a number of confounding factors that influence the effect of the polymorphism in rs1360780 on HPA axis activity. Therefore, subgroup-specific analyses that account for the effects of confounders should be undertaken in order to provide a better understanding of the modulating effects of rs1360780 on HPA axis activity.

Research conducted by Pomerleau et al. (1993) found that female smokers with high cognitive restraint and high disinhibition in relation to eating, both of which are associated with high stress perception, smoke cigarettes to try to avoid obesity and cope with stress. Additionally, we have shown in a previous study that females were overrepresented in a sample of nicotine-dependent patients that exhibit both high cognitive restraint and high disinhibition in relation to eating. These participants scored significantly higher on the Perceived Stress Scale (PSS) and the Beck Depression Inventory (BDI) in comparison to the control group, who exhibited no pathologies in relation to eating behavior (Koopmann et al., 2011). Taken together, these two studies suggest that some individuals, especially females, use smoking to cope with negative affective states and stress.

As discussed above, HPA axis activity is modified by the polymorphism in rs1360780. It could therefore be hypothesized that this polymorphism modulates the effect of smoking on HPA axis activity.

This study aimed to investigate whether the polymorphism in rs1360780 modifies the effect of nicotine consumption on plasma levels of cortisol, in a sample of 296 nicotine-dependent females who currently smoked.

Material and Methods

Participants

We recruited female smokers to participate in this study in seven different recruitment centers throughout Germany (Aachen, Berlin, Bonn, Dusseldorf, Erlangen, Mainz, and Mannheim). All participants were non-treatment-seeking individuals. They were randomly selected from an official register of local residents, and were invited to participate in the study via post. Respondents underwent an initial 10-min prescreening interview conducted by telephone.

Only individuals who met the inclusion and exclusion criteria were invited for a final screening in our lab. The inclusion criteria for participation were: female gender, aged 18−65 years, current smoker (minimum of seven cigarettes per week or one cigarette per day) and of Caucasian origin. The exclusion criteria were as following: male gender, alcohol or substance abuse or dependence (as defined in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition; DSM-IV) or any other DSM-IV axis-I psychiatric diagnosis within the previous 6 months, pregnancy, treatment with psychotropic medication within the previous 6 months, or any neurological illness during the patient's lifetime.

The final laboratory screening involved a medical examination, a standardized psychiatric interview (Structured Clinical Interview for DSM Disorders; SCID-I), a drug screening, and a breathalyzer test to measure alcohol levels. Given that previous findings suggest that minor T-allele carriers have an increased risk of stress-related disorders, we divided our sample into two study groups: a group of *C-allele carriers* and a homozygote group of minor T-allele carriers.

All participants received a monetary reward of €50 for their participation. Written, informed consent was sought from each of the participants before any procedures were carried out. The study was approved by the ethics committee of the local university at each study site, and was conducted in accordance with the Declaration of Helsinki.

Testing Procedures

The assessment sessions, which were held in the laboratories of each study site, followed a prearranged timetable of data collection and included a 1-h lunch break at 12 p.m., during which participants were provided with a standardized 600 kcal meal. The same assessment procedure was used for all participants, starting at 8:30 a.m. with a medical examination, followed by a standardized psychiatric interview (SCID-I), a drug screening and a breathalyzer test to measure alcohol levels, and lasted until 4:30 p.m. The participants smoked according to a fixed schedule of one cigarette at 9 a.m. and one cigarette at 12 noon, before the standardized meal. In order to conduct neuroendocrine and genetic analyses, a blood sample of 30 ml was collected from participants 3 h after nicotine consumption, at 3 p.m. Finally, in order to quantify nicotine consumption using a biological marker, we measured participants' exhaled air CO levels at 8:30 a.m.

Measurements of Cortisol Plasma Levels

We collected blood samples from participants at 3 p.m. using venipuncture; samples were anticoagulated with sodium EDTA (1 mg/ml whole blood) and immediately cooled on ice. We separated blood plasma using centrifugation (4000 g). Aliquots were immediately frozen, and stored at −80ºC until the analyses were carried out (maximum six months after collection). Hormonal analyses were performed at the Neurobiological Laboratory of the Department of Psychiatry at the University Hospital of Hamburg.

Plasma cortisol was measured by modified commercial radioimmunoassay using coated tube techniques (DRG Instruments, Marburg, Germany). The interassay coefficient of variation was 7% and the intra-assay coefficient was 5%. The minimum detectable quantity of cortisol was 1 ng/ml plasma.

DNA Preparation and Genotyping

Genotyping was undertaken at the Cologne Center for Genomics at the University of Cologne. DNA from fresh frozen EDTA blood was prepared using a Qiagen FlexiGene DNA Kit, in accordance with the manufacturer's instructions and normalized based on RNase P copy number measurement using the TaqMan RNase P assay from Applied Biosystems (Foster City, CA, USA). The SNP rs1360780, a regulator of the expression of FKBP5, was chosen to cover the influence of FK506-binding protein on HPA axis activity. Genotyping was performed using SNP stream SNP genotyping assays. Genotyping call rates were 99%.

Psychometric Measures

We used Structured Clinical Interviews (SCID; Wittchen et al., 1993) to confirm the absence of any psychiatric comorbidities in the participants, conducted in accordance with DSM-IV criteria (American Psychiatric Association, 1994).

The severity of participants' nicotine dependence was measured using the Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991; Schumann et al., 1991).

We measured participants' anxiety using the State-Trait Anxiety Inventory (STAI; Spielberger, 1970; Laux et al., 1981), whereas depressive symptoms were evaluated using the BDI (Beck et al., 1961; Hautzinger et al., 1995). Participants' subjective experiences of stress were measured using the PSS (Cohen et al., 1983).

Finally, we used the Alcohol Use Disorder Identification Test (AUDIT; Saunders et al., 1993) to measure participants' risk in relation to alcohol abuse problem behaviors.

Statistical Analysis

We used Predictive Analytics Software (PASW 21) and Statistical Analysis System (SAS 9.3) for Windows to perform our data analyses. The genotypes were divided into two groups,

one comprised *TT-homozygotes* and the other of *C-allele carriers (CC/CT)*. We tested for deviation from the Hardy-Weinberg equilibrium (HWE) using the χ^2Goodness-of-Fit Test for HWE.

Psychometric and hormonal data are reported using the mean and standard deviation (SD) for each of the two independent samples, which were also analyzed for differences using t-tests. In each of the study groups, we looked for potential correlations between psychometric data and cortisol plasma levels using Pearson's correlation coefficient. Statistical significance was accepted if a p-value of less than 0.05 was obtained.

Finally, we used linear regression analysis to assess the influence of the severity of nicotine dependence or level of depression on cortisol plasma concentrations in each study group.

Results

Group Characteristics

After completing the above-mentioned screening procedures, we obtained a sample of 296 female smokers, of which 36 were *TT-homozygotes* for rs1360780 and 260 were *C-allele carriers* (132 *CC*/128 *CT*). The genotype distribution was in the HWE (χ^2= 0.3316, $p = 0.5647$).

On average, participants were found to consume 12.95 cigarettes per day (SD = 9.30) and had been smoking for 19.36 years (SD = 12.12). Their average score on the FTND was 2.95 (SD = 2.62). No significant differences were found in smoking behavior between *C-allele carriers* and *TT-homozygotes*.

Table 1 provides participant characteristics such as age, weight, body mass index (BMI), CO level in exhaled air, and education level. We did not find any significant differences between the groups in relation to these variables.

Cortisol Plasma Concentration

The average cortisol plasma concentration in *C-allele carriers* was 209.63 ng/ml, compared to 218.49 ng/ml in *TT-homozygotes*. The difference in plasma concentrations between the two study groups were not statistically significant (t[296] = 0.441, $p = 0.66$).

Psychometric Testing

Participants were found to have an average state anxiety of 34.98 (SD = 8.31) and an average trait anxiety of 36.71 (SD = 9.16), measured using the STAI. Their average level of depression was 5.04 (SD = 5.20) as assessed by the BDI. Participants' mean score on the PSS was 20.89 (SD = 7.55). Their mean score on the AUDIT was 4.04 (SD = 3.1). There were no statistical differences between the two study groups in relation to all these measures.

Relationship of Cortisol Plasma Concentrations to Smoking Behavior and Psychological Symptoms

In the group of *TT-homozygotes,* we found a significant negative correlation between the severity of nicotine dependence (measured by the FTND) and cortisol plasma levels. However, there was no correlation between cortisol plasma levels and the sum scores of the STAI State with respect to the STAI Trait, as well as of the sum score of the BDI with respect to the PSS, or in relation to participants' AUDIT sum score (see Table 2).

In the group of *C-allele carriers,* we found a significant negative correlation between participants' depression levels (measured by the BDI) and cortisol plasma concentrations. However, we did not find any correlation between cortisol plasma levels and the sum scores of the STAI State with respect to the STAI Trait, or in relation to the sum score of the FTND with respect to the PSS. Similarly, there was no correlation between the sum score of the AUDIT and cortisol plasma levels (see Table 2).

Regression Analyses

We employed four distinct linear regression models in order to analyze any potential effects of depression (measured by the BDI sum score) and nicotine dependence (measured by the FTND sum score) on cortisol plasma levels in *C-allele carriers* and *TT-homozygotes*.

In *TT-homozygotes,* the FTND sum score accounts for 12.4% of the variance in cortisol plasma concentrations (adjusted $R^2 = 0.098$, $p = 0.038$). In *C-allele carriers,* however, a linear regression model assessing the effects of the FTND sum score on cortisol plasma concentration did not produce a significant result (adjusted $R^2 = 0.009$, $p = 0.069$).

In *C-allele carriers,* the BDI sum score accounts for 2.3% of the variance of cortisol plasma concentrations (adjusted $R^2 = 0.019$, $p = 0.016$). However, linear regression analysis assessing the effects of the BDI sum score on cortisol plasma concentrations in *TT-homozygotes* did not produce a significant result (adjusted $R^2 = 0.006$, $p = 0.657$).

Table 1 Sample characteristics.

Variables	Overall (n = 296)	*C-allele carriers* (n = 260)	*TT-homozygotes* (n = 36)
Age in years (mean)	35.45	34.76	38.31
(SD)	(12.37)	(12.46)	(12.62)
Weight in kg	68.89	67.57	68.55
(SD)	(15.65)	(15.08)	(17.22)
BMI (kg/m^2; mean)	24.43	23.95	24.35
(SD)	(5.48)	(5.18)	(6.47)
CO level in exhaled air (mean)	12.32	11.93	15.12
(SD)	(10.61)	(10.76)	(12.34)
Level of education in years (mean)	11.2	10.8	12.2
(SD)	(0.65)	(0.85)	(0.64)

BMI, body mass index; SD, standard deviation.

Table 2 Relationship of cortisol plasma concentration to smoking behavior and psychological symptoms.

	C-allele carriers		*TT-homozygotes*	
	Cortisol		Cortisol	
	r	*p*	*r*	*p*
FTND sum score	−0.114	0.069	−0.353*	0.038
BDI sum score	−0.152*	0.016	−0.079	0.657
STAI-State	−0.064	0.314	0.150	0.390
STAI-Trait	−0.088	0.16	0.123	0.483
PSS	−0.052	0.406	0.018	0.918
AUDIT	0.039	0.534	−0.179	0.305

FTND, Fagerström test for nicotine dependence; BDI, beck depression inventory; STAI-State, state anxiety subscale of the state-trait anxiety inventory; STAI-Trait, trait anxiety subscale of the state-trait anxiety inventory; PSS, perceived stress scale; AUDIT, alcohol use disorder identification test; *r*, Pearson correlation coefficient; *p*, level of significance.
*Indicates that $p < .05$.

Discussion

The principal result from this study is the finding of a significant negative association between the FTND sum score and cortisol plasma concentrations in the group of *TT-homozygotes*. No such association was found in *C-allele carriers*. In the group of *TT-homozygotes,* the FTND sum score was found to account for 12.4% of the variance in cortisol plasma levels. The second significant result we obtained was a negative correlation between levels of depressive symptoms (as measured by the BDI sum score) and cortisol plasma concentrations in *C-allele carriers*. This was not detected in *TT-homozygotes*. In the *C-allele carriers,* the BDI sum score accounted for 2.3% of the variance in cortisol plasma levels.

Previous research on the role of the polymorphism rs1360780 in regulating HPA axis activity in humans, as well as on its role in the development and maintenance of psychiatric disorders, has produced heterogeneous results.

Research has found that in healthy individuals, *T-allele carriers* show a suppressed cortisol response to stress and significant age-related changes in the glucocorticoid receptor and in *FKBP5* mRNA expression levels (Fujii et al., 2014). Additionally, Minelli et al. (2013) found that *T-allele* carriers more frequently exhibit personality traits associated with an increased vulnerability to anxiety.

Various studies in the literature have found evidence linking rs1360780 to depressive symptoms. For example, in research focusing on major depression, Binder et al. (2004), Lekman et al. (2008), Brent et al. (2010), Shinozaki et al. (2011), and Szczepankiewicz et al. (2014) found that the *T-allele* is associated with increased severity of depression. Additionally, Minelli et al. (2013) have shown that the *T-allele* is associated with higher rates of comorbid anxiety disorders. A further study by VanZomeren-Dohm et al. (2015) found a gender-specific effect of the polymorphism in rs1360780: Peer-victimized female *T-allele carriers* exhibited the highest

risk of depressive disorders, whereas male carriers of the minor allele were found to have the lowest risk. Appel et al. (2011) have found a gene environment interaction between the *FKBP5* functional polymorphism in rs1360780 and childhood physical abuse, in relation to the risk of depressive disorders and symptoms. *TT-homozygotes* were found to exhibit significantly higher rates of major depression than *C-allele carriers*.

By contrast, Gawlik et al. (2006) and Sarginson et al. (2010) observed no link between rs1360780 and disease variables of depressive disorders, nor to the success of clinical outcomes following treatment for depression.

Research has proved more homogenous in relation to posttraumatic stress disorders. No studies have found any direct effect of the polymorphism in rs1360780 on the level of PTSD symptoms; however, the literature does document a significant gene-environment interaction, whereby the combination of the minor *T-allele* and child abuse severity can predict the level of PTSD symptoms in adults (Binder et al., 2008), as well as the risk of attempting suicide (Roy et al., 2010). Additionally, Buchmann et al. (2014) concluded that there is a significant interaction between genotype and childhood adversity in relation to the body's cortisol response to stress. The severity of childhood maltreatment was significantly associated with reduced cortisol levels in *CC-homozygotes*, while no such effects were observed in *T-allele carriers*.

Although addictive legal and illegal substances such as alcohol, nicotine, and heroin are often used to cope with negative affective states and depression, research exploring the link between this behavior and the polymorphism in rs1360780 is both rare and inconsistent. A study by Levran et al. (2014) indicates that the polymorphism in rs1360780 contributes to the development of heroin dependence by modulating stress response. By contrast, Huang et al. (2014) found that the minor *T-allele* is associated with less severe symptoms of alcohol withdrawal in alcohol-dependent patients.

Taken together, most of the studies we have examined identify the minor *T-allele* as a risk factor for psychiatric disorders. However, a number of other studies have been unable to confirm these findings, and others even identified the *T-allele* as a protective factor against the development of psychiatric disorders (Huang et al., 2014). The discrepancies between these conflicting findings could be explained by factors such as participants' ethnicities, gender effects, or environmental factors.

As discussed above, some individuals, particularly females, use smoking to cope with negative affective states and stress (Pomerleau et al., 1993; Koopmann et al., 2011). As such, smoking is very common in patients suffering from affective and anxiety disorders. However, no previous studies have considered smoking as a confounding factor in their analysis of the polymorphism in rs1360780. This is the first pilot study that attempts to investigate the interactive effects of smoking and the polymorphism in rs1360780 on HPA axis activity. To control for any other confounding factors, we only included female smokers of Caucasian origin without a psychiatric comorbidity in our study sample.

Our results suggest that the effect of nicotine on HPA axis activity is modified by the polymorphism in rs1360780. In *TT-homozygotes,* nicotine seems to attenuate HPA axis activity; this was not observed in *C-allele carriers*. By contrast, in *C-allele carriers,* HPA axis activity is more strongly influenced by the affective state of the participant. These preliminary results are based on a small study sample, and the cross-sectional design of the study only analyzes a single measurement of cortisol at one point in time. However, they can help to understand and interpret the partly conflicting research detailed above.

Our results suggest that nicotine is a very important confounder in the modulation of HPA axis activity by *FKBP5*, which is regulated by the polymorphism in rs1360780. In light of these findings, further research is needed to investigate the modulating effects of *FKBP5* on affective and anxiety disorders, taking into account nicotine consumption as a confounder.

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Conflicts of Interests

None of the authors have any conflicts of interests.

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