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Methylation of HPA axis related genes in men with hypersexual disorder

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ABSTRACT

Hypersexual Disorder (HD) defined as non-paraphilic sexual desire disorder with components of compulsivity, impulsivity and behavioral addiction, and proposed as a diagnosis in the DSM 5, shares some overlapping features with substance use disorder including common neurotransmitter systems and dysregulated hypothalamic-pituitary-adrenal (HPA) axis function. In this study, comprising 67 HD male patients and 39 male healthy volunteers, we aimed to identify HPA-axis coupled CpG-sites, in which modifications of the epigenetic profile are associated with hypersexuality.

The genome-wide methylation pattern was measured in whole blood using the Illumina Infinium Methylation EPIC BeadChip, measuring the methylation state of over 850 K CpG sites. Prior to analysis, the global DNA methylation pattern was pre-processed according to standard protocols and adjusted for white blood cell type heterogeneity. We included CpG sites located within 2000 bp of the transcriptional start site of the following HPA-axis coupled genes: Corticotropin releasing hormone (CRH), corticotropin releasing hormone binding protein (CRHBP), corticotropin releasing hormone receptor 1 (CRHR1), corticotropin releasing hormone receptor 2 (CRHR2), FKBP5 and the glucocorticoid receptor (NR3C1). We performed multiple linear regression models of methylation M-values to a categorical variable of hypersexuality, adjusting for depression, dexamethasone non-suppression status, Childhood Trauma Questionnaire total score and plasma levels of TNF-alpha and IL-6.

Of 76 tested individual CpG sites, four were nominally significant ($p < 0.05$), associated with the genes CRH, CRHR2 and NR3C1. Cg23409074–located 48 bp upstream of the transcription start site of the CRH gene – was significantly hypomethylated in hypersexual patients after corrections for multiple testing using the FDR-method. Methylation levels of cg23409074 were positively correlated with gene expression of the CRH gene in an independent cohort of 11 healthy male subjects. The methylation levels at the identified CRH site, cg23409074, were significantly correlated between blood and four different brain regions.

CRH is an important integrator of neuroendocrine stress responses in the brain, with a key role in the addiction processes. Our results show epigenetic changes in the CRH gene related to hypersexual disorder in men.

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1. Introduction

Hypersexual Disorder (HD) defined as non-paraphilic sexual desire disorder with components of impulsivity, compulsivity and behavioral addiction, was proposed as a diagnosis for the DSM 5 (Kafka, 2010). Even though limited, recent research demonstrated some overlapping features between HD and substance use disorders, including common neurotransmitter systems and

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dysregulated hypothalamic-pituitary-adrenal (HPA) axis function (Kraus et al., 2016; Chatzittofis et al., 2016). While the neurobiological underpinnings of hypersexual disorder largely remain to be elucidated, it has been proposed that HD may share characteristics with addictive behaviors with a reward deficiency component, in turn partly affected by both genetic and epigenetic factors (Blum et al., 2015).

Increasing evidence suggests that DNA methylation is implicated in the pathophysiology of most psychiatric disorders (Menke and Binder 2014; Mill et al., 2008) and alterations in epigenetic patterns have an effect on brain functions (Ma et al., 2009). Variations in DNA methylation (the addition of a methyl group to the 5′-position of the cytosine pyrimidine ring) modify gene expression by inhibiting transcription or by recruiting specific proteins that alter the chromatin state. Epigenetic modifications, including DNA methylation and histone acetylation (involved in chromatin remodeling), are involved not only in brain functioning but also in sexual behavior. In animal models, epigenetic modifications have been shown to affect sexual behavior, influencing sociosexual behavior, partner preference and post-copulatory sexual selection (Matsuda, 2014; Zeh and Zeh, 2008; Wang et al., 2013). Direct evidence of epigenetic mechanisms in human sexual behavior is sparse and research has mainly focused on the role of epigenetics in sexual orientation (Ngun and Vilain, 2014). To our knowledge, no studies thus far have investigated epigenetic changes in hypersexual disorder.

Substantial translational evidence implicates impairments in glucocorticoid signaling in stress related psychiatric disorders, such as major depressive disorder (MDD) and post-traumatic stress disorder (PTSD). Early life adversity increases risk for developing psychopathology (Teicher and Samson, 2013) through epigenetic modification of stress reactivity genes (Turecki and Meaney, 2016). We have previously reported that hypersexual disorder was associated to hyperactive HPA axis in male patients, irrespective of early life adversity (Chatzittofis et al., 2016) and men with HD had higher TNFα levels compared to healthy male volunteers (Jokinen et al., 2016).

In this study, we aimed to identify HPA-axis coupled CpG-sites, in which modifications of the epigenetic profile are associated with hypersexual disorder. Further, associations of methylation to gene expression were tested in an independent sample of healthy controls.

2. Methods

2.1. Ethics

The study protocols were approved by the Regional Ethical Review Board in Stockholm (Dnrs: 2013/1335-31/2) and the participants gave their written informed consent to the study.

2.2. Patients

The study population has been described previously in detail (Chatzittofis et al., 2016). Sixty-seven male patients with hypersexual disorder seeking psychotherapeutic treatment were recruited to the study at the Center for Andrology and Sexual Medicine (CASM) at the Karolinska University Hospital, a multidisciplinary center for diagnostics and treatment of patients with sexual dysfunctions. Inclusion criteria were a diagnosis of hypersexual disorder, age of 18 years or older and available contact information. Exclusion criteria were as follows: current psychotic illness, other psychiatric disease requiring immediate treatment, current alcohol/drug abuse or serious physical illness. A trained psychiatrist and psychologist established the diagnosis of hypersexual disorder according to proposed DSM 5 criteria (Kafka, 2010) and psychiatric diagnoses using the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998).

2.3. Healthy volunteers

Healthy male volunteers were recruited from the Karolinska Trial Alliance (KTA) database. Subjects were included if they had no previous or current psychiatric illness, no serious physical illness, no first degree relative with completed suicide, bipolar disorder, or schizophrenia; and no previous exposure to serious trauma (natural disaster, assault). Individuals screened positive for HD or pedophilic disorder were also excluded. An effort was made to age match the healthy volunteers and HD patients, as well as to match the time of blood sampling to either fall or spring to minimize potential seasonal variations. In total, 40 male volunteers were included in the study; one healthy volunteer was excluded due to physical illness detected in the laboratory results.

2.4. Assessments

Both HD patients and healthy volunteers were assessed by using the Mini-International Neuropsychiatric Interview (MINI 6.0) (Sheehan et al., 1998), the Hypersexual Disorder Screening Inventory (HDSI) (www.dsm5.org), the Hypersexual Disorder: Current Assesssment Scale (HD:CAS), the Sexual Compulsivity Scale (SCS) (Kalichman and Rompa, 1995), the Montgomery-Åsberg Depression Rating Scale Self rating (MADRS-S) (Svanborg and Asberg, 2001) and the Childhood Trauma Questionnaire (Bernstein and Fink, 1998). For further details please see Supplementary material 1.

2.5. Blood sample collection and analysis

Blood samples from non-fasting participants were collected in the morning according to standard procedures. Analyses of plasma ACTH and Cortisol assays were performed directly after sampling at the laboratory of the Karolinska University Hospital using a chemiluminescence immunoassay. To test HPA axis function, low dose (0.5 mg at 23:00 h) dexamethasone suppression test was performed in all participants the same day after the baseline plasma samples of ACTH and Cortisol were gathered. Post dexamethasone suppression test blood samples were collected the next day at approximately 08.00 h and analysed using the same method as for baseline ACTH and Cortisol. Individuals who are not capable to suppress serum cortisol levels to a certain limit (=impaired feedback inhibition) are considered to have a dysfunctional HPA axis and are denoted non-suppressors. A plasma Cortisol level of 138 nmol/l (=5 g/dl) or higher in the morning sample after dexamethasone administration was classified as non-suppression.

2.6. Methylation profiling

Genomic DNA was extracted from 110 samples using the phenol-chloroform method (Sambrook et al., 1989). Subsequently, the EZ DNA Methylation – GoldTM kit (ZymoResearch, USA) was used for bisulfite conversion. Bisulfite converted DNA was thereafter hybridized to the Illumina Infinium Methylation EPIC BeadChip, representing the methylation state of over 850 K CpG sites. The array was imaged using the Illumina iScan system (Illumina, San Diego, CA, USA) in which the percent methylation state of each CpG site was quantified for the entire study group.

2.7. Data processing

Preprocessing of the methylation data was performed by background correction, adjustment of probe type differences, removal of batch effects and probe exclusion. Subsequently, the global DNA methylation pattern was adjusted for white blood cell type heterogeneity. Principal component analysis (PCA) was used to identify sample outliers in the methylation data. Methylation preprocessing steps were performed using the minfi (Aryee et al., 2014), watermelon (Schalkwyk, 2013), sva (Leek et al., 2012), ChAMP (Morris et al., 2014) and FactoMineR (Lê et al., 2008) packages of the Bioconductor project operable in R, version 3.3.0. And for all other statistical analysis please see Supplementary material concerning background correction, adjustment of type I and type II probes, removal of batch effects and probe exclusion and correction for white blood cell type heterogeneity as well as the correlation analysis of methylation levels between blood and four different brain regions, Supplementary material 2.

2.8. Criteria of sample exclusion

To investigate the global DNA methylation pattern for sample outliers, the 'PCA' function of the FactoMineR package was used (Lê et al., 2008). 7547 probes were further studied and included in the covariance matrix based on a threshold of 0.2 and a 95% reference range, as performed by Voisin et al. (Voisin et al., 2015). The first principal component explained 20.4% of the total variance and successively studied vectors did not add significantly to the total variance. Outliers were identified by visual inspection of the graphical display of the first principal component, resulting in seventeen samples being excluded from further analysis.

2.9. CpG site annotation and selection of HPA-axis coupled probes

90% of the probes on the Illumina EPIC BeadChip array are also present on the Illumina 450 K Methylation Beadchip. Therefore we used the expanded annotation produced by Price et al., originally designed for the 450 K array, to define, for each CpG site, the distance to the closest transcriptional start site (TSS) and the associated gene (Price et al., 2013). As such, only CpG-sites present on the Illumina 450 K methylation beadchip were considered for further analysis. In addition, we only considered CpG sites located within 2000 base pairs (bp) up and downstream of the TSS. Wagner et al. demonstrated that DNA methylation and gene expression is closely related within this region (Wagner et al., 2014).

Based on the hypothesis that HPA-axis is associated with hypersexuality, we considered the following HPA-axis coupled genes: Corticotropin releasing hormone (CRH), corticotropin releasing hormone binding protein (CRHBP), corticotropin releasing hormone receptor 1 (CRHR1), corticotropin releasing hormone receptor 2 (CRHR2), FKBP5 and the glucocorticoid receptor (NR3C1). After the preprocessing steps outlined above, all CpG sites annotated to any of the aforementioned genes were included in the study, resulting in 76 CpG sites investigated in the subsequent analysis.

2.10. Characterization of the expression data set

The expression data set was established by combining two independent samples of healthy controls.

2.10.1. Non-fasting healthy controls

Eleven healthy male volunteers aged between 18 and 40 years were recruited from the region of Uppsala, Sweden, between 2013 and 2014. These eleven men were not part of the 40 healthy volunteers forming an independent expression data set. Blood analyses were performed before and after a meal intake. For the purpose of this study, only the non-fasting blood samples were further studied to match the prandial state. The genome-wide DNA methylation pattern was measured using the Illumina Infinium 450 K BeadChip. RNA microarray expression was measured and analysed using the Affymetrix GeneChip Human Gene 2.1 ST array (Li and Wong, 2001). More details on the sample and preprocessing of the methylation and RNA specimens have been previously published (Rask-Andersen et al., 2016).

2.11. Data analysis

All statistical analyses were performed using R statistics, version 3.3.0.

After the preprocessing steps, 93 samples remained to be included in the subsequent analysis of the 76 HPA-axis coupled CpG sites. In the association analysis between DNA methylation of HPA-axis coupled CpG sites and hypersexuality, we only included male subjects. After exclusion of 6 women, 87 individuals were thus further studied.

2.11.1. Evaluation of the distribution of continuous variables

Skewness and kurtosis of the distribution of continuous variables were evaluated with the Shapiro-Wilks test. Baseline cortisol levels and HbA1C (mml/mol) were normally distributed both in hypersexuality patients and healthy volunteers, whereas the other clinical variables were not. The *t*-test and Kruskal-Wallis' test were subsequently used to investigate group differences in continuous variables between patients with HD and healthy volunteers. Chi-squared tests were used to detect differences in categorical variables, e.g. depression and DST non-suppression status.

2.11.2. Determination of optimal covariates

There were many potential covariates in the association analysis between DNA methylation and hypersexuality, e.g. depression, DST non-suppression status, CTQ Total, plasma levels of TNF-alpha and IL-6. To avoid overfitting by including too many covariates, we investigated each individual covariate against the phenotype of interest in binomial logistic regression models using the 'glm' function in R. Covariates were incrementally and independently selected. Using the computed analysis of variance, we tested whether the addition of a particular covariate resulted in a better fit to the model and only included variables with a p-value < 0.05. The best linear model for hypersexuality included the CpG sites, depression ($p < 0.01$), DST non-suppression status ($p < 0.05$), CTQ ($p < 0.05$), TNF-alpha ($p < 0.01$) and IL-6 ($p < 0.001$).

Beta values of methylation were used for graphical illustration. For statistical analysis, we transformed the beta values to M-values, which have been shown to be statistically more robust (Du et al., 2010).

2.11.3. Main analysis

The association between DNA methylation and hypersexuality were tested with linear models using the 'limma' package for R, applying an empirical Bayes method based on a moderated t-statistic (Smyth 2004). We assumed a linear model where the M values of each CpG site were used as a quantitative dependent trait and the categorical variables, e.g. hypersexual disorder, depression, DST non-suppression status, together with the continuous variables, e.g. TNF-alpha and IL-6, were included as covariates. All analyses were accounted for multiple testing using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995).

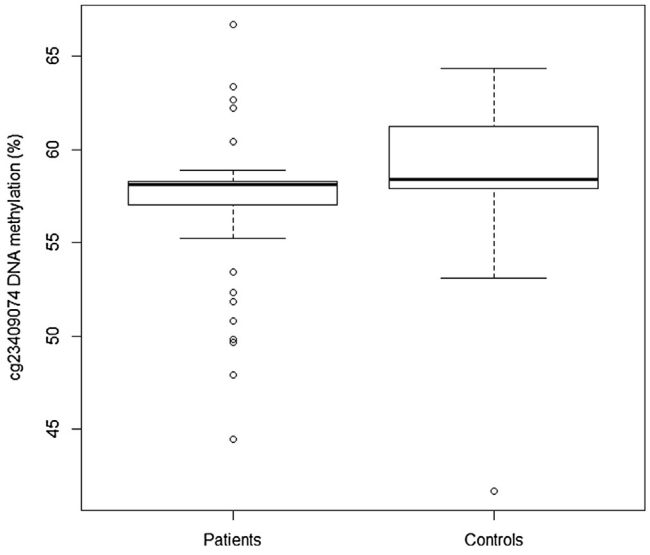

Fig 1. Boxplot of cg23409074% DNA methylation (β-values*100) in HD subjects and in healthy volunteers.

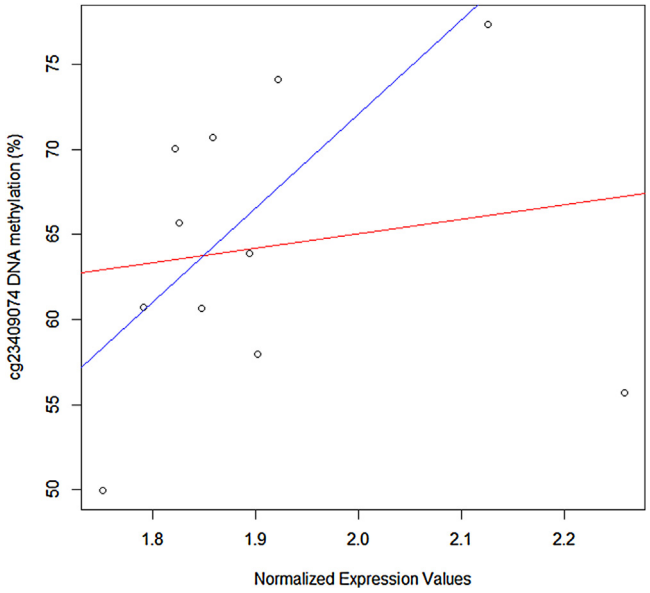

Fig. 2. Scatterplot and regression lines of cg23409074% DNA methylation (β-values*100) and normalized CRH gene expression values.
Legend: The blue line represents the regression line of the robust linear regression model. The red line represents the regression line of the pearson correlation.

2.11.4. Investigation of methylation and expression correlations in an independent cohort

Candidate CpG sites were further investigated with regard to their association with transcriptional expression of the respective gene in the expression data set. Methylation M-values were correlated with normalized gene expression data in two separate regressions, using both Pearson's product moment correlation method and robust linear regressions from the 'lmRob' function of the "robust" package for R (Hampel et al., 1986).

3. Results

3.1. Behavior of the clinical outcome variables

In this study, comprising 54 patients diagnosed with hypersexual disorder and 33 healthy volunteers, we initially aimed to identify HPA-axis coupled CpG-sites, in which modifications of the epigenetic profile are associated with hypersexual disorder. The study group included only male subjects. Patients with HD scored significantly higher on the CTQ ($p < 0.001$) and had higher levels of plasma DST ACTH ($p < 0.01$) and TNF-alpha ($p < 0.0001$), and lower levels of plasma IL-6 ($p < 0.01$). In addition, HD patients tended to be more depressed ($p = 0.052$) and DST non-suppressors ($p = 0.069$) to a larger degree than controls. There were no significant differences between group in age, plasma testosterone levels, TSH/T4-quota, HbA1C, baseline cortisol, DST cortisol and baseline ACTH (Table 1).

3.2. Investigation of 76 HPA-axis coupled probes reveals a CRH associated CpG site to be significantly hypomethylated in hypersexual patients

We performed multiple linear regression models of methylation M-values to a categorical variable of hypersexual disorder in 87 male subjects, adjusting for depression, DST non-suppression status, CTQ total score, and plasma levels of TNF-alpha and IL-6. 76 individual CpG sites were tested, and four of these were nominally significant ($p < 0.05$), associated with CRH, CRHR2 and NR3C1 genes. Furthermore, we identified on locus Cg23409074–located 48 bp upstream of the transcription start site (TSS) of the CRH gene – that was significantly hypomethylated in hypersexual patients after corrections were made for multiple testing using the FDR-method (Table 2), (Fig. 1).

3.3. Methylation levels of cg23409074 is positively correlated with gene expression of the CRH gene in an independent cohort of 11 healthy male subjects

Methylation M-values of cg23409074 were correlated with normalized expression values of the CRH gene inter-individually, by Pearson correlations and robust linear regression models, respectively. This methylation loci was significantly positively correlated with CRH gene expression in the robust linear regressions ($p < 0.05$), but not in the Pearson correlation analysis (Table 3), (Fig. 2).

3.4. Blood-brain correlation of the CRH CpG site

Methylation levels at cg23409074 in blood were positively correlated to cg23409074 methylation levels in all four different brain regions (prefrontal cortex; $r = 0.29$, $p = 0.01$; entorhinal cortex; $r = 0.27$, $p = 0.02$; superior temporal gyrus; $r = 0.26$, $p = 0.02$; cerebellum; $r = 0.24$, $p = 0.03$), in linear regression models. Fig. S3 Supplementary.

4. Discussion

In this study, we found that male patients with hypersexual disorder had reduced levels of methylation in a methylation locus (cg23409074) site located 48 bp upstream of the transcription start site of the CRH gene. Furthermore, this methylation locus was significantly positively correlated with CRH gene expression in an independent cohort of healthy male subjects. To our knowledge, this is the first report on epigenetic changes related to hypersexual disorder. We used genome-wide methylation chips with over 850 K CpG sites, however, based on our earlier findings on HPA dysregulation in men with hypersexual disorder (Chatzittofis et al., 2016), we applied a targeted approach on candidate genes of the HPA axis.

CRH is an important integrator of neuroendocrine stress responses in the brain, modulating behavior and the autonomic nervous system (Arborelius et al., 1999), as well as in neuroplasticity (Regev and Baram, 2014). Considering hypersexual disorder in the frame of addiction neurobiology, it is well established that CRH has a key role in the addiction process (Zorrilla et al., 2014).

Table 1
Clinical characteristics of patients with hypersexual disorder and healthy volunteers (without global DNA methylation pattern for sample outliers).

	Patients	Healthy volunteers	Statistics (t-test, Kruskall-Wallis, Chisquared), *p*-value
N	54	33	
Age (years)	39.7 (12.3)	37.4 (11.3)	*ns*
Men:Women, (n (%))	54 (100.0): 0 (0.0)	33 (100.0): 0 (0.0)	*ns*
Diagnosis depression (n(%))	8 (14.8)	0 (0.0)	5.26E-02
DST non-suppressors (n(%))*	15 (27.7)	3 (9.1)	6.95E-02
CTQ Total	39.98 (12.33)	32.85 (9.39)	**7.56E-04**
TSH (mE/L)/T4 (nmol/L)	0.019 (0.099)	0.027 (0.033)	*ns*
HbA1C (mmI/mol)	39.98 (12.34)	32.82 (3.96)	*ns*
Cortisol (nmol/L)	469.09 (130.11)	474.49 (148.32)	*ns*
DST Cortisol (nmol/L)	101.28 (102.78)	62.54 (48.54)	ns
ACTH (pmol/L)	6.44 (3.10)	5.82 (2.99)	*ns*
DST ACTH (pmol/L)	2.16 (1.61)	1.26 (0.90)	**6.03E-03**
Testosterone (nmol/L)	15.19 (4.59)	14.24 (4.37)	*ns*
TNF-alpha (ng/L)	7.18 (1.74)	5.84 (2.41)	**1.21E-05**
IL-6 (ng/L)	2.09 (0.43)	2.53 (1.32)	**1.16E-03**

Values are shown as mean (SD) unless otherwise specified. P-values were calculated by means of *t*-test, Kruskall-Wallis' test or chi-squared tests, contrasting values for patients with hypersexuality disorder and healthy volunteers. A one-tailed p-value <0.05 was considered significant.
Abbreviations: CTQchildhood trauma questionnaire; DST ACTHACTH levels after the dexamethasone suppression test; DST Cortisolcortisol levels after the dexamethasone suppression test; DST non-suppressorsnon-suppression status defined as DST cortisol levels >138nmol/l; *ns*not significant.

Table 2
Hypersexuality associated methylation changes in HPA-axis coupled CpG sites.

Gene	Transcript	Illumina ID	logFC	*p*	*p* (FDR)
CRH	NM_000756	cg23409074	−0.11	1.15E-04	8.76E-03
NR3C1	HQ450644	cg08845721	−0.03	2.33E-02	*ns*
NR3C1	NM_001204265	cg18146873	−0.01	3.71E-02	*ns*
CRHR2	EU012442	cg09516959	−0.03	4.55E-02	*ns*
CRHR1	EU012435	cg07778819	−0.04	6.00E-02	*ns*
FKBP5	NM_001145775	cg11845071	0.00	6.81E-02	*ns*
CRHR1	EU012435	cg13521908	0.00	7.47E-02	*ns*
CRHR2	NM_001202481	cg03012028	−0.05	1.06E-01	*ns*
FKBP5	NM_004117	cg08636224	0.02	1.08E-01	*ns*
FKBP5	NM_001145775	cg25114611	−0.02	1.29E-01	*ns*

Analysis by multiple linear regression models of methylation M-values to a binary outcome variable of Hypersexuality (Y/N), adjusting for Depression (Y/N), DST non-suppression status (Y/N), CTQ total score, TNF-alpha (ng/L) and IL-6 (ng/L). 76 CpG-sites were analysed, located within 2000 bp of the transcriptional start site of known HPA-axis genes CRH, CRHR1, CRHR2, CHRBP, FKBP5 and NR3C1. P-values were corrected for multiple-testing using the false discovery rate (FDR)-method.
Abbreviations: logFC, log fold change; *ns*, not significant; *p*, p-value; *p* (FDR), FDR-adjusted p-value.

Table 3
Methylation/transcription correlations of CpG sites differentially methylated in hypersexuality patients.

			FTO-cohort (n = 11)			
			Robust linear regression		Pearson correlation analyses	
Gene	Transcript	Illumina ID	Coef.	*p*	Coef.	*p*
CRH	NM_000756	cg23409074	3.70	2.69E-02	–	*ns*

The table lists CpG-sites located within the TSS2000 of HPA-axis genes with significant hypersexuality-dependent methylation changes. These methylation probes are investigated for a correlation with transcription in a separate cohort of 11 healthy non-fasting controls (FTO-cohort). Methylation M-values were correlated with expression values inter-individually, by pearson correlations and robust linear regression models, respectively.
Abbreviations: Coef., regression coefficient; *ns*, not significant; *p*, p-value.

In rodent models, the CRF system drive addiction via actions in the central extended amygdala, producing anxiety-like behavior, reward deficits, compulsive-like drug self-administration and stress-induced drug seeking behavior (Zorrilla et al., 2014). Furthermore, activation of CRF neurons in the medial prefrontal cortex may contribute to the loss of control seen in HD subjects. It has been shown that the chronic drug use leads to a hyperactive HPA-axis with increased ACTH levels while the CRH plays a central role in mediating negative affective responses to stress during drug withdrawal (Kakko et al., 2008; Koob et al., 2014). Similarly, a hyperactive HPA-axis with higher ACTH levels and epigenetic changes in the CRH gene in male patients with hypersexual disorder may lead to a circle of craving and relapse, with a new negative emotional allostatic state, maintaining hypersexual behavior in a futile effort to compensate for a dysphoric emotional state. To repetitively engage in sexual fantasies, urges or behaviors in response to dysphoric mood states and/or in response to stressful life events are key symptoms in the proposed diagnostic criteria of hypersexual disorder (Kafka, 2010). Our findings of hypomethylation of a CRH gene associated methylation locus couple which was associated with gene expression in an independent cohort, adds to the previous findings of HPA axis dysregulation in male patients with hypersexual disorder on a molecular level. Heroin self-administration behavior was associated with differential CRH signaling gene expression partly regulated by methylation shifts in an animal model (McFalls et al., 2016) and promoter methylation has been reported to impact on the expression pattern of CRH (Chen et al., 2012). However, the magnitude of methylation difference in CRH gene locus (cg23409074) was quite low (mean difference approximately 1.60%), and physiological relevance of subtle methylation changes is not fully elucidated. There is though, a growing body of literature on specific genes, suggesting wide ranging tran-

scriptional and translational consequences of subtle methylation changes (1–5%), especially in complex multifactorial syndromes like depression or schizophrenia (Leenen et al., 2016).

In this study, we took the most relevant confounders, such as depression, DST non-suppression status, CTQ total score and plasma levels of TNF-alpha, into consideration, on the association analyses between methylation of HPA-axis related genes and hypersexual disorder. Interestingly, patients with hypersexual disorder had significantly higher (TNF)-α levels compared to healthy volunteers (Jokinen et al., 2016). Due to the interplay between glucocorticoids and inflammation and the group differences in TNF-alpha and IL-6 levels between patients and healthy controls, we used inflammatory markers as covariates to take into account potential confounding of low grade neuroinflammation. Immune dysregulation is of importance in the pathophysiology underlying several psychiatric disorders including major depression, bipolar disorder and schizophrenia (Dantzer et al., 2008). Low grade neuroinflammation is often seen in patients with HPA axis dysregulation (Horowitz et al., 2013) and the inflammatory hypothesis emphasizes the role of psycho-neuroimmunological dysfunctions (Zunszain et al., 2013). It is possible that inflammation and glucocorticoid signaling may act independently on the same structures and processes without direct interaction results in an additive damage effect; in this cohort male patients with HD had higher TNFα levels compared to healthy male volunteers irrespective of HPA-axis dysregulation (Jokinen et al., 2016). As earlier reported (Chatzittofis et al., 2016), antidepressant medication or depression severity were not significantly associated with HPA function measures in this study population.

Further in this study, due to the fact that patients reported more early life adversity compared to healthy controls and the well-known effects of childhood trauma on epigenome (Szyf and Bick, 2013), we used early life adversity in the regression models to take into account possible confounding effect of childhood trauma on methylation patterns. HPA-axis dysregulation related to early life adversity reflects vulnerability and the effort for compensation of the effects of childhood adversity (Heim et al., 2008) and early life adversity is related to epigenetic changes of HPA-axis related genes (Turecki and Meaney, 2016).

The conceptualization of the hypersexual disorder has been intensively debated and even though the diagnosis was not included in the DSM-5, the field of study has shown a high degree of reliability and validity for the proposed diagnostic criteria for hypersexual disorder (Reid et al., 2012).

The strengths of the study are a relatively homogenous patient population with thorough diagnostics of the hypersexual disorder, the age matched control group of healthy volunteers, without present or past psychiatric disorders as well as without family history of major psychiatric disorders and severe traumatic experiences. Moreover, the consideration of possible confounders such as childhood adversity, depression, neuroinflammatory markers and dexamethasone test results can be seen as strength.

Some limitations: self-report of early life adversity and the cross sectional design of the study, that does not allow any conclusions about causality. Furthermore, since this is the first study investigating epigenomics in men with hypersexual disorder, it would be of value to replicate our findings in an independent cohort of HD subjects. In addition, while cg23409074 was demonstrated to correlate with gene expression of the CRH gene in healthy controls, it is still not demonstrated to what extent this could reflect modifications occurring in HD subjects and a measure of CRF would have been of value for the study. Further studies are needed to investigate the potential differential expression pattern of CRH in men with HD. An important question is if the whole blood CRH component methylation reflects the effects on the brain.Using a reliable tool to compare methylation between whole blood and brain, the methylation levels at the identified CRH site, cg23409074, were significantly correlated between blood and four different brain regions, with the strongest correlation for prefrontal cortex, a key regulator of stress response. This provides some support that the differential methylation status observed in whole blood can reflect modifications occurring in certain brain regions. Furthermore, the association analysis of methylation and expression was performed in a relatively small group of healthy volunteers and were significant in the robust models, but not by Pearson correlations. This conflicting result could be explained in that robust linear models are recommended to be used in case of a small sample size, to account for any outliers or heteroscedasticity in the data which could bias results (Joubert et al., 2012). In addition, by performing correlation analyses intra-individually, we greatly reduce the likelihood of confounding due to interindividual variance, Other unaccounted potential confounding factors could also induce changes in methylation patterns, e.g. dietary patterns or prandial states (Rask-Andersen et al., 2016) and not controlling for the dexamethasone plasma concentrations during the DST (Menke et al., 2016).

In conclusion our finding of epigenetic state in CRH gene, connecting to the literature on addiction neurobiology, in men with hypersexual disorder, may contribute to elucidating the pathophysiological biological mechanisms of hypersexual disorder.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.03.007.

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