Sex-Dependent Association of DNA Methylation in the Coding Region of the Corticotropin-Releasing Hormone Gene and Schizophrenia

Lili Qing
Kunming Medical University https://orcid.org/0000-0002-3458-4222
Peng Xiong
Kunming Medical University First Affilliated Hospital
Yumei Lu
Kunming Medical University
Hongyan Jiang
Kunming Medical University First Affilliated Hospital
Shengjie Nie (nieshengjie@126.com)
Kunming Medical University https://orcid.org/0000-0001-6414-6621

Research Article

Keywords: Schizophrenia, HPA axis, CRH, DNA methylation

Posted Date: March 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-277493/v1

Sex-dependent association of DNA methylation in the coding region of the corticotropin-releasing hormone gene and schizophrenia

Lili Qing[1#], Peng Xiong[2#], Yumei Lu[1], Hongyan Jiang[2]*, Shengjie Nie[1]*

1. School of Forensic Medicine, Kunming Medical University, Kunming, Yunnan Province, People's Republic of China

2. Department of Psychiatry, the First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan Province, People's Republc of China

*Corresponding author

Correspondence to Shengjie Nie (Email address: nieshengjie@126.com)

# These authors contributed equally to this work.

Abstract

Schizophrenia (SCZ) is a common mental disease causing severe chronic disability. Epigenetic changes in HPA axis-related genes are considered to play an important role in SCZ pathogenesis. Unfortunately, the methylation status of the corticotropin-releasing hormone (*CRH*) gene, which is the central driving force in the HPA axis, has not been reported in SCZ patients. Here, we used the sodium bisulfite and MethylTarget methods to detect the DNA methylation status of the *CRH* gene coding region in peripheral blood samples from 70 schizophrenic patients and 68 healthy controls. The results showed that the methylation level of the *CRH* gene CDS was significantly increased in SCZ patients, especially in the male subgroup. In conclusion, this study showed that differences in *CRH* gene CDS methylation were detectable in the peripheral blood of SCZ patients and that epigenetic abnormalities in the *CRH* gene were closely related to SCZ, revealing that epigenetic processes may mediate the pathophysiology of SCZ. Further research should address the underlying mechanism whereby *CRH* gene methylation regulates the occurrence and development of SCZ.

Keywords: Schizophrenia; HPA axis; *CRH*; DNA methylation

Introduction

Schizophrenia (SCZ) is a common severe, chronic, disabling mental illness with affective disorder, cognitive disorder and volitional behavior disorder symptoms (Zhu et al. 2019) affecting approximately 1% of the worldwide population (McGrath et al. 2008). SCZ is characterized by a slow onset, prolonged disease course and poor prognosis, which not only cause great harm to an individual's life and health but also imposes heavy economic and psychological burdens on families. It imposes one of the heaviest disease burdens with the highest costs worldwide (Rössler et al. 2005). SCZ is a complex disease that is affected by both genetic and environmental factors (Nishioka et al. 2012; Misiak et al. 2018), but its pathogenesis is still unclear.

In fact, mental disorders are closely associated with stress responses in the brain (Watkeys et al. 2018; Palma-Gudiel et al. 2015). In normal organisms, appropriate responses to stress are the basis of survival and adaptation (de Kloet et al. 2005), while inappropriate and long-term stress responses can lead to the occurrence and symptoms of mental disorders, including SCZ and bipolar disorder (Sinclair et al. 2012). The hypothalamic-pituitary-adrenal (HPA) axis is an important stress response system that maintains homeostasis through the responses to environmental stressors (Russo et al. 2012). Our previous studies have shown that DNA methylation of the *NR3C1* and *NR3C2* genes in the HPA axis leads to a risk of mental illness, including SCZ and aggressive behavior (Liu et al. 2020a and 2020b; Qing et al. 2020; Qing et al. 2021). As the central driving force in the HPA axis (Zhou and Fang, 2018), the *CRH* gene has attracted our attention.

CRH plays a key role in the regulation of neuroendocrine, autonomic and behavioral adaptation to stress (de Kloet et al. 2005; Ishiwata et al. 2020). The *CRH* gene consists of two exons and one intron, and the coding region is located in the second exon (Figure 1) (Cramer et al. 2019). *CRH* is mainly expressed by the parvocellular neurons of the hypothalamic paraventricular nucleus (PVN), which drives the activation of the HPA axis, thus regulating the stress response (Zhou & Fang, 2018; Ishiwata et al. 2020). Elevated *CRH* levels in the central nervous system are associated with stress-related physiological and behavioral disorders (Linthorst et al.1997). It has been confirmed in a large number of studies that *CRH* levels are upregulated in patients with depression disorders and suicide victims, *CRH* concentrations return to normal after recovery from

depression (Naughton et al. 2014). The *CRH* concentration is also upregulated in individuals with posttraumatic stress disorder (Sautter et al. 2003). *CRH* also plays an important role in anxiety (Dedic et al. 2018), addiction (Carboni et al. 2018) and sleep (Kimura et al. 2010). The most recent study on this topic showed that the concentration of *CRH* in the cerebrospinal fluid (CSF) of schizophrenic patients was lower than that in the control group, and a lower *CRH* concentration was associated with negative symptoms of the illness (Ishiwata et al. 2020). This evidence suggests that *CRH* expression may be related to the pathogenesis of SCZ and other mental diseases.

As a mechanism of gene expression mediated by environmental factors, methylation plays a particularly important role in mental diseases. *CRH* methylation studies support this view. Under conditions of prenatal stress (Xu et al. 2014; Mueller and Bale, 2008), chronic variable mild stress (CVMs) (Sterrenburg et al. 2011), social stress (Elliott et al. 2010), and a lack of maternal love (Chen et al. 2012), *CRH* methylation decreases and *CRH* expression increases, leading to corresponding mental disorders or symptoms. Two *CRH*-related CpG sites (cg19035496 and cg23409074) were found to be significantly demethylated in attempted suicide patients. In subsequent adolescent cohort studies, one of the above cg19035496 sites was shown to be hypermethylated in subjects with higher general psychiatric risk scores (Jokinen et al. 2018). The results suggest that *CRH* methylation may be involved in the pathogenesis of mental disorders.

Previous studies on *CRH* methylation have focused on the promoter region (Xu et al. 2014; Mueller and Bale, 2008; Sterenburg et al. 2011; Elliott et al. 2010; Chen et al. 2012; Jokinen et al. 2018). With ongoing, increasingly in-depth research, it has been found that some CpGs may be located in exons or coding regions, which can affect promoter activity by affecting transcriptional elongation, and the DNA methylation of CDs is more effective than promoter methylation in inhibiting gene expression (Zhu et al. 2005; Hisano et al. 2003). CpG methylation in the coding region of MCT3 inhibits its expression (Zhu et al. 2005). Testicular germ cell-specific CD demethylation of the *TACT1*/actl7b and *PDHA2* genes can inhibit their expression (Hisano et al. 2003; Pinheiro et al. 2012). The methylation of + 10 and + 88 CpG sites in the CDS of the *MAT1A* gene can reduce promoter activity by 60% (Tomasi et al. 2012). These results indicate that CpG-site or CpG-island methylation in the CDS region is also very important for gene expression regulation. Unfortunately, there have been no studies on the methylation of the *CRH* CDS in SCZ.

The purposes of this study were to analyze the methylation level of CpG islands in the CDS of the *CRH* gene in the SCZ population and to explore the relationship between the methylation level of the *CRH* gene CDS and SCZ.

Materials and methods

Study participants

Blood samples were collected from 70 schizophrenic patients and 68 healthy controls living in Yunnan Province, China. Their demographic characteristics are shown in Table 1. Schizophrenic patients who were admitted to the Psychiatric Hospital of Yunnan from December 2012 to August 2013 and agreed to participate were included. All patients were diagnosed by at least two professional attending psychiatrists according to the standards of the Diagnostic and Statistical Manual of Mental Disorders, fourth revised edition (DSM-IV) and clinical records. The healthy controls were unrelated individuals from the Center for Disease Prevention and Control of Kunming. They were recruited and interviewed by psychiatrists. The exclusion and inclusion criteria of the disease group and the control group were described previously (Qing et al. 2020). The study was approved by the ethics committee of Kunming Medical University. All subjects signed informed consent forms and donated peripheral blood.

DNA sample preparation and bisulfite modification

Blood samples were collected from all schizophrenic patients and controls. Genomic DNA was isolated from whole blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genomic DNA integrity was evaluated by agarose gel electrophoresis. Genomic DNA was quantified with a NanoDrop 2000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and the absorbance ratios at 260/280 and 260/230 were determined to assure DNA purity. The samples were treated with sodium bisulfite and processed by using the EZ DNA Methylation Gold Kit (Zymo, CA, USA) according to the manufacturer's instructions. Cytosine (C) residues without genomic DNA methylation were transformed into uracil (U) residues.

DNA methylation analysis

EBI database boss cpgplot software was used to predict CpG islands (http://emboss.bioinformatics.nl/cgi-bin/emboss/cpgplot) (Gardiner-Garden and Frommer, 1987),

and the results predicted a CpG island containing 17 CpG sites in the CDS of the *CRH* gene (NCBI reference sequence: NG_016127.1 (+6013 to + 6231), Figure 1). By designing methylation primers for amplification with primer design software, the following primers were obtained: forward 5'-GGGAGTTTTGTTGGTGGTTTT-3', reverse 5' CTCTTATTAAAATTCCCCAAACRAAAA -3'.

DNA methylation levels were analyzed by using MethylTarget® (Genesky Biotechnologies Inc., Shanghai, China) based on next-generation sequencing (NGS). The optimized multiplex PCR primer panel was used to amplify the target fragment from the sample with the sulfite-transformed sample genome as the template. After the application of quality control procedures, the amplification products of all multiplex PCR primers in the panel from the same sample genomic DNA template were mixed together, and the quantity of the amplification products from each primer was equal. Primers with index sequences were used to amplify the specific tag sequences compatible with the Illumina platform. All samples were mixed with the same amount of index PCR products, and the final MethylTarget library was obtained by tapping and recycling. The fragment length distribution of the library was verified with an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). After the accurate quantification of library molarity, high-throughput sequencing was performed on the Illumina MiSeq (Illumina, CA, USA) platform in 2*150 bp double-ended sequencing mode to obtain FASTQ data.

Statistical analyses

FastQC software was used to control the quality of the sequencing data. The FASTX Toolkit (http://hannonlab.cshl.edu/fastx_ toolkit/index.html) was used for the FASTA formatting steps of the FASTQ data. The FASTA format reads were mapped to the target bisulfite genome (hg19) using BLAST (Camacho et al. 2009). Methylation analysis was conducted with Perl scripts. T-tests ere used for statistical analysis, and differences at the $P<0.05$ level were considered statistically significant. All statistical analyses were performed using SPSS 22.0; all graphical analyses were performed using GraphPad Prism version 8.

Results

In this study, we selected predicted CpG islands in the coding region for analysis, including

17 CpG sites. Through sequencing, we successfully obtained signals from 68 control subjects (47 males, 21 females) and 70 schizophrenic patients (46 males, 24 females). The sequencing depth and quality of all samples met the standards required for subsequent analysis.

Hypermethylation of *CRH* gene in SCZ

Overall analysis of CpG-island methylation

The analysis of the DNA methylation status of CpG islands in the *CRH* gene CDS showed that compared with the control group (0.0204±0.0062), methylation was significantly increased in the case group (0.0242±0.0105) (p=0.0092). When the subgroups were further divided according to sex, significance was maintained in the male subgroup (p=0.0105). Although a trend of hypermethylation remained in the female disease subgroup, it was not significant (p=0.4632) (Figure 2 and Table S1).

Methylation analysis of CpG sites

A total of 17 CpG sites were detected in the CpG island (Figure 1 and Table S2). The methylation values of all CpG sites were below 6% in both the disease group and the control group. Compared with the control group, the DNA methylation levels of 7 CpG sites in the disease group were significantly higher (CpG3, P=0.0469; CpG9, P=0.0045; CpG11, P=0.0123; CpG12, P=0.0170; CpG14, P=0.0328; CpG16, P=0.0278; CpG17, P=0.0027) (Figure 3A and Table S2).

For individual CpG sites, we also grouped the results by sex and compared the DNA methylation levels of all CpG sites according to sex. Compared with the control group, the methylation levels of 6 CpG sites (CpG9, P=0.0083; CpG11, P=0.0163; CpG12, P=0.0233; CpG14, P=0.0154; CpG16, P=0.0081; CpG17, P=0.0031) in the male group were still significantly higher than those in the control group, while the CpG3 methylation level was not significantly different (Figure 3B and Table S2). Surprisingly, compared with the female control group, only the CpG3 site presented a significant difference in the female disease group (p=0.0383), and no significant difference was found at the other sites (Figure 3C and Table S2).

In addition, we compared and analyzed the methylation levels of all male (n=93) and female (n=45) samples. The results showed that there was no significant difference in the methylation levels of either the whole fragments or the single CpG sites (Figure 3D).

Discussion

A large number of previous studies have confirmed that because *CRH* is the central driving force of the HPA axis (Zhou et al. 2018) and therefore plays a particularly important role in body stress, *CRH* promoter region methylation can cause excessive activation of the HPA axis by regulating the expression of mRNA and protein and, thus, participates in the pathogenesis of SCZ and other mental diseases (Ishiwata et al. 2020; Xu et al. 2014; Mueller and Bale, 2008; Sterenburg et al. 2011; Elliott et al. 2010; Chen et al. 2012; Jokinen et al. 2018). It is worth noting that CDS DNA methylation is more effective than promoter methylation in inhibiting gene expression (Zhu et al. 2005; Hisano et al. 2003). Unfortunately, there is no available analysis of the correlation between *CRH* coding region methylation and SCZ. This study is the first to examine the methylation status of the *CRH* gene CDS in the peripheral blood of schizophrenic patients, with the aim of filling the gap in knowledge about how CDS of *CRH* gene CDS methylation is related to SCZ. As mentioned above, we selected CpG islands located in the CDS of the *CRH* gene, including 17 CpG sites (Figure 1), and analyzed them by sodium bisulfite treatment combined with the MethylTarget method. The results showed that the methylation level of the *CRH* gene CDS patients was significantly increased in schizophrenic (Figure 2 and Table S1). After division into subgroups according to sex, the significance of this increase was only maintained in the male subgroup. Among the individual CpG sites, a total of 7 CpG sites (CpG3, CpG9, CpG11, CpG12, CpG14, CpG16, and CpG17) were found to be significantly associated with SCZ. When the samples were divided into male and female subgroups, the CpG3 site showed significant differences only in the female subgroup, while the other 6 CpG sites maintained significant differences only in the male subgroup (Figure 3 and Table S2).

DNA methylation is an important link between environmental factors and genetic background, and differences in DNA methylation may lead to SCZ and other mental diseases (Popov et al. 2012; Gürel et al. 2020). Previous studies have focused mainly on the promoter regions of genes and less on the methylation of CDS regions. This study confirmed that the methylation level of the *CRH* gene coding region is associated with SCZ. This phenomenon has been observed not only in human and mouse studies (Zhu et al. 2005; Hisano et al. 2003;Pinheiro et al. 2012; Tomasi et al. 2012) but also in studies on fungi, viruses and cells. In fungi, the effect

of coding region methylation on transcriptional extension is greater than the effect on transcription initiation (Barry et al. 1993; Rountree and Selker, 1997). The methylation of the CDS of the herpes simplex virus TK gene has been shown to inhibit TK expression (Graessmann et al. 1994). At the cellular level, Hsieh (Hsieh, 1997) and Irvine (Irvine et al. 2002) demonstrated that the methylation of the coding region of the luciferase gene inhibited transcription 5 times more effectively than that of the long terminal repeat promoter of ROS sarcoma virus in 293 cells. CDS methylation may control genes by inducing local inhibitory chromatin structures via CpG methylation and thereby preventing promoter activity (Pinheiro et al. 2012). Some studies have suggested that gene coding region DNA methylation can initiate chromatin structure formation, reduce the efficiency of Pol II extension, and lead to transcriptional silencing (lorincz et al. 2004). Although the mechanism by which coding region methylation affects gene expression is not completely clear, more attention should be paid to the important role of coding region methylation.

In the past, most of the obtained evidence has shown that the methylation level of *CRH* decreases under a lack of maternal love and prenatal stress, leading to the upregulation of *CRH* mRNA or protein expression, which drives the HPA axis and eventually leads to mental or stress disorders (Chen et al. 2012; Sterenburg et al. 2011; Xu et al. 2014; Mueller and Bale, 2008；Elliott et al. 2010). However, we found that the methylation level of the SCZ group was higher than that of the control group, suggesting low *CRH* expression in the disease group. Similar results were found in a recent study in which the concentration of *CRH* in the CSF of schizophrenic patients was shown to be lower than that in the control group, and a lower *CRH* concentration was associated with negative symptoms of the disease (Ishiwata et al. 2020). Similarly, in an adolescent cohort study, the cg19035496 *CRH* gene was found to be hypermethylated in subjects with higher psychiatric risk scores (Jokinen et al. 2018). Previous studies have shown that the expression of *CRH* in SCZ patients is not consistent. The expression of *CRH* in disease versus control groups has been found to be downregulated or upregulated or to shown no difference (Ishiwata et al. 2020). The results regarding the methylation and expression of the *CRH* gene are not consistent among different mental disorders or stress disorders, suggesting that the regulatory network associated with *CRH* may be highly complex.

In this study, we found that the methylation level of the *CRH* gene coding region was

associated with SCZ. It is worth noting that this association was male specific. Previous studies have reported that males showing sex-specific differences in SCZ are more likely to be diagnosed and develop cognitive impairment than females (Gershon, 2002; Mendrek and Mancini-Marïe, 2016). Sex dependence of *CRH* function has also been found in previous studies, although the results are inconsistent. After adrenalectomy (ADX), the potent androgen dihydrotestosterone can promote a rapid *CRH* mRNA response in male rats (Heck and Handa, 2019). Male mice are more susceptible than female mice to memory impairment caused by *CRH* in the medial septum (Kimberly et al. 2019). Negative feedback from PVN *CRH* mediated by the glucocorticoid receptor (GR) shows a significant sex difference, especially in female mice (Heck et al. 2020). This indicates that the mechanism of *CRH* action in stress responses and mental disorders may be sex-dependent. We speculate that data on sex-dependent DNA methylation may contribute to the understanding of sex differences in *CRH* gene expression, HPA axis activation and the pathogenesis of SCZ.

Similar to most previous studies on methylation in humans, DNA extracted from peripheral blood was used for methylation analysis in the present study. In the study of SCZ, although most DNA methylation markers in peripheral blood cannot reliably predict DNA methylation status in the brain, data from peripheral tissue can reflect the methylation status in brain tissue to some extent (Walton et al. 2016). A previous study on *CRH* methylation supports this view. *CRH* methylation in peripheral blood is significantly correlated with methylation in four different brain regions, indicating that *CRH* methylation detected in peripheral blood can reflect the situation in the central nervous system and brain (Jokinen et al. 2018). To verify this hypothesis, future studies could directly compare methylation levels in brain tissue and peripheral blood after death.

Our previous studies on the methylation of the *NR3C1* and *NR3C2* genes in the HPA axis and SCZ patients have produced very significant results. The methylation status of the *NR3C1* and *NR3C2* genes is significantly associated with SCZ and is sex dependent to some extent (Liu et al. 2020a and 2020b; Qing et al. 2020; Qing et al. 2021). Based on the results of this study, we strongly believe that HPA axis methylation can mediate environmental and genetic interactions and affect the pathogenesis of SCZ.

In conclusion, this study revealed differences in the methylation of the *CRH* gene coding region in the peripheral blood of schizophrenic patients. Although the functional significance of

this methylation difference is not clear, it suggests that epigenetic abnormalities in the *CRH* gene are related to the pathophysiology of SCZ. This is the first study to show an association between SCZ and CpG-island methylation in the *CRH* gene coding region in peripheral blood. These results improve our understanding of the role of the HPA stress response system in the pathological development of mental disorders (especially SCZ) and fill in a gap in research on the methylation of the *CRH* gene coding region in SCZ. Our results suggest that epigenetic processes may mediate psychopathology by influencing HPA axis activity. The effect of *CRH* methylation differences on gene expression and function needs further study, which may help to clarify the pathophysiology of SCZ.

Ethical Statement

Ethics approval and consent to participate

The ethics committee of Kunming Medical University approved this study. Informed consent was obtained from all individual participants included in the study.

Consent for publication

Manuscript is approved by all authors for publication.

Availability of data and materials

Not Applicable.

Conflicts of interest

None

Funding

This work was supported by grants of the National Natural Science Foundation of China (31860300, 31660310), Yunnan Provincial Young Academic and Technical Leader Reserve Talent Project (2018HB104), Science and Technology Innovation Group of Kunming Medical University (CXTD201803), Training of high-level health technical personnel in Yunnan Province, Medical academic leader (D-2018047).

Authors' contributions

Shengjie Nie and Lili Qing participated in the conception and design of this study. Sample collection and material preparation were performed by Peng Xiong and Hongyan Jiang, and data collection and analysis were performed by Lili Qing, Peng Xiong, Hongyan Jiang and Yumei Lu. The first draft of the manuscript was written by Lili Qing. Shengjie Nie performed manuscript review. All authors read and approved the final manuscript.

Acknowledgements

The authors acknowledge Center for Genetic & Genomic Analysis, Genesky Biotechnologies Inc. (Shanghai, 201315) for their technical support in methylation sequencing.

References

Barry C, Faugeron G, Rossignol JL (1993) Methylation induced premeiotically in Ascobolus: coextension with DNA repeat lengths and effect on transcript elongation. Proc Natl Acad Sci U S A 90(10):4557-61.

Camacho C, Coulouris G, Avagyan V et al (2009) BLAST+: architecture and applications. BMC Bioinformatics 10:421.

Carboni L, Romoli B, Bate ST et al (2018) Increased expression of CRF and CRF-receptors in dorsal striatum, hippocampus, and prefrontal cortex after the development of nicotine sensitization in rats. Drug Alcohol Depend 189:12-20.

Chen J, Evans AN, Liu Y et al (2012) Maternal deprivation in rats is associated with corticotrophin-releasing hormone (CRH) promoter hypomethylation and enhances CRH transcriptional responses to stress in adulthood. J Neuroendocrinol 24(7):1055-64.

Cramer T, Rosenberg T, Kisliouk T et al (2019) Early-life epigenetic changes along the corticotropin-releasing hormone (CRH) gene influence resilience or vulnerability to heat stress later in life. Mol Psychiatry 24(7):1013-1026.

de Kloet ER, Joëls M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6(6):463-75.

Dedic N, Kühne C, Jakovcevski M et al (2018) Chronic CRH depletion from GABAergic, long-range projection neurons in the extended amygdala reduces dopamine release and increases anxiety. Nat Neurosci 21(6):803-807.

Elliott E, Ezra-Nevo G, Regev L et al (2010) Resilience to social stress coincides with functional DNA methylation of the Crf gene in adult mice. Nat Neurosci 13(11):1351-3.

Gardiner-Garden M, Frommer M (1987) CpG islands in vertebrate genomes. J Mol Biol 196(2):261-82.

Gershon J (2002) A meta-analytic review of gender differences in ADHD. J Atten Disord 5(3):143-54.

Graessmann A, Sandberg G, Guhl E et al (1994) Methylation of single sites within the herpes simplex virus tk coding region and the simian virus 40 T-antigen intron causes gene inactivation. Mol Cell Biol 14(3):2004-10.

Gürel Ç, Kuşçu GC, Yavaşoğlu A et al (2020) The clues in solving the mystery of major psychosis:

The epigenetic basis of schizophrenia and bipolar disorder. Neurosci Biobehav Rev 113:51-61.

Heck AL, Handa RJ (2019) Androgens Drive Sex Biases in Hypothalamic Corticotropin-Releasing Hormone Gene Expression After Adrenalectomy of Mice. Endocrinology 160(7):1757-1770.

Heck AL, Thompson MK, Uht RM et al (2020) Sex-Dependent Mechanisms of Glucocorticoid Regulation of the Mouse Hypothalamic Corticotropin-Releasing Hormone Gene. Endocrinology 161(1):bqz012.

Hisano M, Ohta H, Nishimune Y et al (2003) Methylation of CpG dinucleotides in the open reading frame of a testicular germ cell-specific intronless gene, Tact1/Actl7b, represses its expression in somatic cells. Nucleic Acids Res 31(16):4797-804.

Hsieh CL (1997) Stability of patch methylation and its impact in regions of transcriptional initiation and elongation. Mol Cell Biol 17(10):5897-904.

Irvine RA, Lin IG, Hsieh CL (2002) DNA methylation has a local effect on transcription and histone acetylation. Mol Cell Biol 22(19):6689-96.

Ishiwata S, Hattori K, Hidese S et al (2020) Lower cerebrospinal fluid CRH concentration in chronic schizophrenia with negative symptoms. J Psychiatr Res 127:13-19.

Jokinen J, Boström AE, Dadfar A et al (2018) Epigenetic Changes in the CRH Gene are Related to Severity of Suicide Attempt and a General Psychiatric Risk Score in Adolescents. EBioMedicine 27:123-133.

Kimura M, Müller-Preuss P, Lu A et al (2010) Conditional corticotropin-releasing hormone overexpression in the mouse forebrain enhances rapid eye movement sleep. Mol Psychiatry 15(2):154-65.

Linthorst AC, Flachskamm C, Hopkins SJ et al (1997) Long-term intracerebroventricular infusion of corticotropin-releasing hormone alters neuroendocrine, neurochemical, autonomic, behavioral, and cytokine responses to a systemic inflammatory challenge. J Neurosci 17(11):4448-60.

Liu L, Li J, Qing L et al (2020b) Glucocorticoid receptor gene (NR3C1) is hypermethylated in adult males with aggressive behaviour. Int J Legal Med 135(1):43-51.

Liu L, Wu J, Qing L et al (2020a) DNA Methylation Analysis of the NR3C1 Gene in Patients with Schizophrenia. J Mol Neurosci 70(8):1177-1185.

Lorincz MC, Dickerson DR, Schmitt M et al (2004) Intragenic DNA methylation alters chromatin structure and elongation efficiency in mammalian cells. Nat Struct Mol Biol 11(11):1068-75.

McGrath J, Saha S, Chant D et al (2008) Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev 30:67-76.

Mendrek A, Mancini-Marïe A (2016) Sex/gender differences in the brain and cognition in schizophrenia. Neurosci Biobehav Rev 67:57-78.

Misiak B, Stramecki F, Gawęda Ł et al (2018) Interactions Between Variation in Candidate Genes and Environmental Factors in the Etiology of Schizophrenia and Bipolar Disorder: a Systematic Review. Mol Neurobiol 55(6):5075-5100.

Mueller BR, Bale TL (2008) Sex-specific programming of offspring emotionality after stress early in pregnancy. J Neurosci 28(36):9055-65.

Naughton M, Dinan TG, Scott LV (2014) Corticotropin-releasing hormone and the hypothalamic-pituitary-adrenal axis in psychiatric disease. Handb Clin Neurol 124:69-91.

Nishioka M, Bundo M, Kasai K et al (2012) DNA methylation in schizophrenia: progress and challenges of epigenetic studies. Genome Med 4(12):96.

Palma-Gudiel H, Córdova-Palomera A, Leza JC et al (2015) Glucocorticoid receptor gene (NR3C1) methylation processes as mediators of early adversity in stress-related disorders causality: a critical review. Neurosci Biobehav Rev 55:520-535.

Pinheiro A, Nunes MJ, Milagre I et al (2012) Demethylation of the coding region triggers the activation of the human testis-specific PDHA2 gene in somatic tissues. PLoS One 7(6):e38076.

Popov NT, Stoyanova VK, Madzhirova NP et al (2012) Epigenetic aspects in schizophrenia etiology and pathogenesis. Folia Med (Plovdiv) 54(2):12-6.

Qing L, Gao C, Ji A et al (2021) Association of mineralocorticoid receptor gene (NR3C2) hypermethylation in adult males with aggressive behavior. Behav Brain Res 398:112980.

Qing L, Liu L, Zhou L et al (2020) Sex-dependent association of mineralocorticoid receptor gene (NR3C2) DNA methylation and schizophrenia. Psychiatry Res 292:113318.

Rössler W, Salize HJ, van Os J et al (2005) Size of burden of schizophrenia and psychotic disorders. Eur Neuropsychopharmacol 15(4):399-409.

Rountree MR, Selker EU (1997) DNA methylation inhibits elongation but not initiation of

transcription in Neurospora crassa. Genes Dev 11(18):2383-95.

Russo SJ, Murrough JW, Han MH et al (2012) Neurobiology of resilience. Nat Neurosci 15(11):1475-84.

Sautter FJ, Bissette G, Wiley J et al (2003) Corticotropin-releasing factor in posttraumatic stress disorder (PTSD) with secondary psychotic symptoms, nonpsychotic PTSD, and healthy control subjects. Biol Psychiatry 54(12):1382-8.

Sinclair D, Fullerton JM, Webster MJ et al (2012) Glucocorticoid receptor 1B and 1C mRNA transcript alterations in schizophrenia and bipolar disorder, and their possible regulation by GR gene variants. PLoS One 7(3):e31720.

Sterrenburg L, Gaszner B, Boerrigter J et al (2011) Chronic stress induces sex-specific alterations in methylation and expression of corticotropin-releasing factor gene in the rat. PLoS One 6(11):e28128.

Tomasi ML, Li TW, Li M et al (2012) Inhibition of human methionine adenosyltransferase 1A transcription by coding region methylation. J Cell Physiol 227(4):1583-91.

Walton E, Hass J, Liu J et al (2016) Correspondence of DNA Methylation Between Blood and Brain Tissue and Its Application to Schizophrenia Research. Schizophr Bull 42(2):406-14.

Watkeys OJ, Kremerskothen K, Quidé Y et al (2018)Glucocorticoid receptor gene (NR3C1) DNA methylation in association with trauma, psychopathology, transcript expression, or genotypic variation: a systematic review. Neurosci Biobehav Rev 95:85-122.

Wiersielis KR, Ceretti A, Hall A et al (2019) Sex differences in corticotropin releasing factor regulation of medial septum-mediated memory formation. Neurobiol Stress 10:100150.

Xu L, Sun Y, Gao L et al (2014) Prenatal restraint stress is associated with demethylation of corticotrophin releasing hormone (CRH) promoter and enhances CRH transcriptional responses to stress in adolescent rats. Neurochem Res 39(7):1193-8.

Zhou JN, Fang H (2018) Transcriptional regulation of corticotropin-releasing hormone gene in stress response. IBRO Rep 5:137-146.

Zhu S, Goldschmidt-Clermont PJ, Dong C (2005) Inactivation of monocarboxylate transporter MCT3 by DNA methylation in atherosclerosis. Circulation 112(9):1353-61.

Zhu X, Li R, Kang G et al (2019) CACNA1C Polymorphism (rs2283291) Is Associated with Schizophrenia in Chinese Males: A Case-Control Study. Dis Markers 2019:8062397.

Figure legends

Figure 1 Schematic diagram of CpG island in the CDS of *CRH*. Upper panel: *CRH* gene structure. The *CRH* gene is composed of 2 coding exons. The coding region is completely within the second exon and is represented by the dotted fill. The diagonal lines represent the regions sequenced in this study. Lower panel: The box surrounded with the dotted line contains the genomic sequences analyzed to identify CpG islands. The examined CpG loci are underlined, presented in bold and numbered (NCBI reference sequence: NG_016127.1).

Figure 2 DNA methylation levels of the CDS of *CRH* in schizophrenia patients and matched healthy controls. Methylation status (mean ± SEM) among all samples (left) and the male (middle) and female (right) groups (when samples were separated by sex). *P < 0.05, **P < 0.01.

Figure 3 CpG site methylation status between schizophrenia patients and matched healthy controls. Methylation status (mean ± SEM) among all samples (A) and male (B), and female (C) groups (when samples were separated by sex). (D) Methylation status between men and women *P < 0.05. *P < 0.05, **P < 0.01.

Table 1 Sample characteristics

		Control (68)	Schizophrenia (70)	p-value
Gender		Male:Female=47:21	Male:Female=46:24	
Age (mean ± SD)	Total	40.13 ±8.30	41.60±9.11	0.3239
	Male	40.04±9.09	42.11±10.21	0.3057
	Female	40.33±6.38	40.63±6.58	0.8809
BMI (mean ± SD)	Total	37.49±5.90	36.26±5.41	0.2064
	Male	38.46±6.17	36.53±5.39	0.1120
	Female	35.33±4.76	35.75±5.52	0.7815

Supplementary material Captions

Table S1 DNA methylation levels of *CRH* in schizophrenia patients and matched healthy controls.

Table S2 All CpG site methylation in patients with schizophrenia and matched healthy controls.

Figures

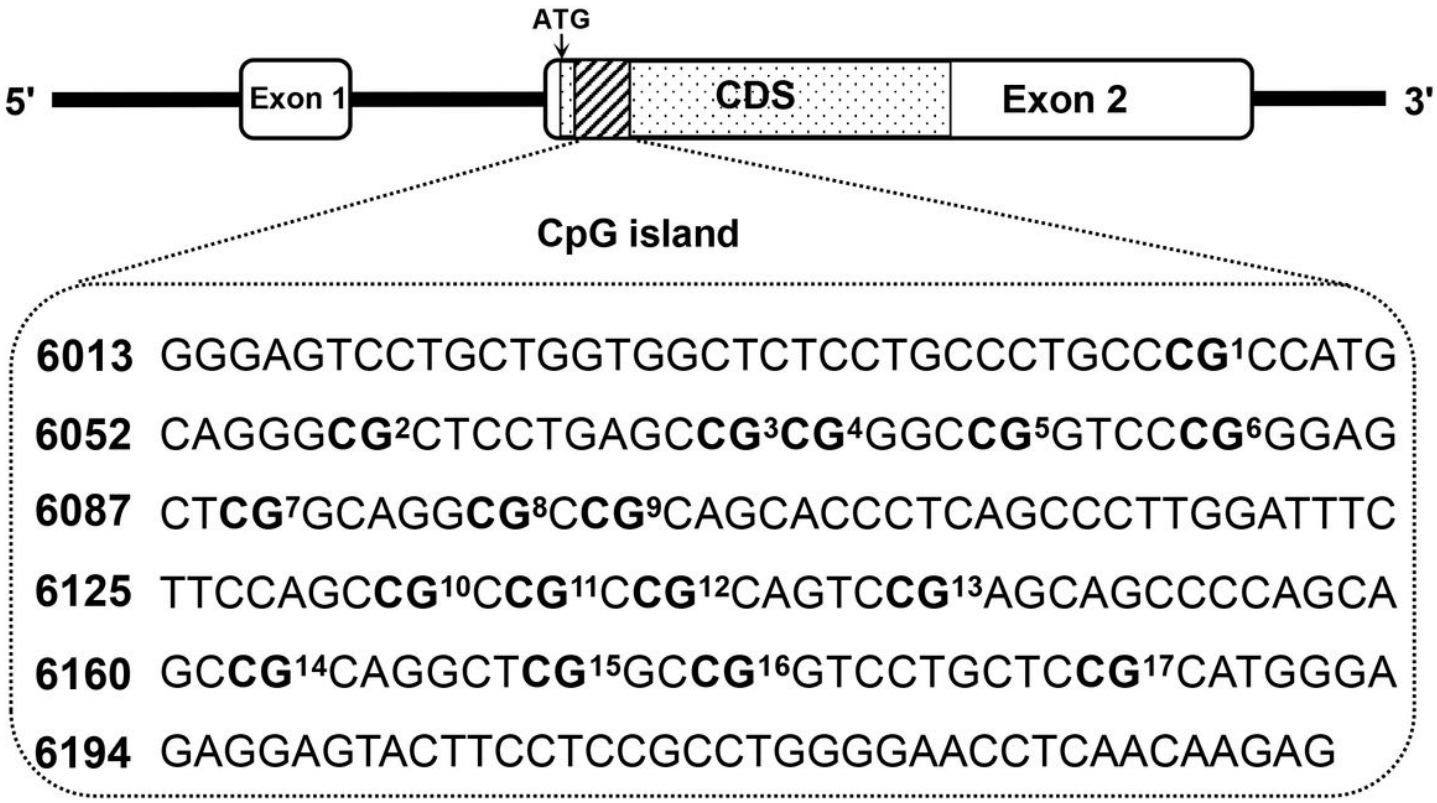

Figure 1

Schematic diagram of CpG island in the CDS of CRH. Upper panel: CRH gene structure. The CRH gene is composed of 2 coding exons. The coding region is completely within the second exon and is represented by the dotted fill. The diagonal lines represent the regions sequenced in this study. Lower panel: The box surrounded with the dotted line contains the genomic sequences analyzed to identify CpG islands. The examined CpG loci are underlined, presented in bold and numbered (NCBI reference sequence: NG_016127.1).

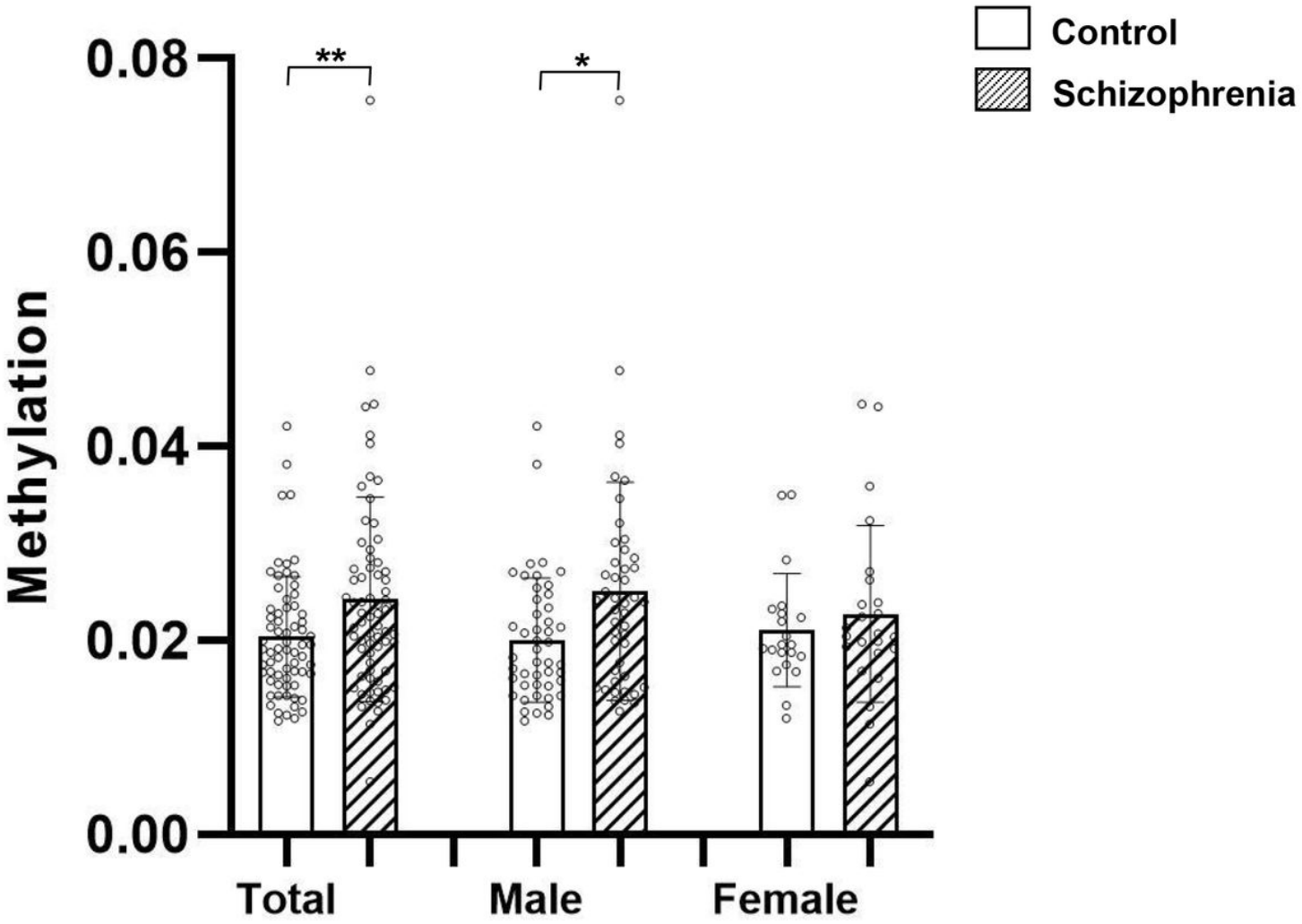

Figure 2

DNA methylation levels of the CDS of CRH in schizophrenia patients and matched healthy controls. Methylation status (mean ± SEM) among all samples (left) and the male (middle) and female (right) groups (when samples were separated by sex). *P < 0.05, **P < 0.01.

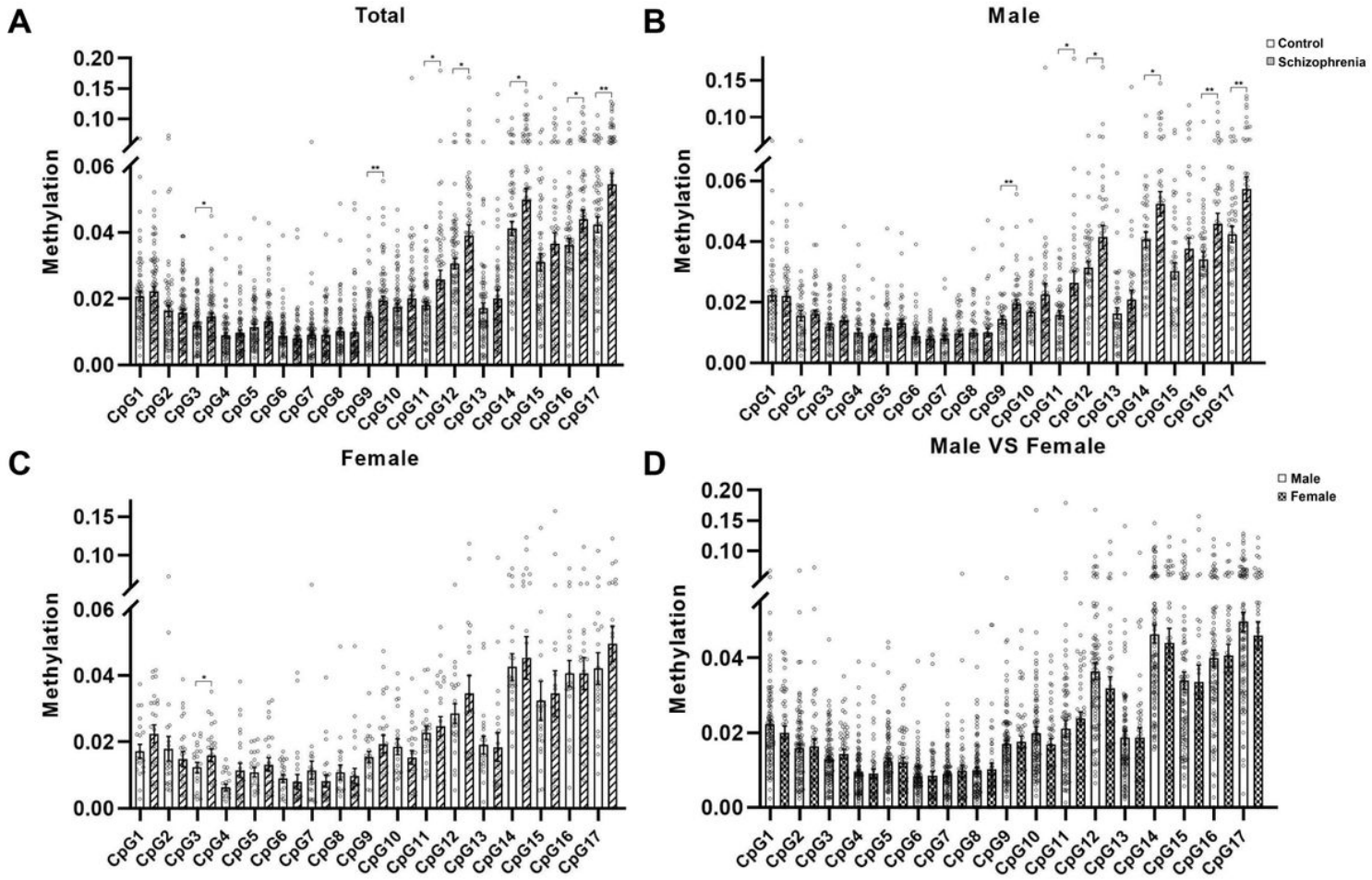

Figure 3

CpG site methylation status between schizophrenia patients and matched healthy controls. Methylation status (mean ± SEM) among all samples (A) and male (B), and female (C) groups (when samples were separated by sex). (D) Methylation status between men and women *P < 0.05. *P < 0.05, **P < 0.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- TableS2.xlsx