Anorexia nervosa (AN) is a subtype of eating disorder characterized by excessive and purposeful restriction of food intake, which leads to substantial weight loss and physiological function decline below normal ranges. The longest duration and greatest fatality rate among mental diseases make AN one of the deadliest and most disabling ailments. While the actual etiology of AN is still unknown, a plethora of data suggests a complicated genesis that involves both environmental and genetic factors. In AN research, epigenetic mechanisms—particularly those influencing gene expression—have acquired relevance as important intermediates in the interaction between genetic predisposition and environmental variables. DNA methylation, in which methyl groups bond to cytosine inside cytosine-guanine dinucleotides (5'-CpG-3' sites), is a well-researched epigenetic modification. Evidence shows that methylation may have a direct influence on transcription factors(TFs).

The investigative stage is presently concentrating on DNA methylation in connection to anorexia nervosa (AN). A recent study, however, reveals a potential relationship between gene methylation and the clinical signs of AN. Notably, GHSR (ghrelin receptor), SNCA (α-synuclein), DAT (dopamine transporter), DRD2 (dopamine receptor D2), OXTR (oxytocin receptor), and LEP (leptin) are among the genes with altered methylation patterns found in AN. These genes are critical for modulating hunger and reward circuits. Moreover, two comprehensive whole-genome sequencing studies (GWAS) on patients with AN have shown a drop in their genome-wide methylation levels.

Anorexia nervosa (AN) appears to be largely caused by dysregulation of appetite control, both in its onset and persistence. The neuropeptide ghrelin, which is released by the gastrointestinal tract, is implicated in the pathophysiology of the central nervous system, cardiovascular system, gastrointestinal tract, reproductive system, and immunological system, in addition to modulating hormones and appetite. P/D1 cells are the key stomach mucosal endocrine cells responsible for generating and releasing the precursor protein ghrelin. It becomes functional ghrelin in the circulation upon acylation. Ghrelin may directly bind to certain receptors in the arcuate nucleus of the hypothalamus (ARC), demonstrating its capacity to traverse the blood-brain barrier. Agouti-Related Protein (AgRP) and Neuropeptide Y (NPY) neurons become more excitable as a result of this interaction, prompting appetite.

Leptin is a peptide hormone produced by white adipose tissue. It impacts several organs, such as the liver, kidneys, skeletal muscles, and hypothalamus, which contain leptin receptors. Leptin stimulates glucose absorption and utilization in white adipose tissue. It controls the intake and release of blood glucose in organs such as the small intestine, liver, and skeletal muscles to ensure glucose balance. Leptin activates the vagus nerve to trigger the secretion of hormones such as glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK) after eating, which reduces hunger. Leptin has a direct impact on certain receptors in the arcuate nucleus, which in turn affects the transcription of proopiomelanocortin (POMC). The produced transcription product attaches to melanocortin receptors (MCR), stimulating neurons linked to satiety and hence reducing appetite. Leptin suppresses the production of Neuropeptide Y (NPY) and Agouti-Related Protein (AgRP) in neurons, which decreases the stimulating impact of AgRP on the central appetite center, resulting in reduced hunger. Leptin influences excitability in reward circuits, decreasing pleasure after eating and suppressing the urge to consume. Leptin boosts sympathetic nervous system activity, which helps break down fat tissue to stabilize lipid metabolism. The dynamic balance between leptin and ghrelin plays a critical role in regulating eating behavior and internal homeostasis, underscoring its relevance to maintaining physiological equilibrium.

Environmental factors can influence the methylation of specific cytosine-phosphate-guanine (CpG) sites, leading to abnormal transcription factor binding and subsequent alterations in gene expression, resulting in the manifestation of clinical symptoms. In recent years, there has been a growing body of research on the relationship between environmental factors and diseases.

A study in 2013 found that the duration of breastfeeding during childhood was negatively correlated with the methylation levels of the Leptin (LEP) gene. Specifically, children who received sufficient breastfeeding exhibited lower methylation of the LEP gene. Breast milk contains higher nutritional content and essential growth factors compared to formula milk. Adequate breastfeeding was associated with a decrease in LEP gene methylation levels and an increase in plasma LEP levels, serving as a significant preventive factor against obesity. The study also identified a lower obesity risk in children who received adequate nutritional support during childhood.

The methylation levels of the Growth Hormone Secretagogue Receptor (GHSR) gene are also influenced by maternal factors. Previous research indicated that maternal exposure to a cold environment during pregnancy led to increased GHSR expression in offspring, which gradually decreased after birth. A study in 2015 demonstrated that the stress levels of lactating mothers during the postpartum period affected the methylation levels of their offspring's GHSR gene. Postpartum depression and low attention to offspring were associated with an increase in GHSR gene methylation levels, resulting in reduced offspring feeding. Abnormal regulation of emotions also impacts the methylation levels of the GHSR gene. A recent study in adolescents with depression found that negative life events during adolescence led to an increase in GHSR gene methylation levels, positively correlating with the severity of depressive symptoms.

Current research on the impact of environmental factors on DNA methylation has been confirmed in various diseases, including tumors, obesity, and diabetes. This suggests that environmental factors play a crucial role in gene methylation, providing valuable insights for further investigating the correlation between the environment and symptoms of anorexia nervosa (AN).

In order to distinguish between the derivative effects of malnutrition (state markers) and the biological processes that may lead and cause in the physiology of anorexia (properties markers), we investigated the differences between the methylation levels of DNA in acute aneurysm patients and in the GHS-R1a and LEP genes in healthy people, alongside the impact of traumatic experiences and family environments on the condition, while controlling the extent of possible age and cultural impact.

Method

2.1. Study participants

This research involved female volunteers diagnosed with acute anorexia nervosa (AN) and healthy controls (HC). 101 critically underweight anorexia nervosa patients fulfilling DSM-IV criteria were recruited from the Eating Disorder Programs at a University Child and Adolescent Psychiatry Department. The control group contained 52 normal-weight, eumenorrheic, healthy female participants (HC) recruited by advertising among middle school, high school, and university students.

Through structured interviews, information was gathered from all participants on exclusion criteria and potential contributing variables, including name, gender, profession, marriage, cultural status, height, weight, etc., and semi-structured interviews and medical records were supplemented.

HC individuals were disqualified if they had a history of any mental disorder. Participants in the AN groups were excluded if they had a lifetime history of psychiatric diagnoses such as organic brain syndrome, schizophrenia, drug dependency, bipolar illness, neurosis, or eating disorders. Additional exclusion criteria for all participants included people with an IQ of less than 85 and with severe physical complications (such as nervous system disease, heart rate abnormalities, severe electrolyte disorders, etc.), with serious negative suicidal thoughts or behavior; a family history of mental illness; taking psychotropic drugs, hormonal drugs, etc., for a month. Through structured interviews, information was gathered from all participants on exclusion criteria and potential contributing variables, including name, gender, profession, marriage, cultural status, height, weight, etc., and semi-structured interviews and medical records were supplemented.

The design of the research was approved by the Shanghai Mental Health Center Institutional Review Board. All participants (or guardians of children) submitted written informed consent after describing the research procedure.

2.3. Blood Collection, Biochemical Assessments, and Bisulfite Sequencing

After a nighttime fast, blood is collected from the arteries with EDTA between 7:30 and 9:30 in the morning. In the AN group, blood collection happened during the first week following the commencement of enhancing medication. Wait for further examination; the plasma sample is kept at -80°C. DNA methylation of the GHS-R1a gene promoter and the LEPR gene promotor was studied, and genomic DNA isolated from external blood mononucleic cells was evaluated using sulfur dioxide transformation and Sanger sequencing procedures.

2.4. Quality Control (QC)

All sequences underwent scrutiny in the Sequence Scanner, and those with a low Quality Value (QV20) were subjected to repeat sequencing. Only samples that could be technically sequenced adequately were retained for analysis. In the analysis, only CpG positions with 95% valid values were included. Similarly, only subjects with 95% valid CpG values were considered for inclusion. CpG positions with a variance of less than 0.001 were excluded from the analysis.

Ultimately, the study included a total of 115 subjects and a collective of 64 CpG positions (24 from GHS-R1a and 40 from LEP) for subsequent analysis.

**3. Results**

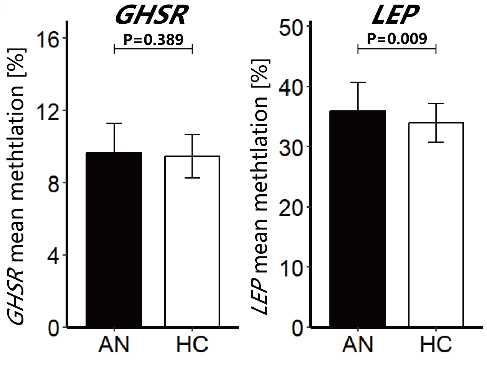
3.1. Sample characteristics

The clinical features of all patients are presented in table 1. As expected, people with acute anorexia nervosa (AN) drastically lowered their BMI and psychiatric levels (measured by EDI-2, EDE-Q).In addition, AN patients were slightly younger than HC patients. In the AN group, 60 patients were classified as restricted type (AN-R) and 41 patients were categorized as/or eliminated type (AN-BP). It is interesting that there were no significant changes in clinical parameters across these subtypes, including body mass index, EDI and EDE-Q.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *变量* | *AN* | *HC* | *t/Z* | *p* |
| Age | 18 [ 3 ] | 21 [ 6 ] | -1.912 † | 0.055 |
| education years | 12 [ 5 ] | 16 [ 5 ] | -3.609 † | <0.001 \*\*\* |
| years | 17 [ 4.5 ] | 21 [ 6 ] | -3.513 † | <0.001 \*\*\* |
| duration | 10 [ 19 ] | 0 [ 0 ] | 10.115 † | <0.001 \*\*\* |
| Weight | 43 [ 12 ] | 46 [ 12 ] | -3.168 † | 0.002 \*\* |
| high | 1.6 [ 0.06 ] | 1.6 [ 0.05 ] | -0.803 † | 0.422 |
| BMI | 16 ± 2.5 | 18 ± 3.5 | -3.774 ‡ | <0.001 \*\*\* |
| Restraint | 1.8 [ 2.2 ] | 0.2 [ 0.8 ] | 5.801 † | <0.001 \*\*\* |
| Eating concern | 1.6 [ 2.6 ] | 0.2 [ 0.2 ] | 7.265 † | <0.001 \*\*\* |
| Shape concern | 2.2 [ 2.4 ] | 0.56 [ 1.7 ] | 5.344 † | <0.001 \*\*\* |
| Weight concern | 2 [ 2.4 ] | 0.2 [ 1.2 ] | 5.972 † | <0.001 \*\*\* |
| Global score | 2 [ 2 ] | 0.33 [ 0.84 ] | 6.758 † | <0.001 \*\*\* |
| EDI | 174 [ 83 ] | 153 [ 32 ] | 2.691 † | 0.007 \*\* |

3.2. Main Analysis - Mean Methylation

The study of the outcomes of the t-test indicates there is no significant difference in the mean methylation level of the GHS-R1a gene promoter among groups (t = 0.647, p = 0.389). However, a significant difference is seen in the average methylation level of the LEP gene promoter among groups (t = 2.615, p = 0.009).

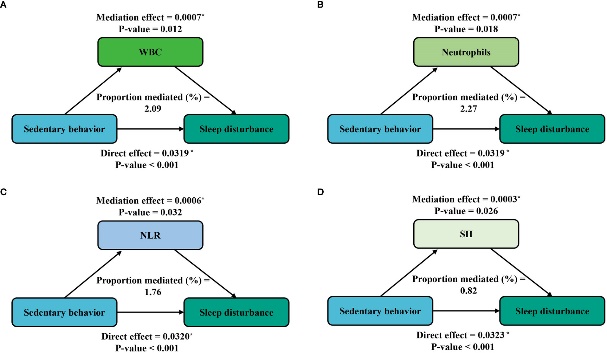


3.3 Relationship between DNA methylation levels and clinical variables

A partial correlation study in the AN group between the mean amount of LEP promoter methylation and the total EDEQ score indicated substantial bilateral correlations (r=0.338,p=0.0006).In addition, the total EDEQ score is substantially connected with the coherence of the FESCV scale and the emotional abuse of the ETI scale.

3.4.mediating effect of LEP gene methylation

Furthermore, mediation analyses were conducted to explore the mediating effect of inflammatory markers. Figure 3 shows the mediating role of inflammatory markers in the relationship between sedentary behavior and sleep disturbance. All four inflammatory markers significantly mediated the association between sedentary behavior and sleep disturbance, with WBC, neutrophils, NLR, and SII explaining 2.09%, 2.27%, 1.76%, and 0.82% of the association, respectively (P < 0.05). Meanwhile, on the basis of the results from Table 4, we also conducted a mediation analysis of the effect of exercise in attenuating sleep disturbance on the severe sedentary group. As shown in Figure 4, WBC, NEU, NLR, and SII explained 1.11%, 1.39%, 0.42%, and 1.39% of the association, respectively. Although the direct effects were all significant in these four cases (P < 0.001), the mediating role of inflammatory markers tended to be non-significant.



**Figure 3** Path diagram of the mediation analysis of inflammatory biomarkers on the relationship between sedentary behavior and sleep disturbance. The graphs in **(A–D)** represented the mediating role of WBC, neutrophils, NLR, and SII, respectively.