Methed

2.1. Study participants

This study enrolled female participants diagnosed with acute anorexia nervosa (AN) and healthy controls(HC). 101 acutely underweight anorexia nervosa patients meeting DSM-IV criteria were recruited from the Eating Disorder Programs at a University Child and Adolescent Psychiatry Department. The control group comprised 52 normal-weight, eumenorrheic, healthy female subjects (HC) recruited through advertising among middle school, high school, and university students.

Information on exclusion criteria and potential confounding variables, including menstrual cycle and contraceptive medication use, was gathered from all participants using the Structured Interview for EDI , supplemented by a semi-structured interview and medical records.

HC participants were excluded if they had any history of psychiatric illness. Participants from the acAN or recAN groups were excluded if they had a lifetime history of clinical diagnoses such as organic brain syndrome, schizophrenia, substance dependence, bipolar illness, bulimia nervosa, or binge-eating disorder. Additional exclusion criteria for all participants included an IQ less than 85, current inflammatory, neurological, or metabolic illnesses, chronic bowel diseases, cancer, anemia, pregnancy, breastfeeding, treatment with cortisone, and the use of psychotropic medications within the past 6 weeks. Subjects with indicated or reported drug use, notably cannabis, were also excluded from the analyses.

This study was conducted in accordance with the latest version of the Declaration of Helsinki, and the study design was approved by the Institutional Review Board of Charité – Universitätsmedizin Berlin. All participants (or their guardians if underage) provided written informed consent after a thorough explanation of the study procedures.

2.3. Blood Collection, Biochemical Assessments, and Bisulfite Sequencing

Venous blood was drawn into vacutainer tubes containing EDTA between 7:30 and 9:30 a.m. after an overnight fasting period. For the acAN group, blood collection occurred within the first week after the initiation of intensive treatment. Plasma samples were stored at -80°C until further analysis. DNA methylation analysis of the GHS-R1a gene promoter and LEPR gene promoter was conducted on genomic DNA extracted from peripheral blood mononuclear cells, employing bisulfite conversion and Sanger sequencing techniques.

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.

Following QC procedures, 7 participants from the acAN group, 4 from the recAN group, and 6 from the HC group were excluded based on the mentioned criteria. Ultimately, the study included a total of 115 subjects and a collective of 64 CpG positions (24 from GHS-R1a and 40 from LEPR) for subsequent analysis.

**3. Results**

*3.1. Sample characteristics*

Table 1 provides a summary of the demographic and clinical characteristics of all participants. As anticipated, individuals with acute anorexia nervosa (acAN) exhibited significantly lower BMI-standard deviation scores (BMI-SDS) and elevated levels of psychopathology (measured by EDI-2, SCL-90-R). While patients in the weight-recovered anorexia nervosa (recAN) group had BMI-SDS similar to that of healthy controls (HC), they still presented with some residual psychopathology. Moreover, recAN patients were slightly older than their HC counterparts. Within the acAN group, 28 patients were classified as restrictive type (AN-R), and 11 patients were categorized as binge/purging type (AN-BP). Notably, there were no significant differences in clinical characteristics between these subtypes, including BMI, EDI-2, 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 analysis of the rank-sum test results indicates that there is no significant difference in the average methylation level of the GHS-R1a gene promoter across groups (Z = 0.647, p = 0.389). However, a significant difference is observed in the average methylation level of the LEP gene promoter among groups (Z = 2.615, p = 0.009).

