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Coordinated epigenetic dysregulation of *CNR1* and *FAAH* genes drives endocannabinoid system dysfunction in anorexia nervosa

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Abstract

Background Anorexia nervosa (AN) is a severe psychiatric disorder with limited biomarkers for diagnosis and treatment monitoring. We investigated endocannabinoid system (ECS) dysregulation in AN through integrated epigenetic and genetic analysis of saliva.

Methods We analyzed DNA methylation patterns of cannabinoid receptor 1 (*CNR1*) and fatty acid amide hydrolase (*FAAH*) genes, characterized genetic polymorphisms, and quantified expression of exosomal microRNAs targeting these genes in AN patients versus healthy controls.

Results We discovered a novel bidirectional epigenetic dysregulation of the ECS: *CNR1* promoter hypermethylation coupled with *FAAH* promoter hypomethylation. This dual-target mechanism systematically impairs endocannabinoid signaling by simultaneously reducing receptor availability while increasing endocannabinoid degradation. Compensatory miRNA responses (upregulated miR-342-3p, miR-23b-3p targeting *CNR1*; upregulated miR-4505, miR-1275 targeting *FAAH*) revealed dynamic regulatory attempts to counterbalance these primary epigenetic changes. This convergent pathway dysfunction demonstrates how multiple molecular mechanisms work in concert to dysregulate appetite regulation in AN. Combined biomarker panels showed superior diagnostic precision compared to individual markers.

Conclusions Our findings establish this bidirectional epigenetic dysregulation as a central mechanism underlying ECS dysfunction in AN, providing mechanistic insights that identify novel therapeutic targets and advance precision medicine approaches for this challenging disorder.

Plain English summary

This study identifies biological markers in saliva that may help detect anorexia nervosa more accurately. We analyzed specific genes involved in the endocannabinoid system—a network in the body that helps regulate appetite, mood, and reward—suggesting patterns that could improve diagnosis and reveal new treatment targets. By identifying these gene patterns in saliva, we offer a non-invasive way to better understand how anorexia

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develops and progresses. Our findings may help improve early diagnosis and open new possibilities for treatment, especially by targeting the biological systems that influence appetite and emotional regulation. This research brings us closer to understanding the underlying biology of eating disorders and may support the development of more personalized and effective care strategies in the future.

Keywords Anorexia nervosa, Psychiatric disorders, Epigenetics, DNA methylation, MicroRNAs, Transcription factors, Endocannabinoid system, Molecular biology, Saliva

Background

Anorexia nervosa (AN) is among the most challenging psychiatric disorders, marked by considerable short- and long-term medical complications, low rates of full recovery, and high mortality [1, 2]. Behavioral symptoms include severe food restriction accompanied by either or both fasting, excessive exercise, and bingeing/purging to avoid weight gain [3]; self-worth is severely influenced by body weight and shape, with poor insight into illness severity. AN affects approximately 0.1–3.6% of women and 0.1–0.3% of men [4–6] and has a multifactorial etiology, involving genetic [5, 7–9], neurobiological [10, 11], environmental [12, 13], metabolic [5] and psychological factors [13].

Diagnosis remains mostly clinical, relying on psychological assessment and physical examination, with few objective biomarkers available to support diagnosis, prognosis, or treatment monitoring. Reliable biomarkers could enable earlier detection and prevention by identifying individuals at risk prior to the full clinical manifestation, improve diagnostic accuracy despite denial or symptom concealment [14, 15], and track disease progression, remission, or relapse [16].

Proposed biomarkers include hormonal, neurotransmitter, inflammatory, and metabolic changes, though clinical utility remains limited due to inconsistent, nonspecific results and complex sampling [17]. More recently, neurobiological circuits or domains targeting appetite control, reward processing, and stress management [18, 19], with the endocannabinoid system (ECS) being a particularly promising area, have been the focus of research [20].

The ECS is a complex endogenous system that includes central and peripheral cannabinoid receptors (CB1, CB2), endogenous cannabinoids (primarily anandamide and 2-arachidonoylglycerol), and the enzymes responsible for their biosynthesis and degradation, like fatty acid amide hydrolase (FAAH) [21, 22]. By modulating regulatory reactions to internal and environmental changes, such as appetite, energy balance, reward processing, emotional control and stress response, the ECS affects several functions specifically dysregulated in AN [23] and could likely be considered a promising therapeutic target in ED [24].

Altered ECS activity, especially in cannabinoid receptor 1 (*CNR1*) and *FAAH* genes has been reported in AN [25]. CB1 receptors are densely expressed in brain regions linked to appetite (hypothalamus), reward (mesolimbic), and emotion (amygdala and prefrontal cortex). These brain regions show structural and functional alterations in AN patients [26]. Alterations in CB1 receptor expression and endocannabinoid levels in central brain regions have been linked to AN-like behavior in humans [27, 28] and in animal models [29], yielding mixed and inconclusive results. ECS pharmacological modulation affects food intake and anxiety-like behavior in these models, supporting its therapeutic relevance [25].

FAAH, the primary enzyme responsible for degrading anandamide (AEA), plays a direct role in endocannabinoid tone and the signal's duration [30]. Studies comparing AN patients to healthy controls (CTRL) have reported inconsistent findings regarding AEA levels: some show elevated levels in AN [31, 32], others report reductions [33], and some observe no significant differences [27, 34].

Environmental factors can modulate gene function through epigenetic mechanisms [35]. Chemical DNA modifications, including DNA methylation, and microRNAs (miRNAs) regulation, can induce transient changes in gene expression [36]. These modifications serve as a dynamic interface between genetic predisposition and environmental influences, playing a crucial role in both normal physiological processes and disease development. DNA methylation adds a methyl group to cytosine residues in CpG nucleotides, typically leading to gene silencing when present in promoter regions [37]. This mechanism is implicated in various neurobiological processes relevant to the development of AN, including energy homeostasis, hunger, and feeding regulation [38]. Epigenetic changes are responsive to environmental pressures like diet, stress, and psychosocial factors, all shown to contribute to AN development [39]. ECS components, especially *CNR1* and *FAAH* genes, are regulated through epigenetic mechanisms and controlled as a function of stress and nutrition [40]. Recently, increased *Cnr1* methylation has been proposed as potentially contributing to dysregulated endocannabinoid signaling in the activity-based anorexia (ABA) rat model of AN [41].

Genetic heterogeneity of ECS components has also been implicated in ED and associated traits. *CNR1* and *FAAH* gene polymorphisms are linked to AN susceptibility, reward sensitivity, and treatment response [42, 43]. Given AN's high heritability (50–80%), further genetic

analysis in these ECS components may clarify individual risk and guide personalized interventions [5].

Here, we assessed the potential of *CNR1* and *FAAH* genes as biomarkers for AN. To this extent, we examined DNA methylation, transcription factor binding, and genetic variation within these loci, as well as the expression of exosomal miRNAs known to target them in individuals with AN compared to healthy controls.

Methods

Ethical aspects and selection of participants

From April 2022 to May 2023, individuals seeking ED care at the Outpatient Unit for Clinical Research and Treatment of Eating Disorders of the University Hospital Renato Dulbecco (Catanzaro, Italy) were screened for eligibility and invited to participate. Age- and sex-matched healthy students from the University of Chieti (Chieti, Italy) volunteered as controls.

Eligibility for the clinical sample included: (a) ongoing diagnosis of AN according to DSM-5 criteria and psychiatric examination; (b) age over 14. Potential participants were interviewed by psychiatrists and formally diagnosed through the Structured Clinical Interview for the DSM-5 (SCID-5 CV) [44] and the Eating Disorder Examination (EDE 17.0 D) [45].

Table 1 Demographic and clinical data comparing healthy controls (CTRL, $n=40$) and patients with anorexia nervosa (AN, $n=38$)

	AN ($N=38$)		CTRL ($N=40$)	
	Mean	**SD**	**Mean**	**SD**
Age	19.9	7.6	19	0.6
Age at onset	16.6	5.6		
Illness duration (years)	3.2	4.2		
BMI (kg/m^2)	18.6	2.3	21.2	2.0
BMI lowest value (kg/m^2)	16.1	1.9		
BMI highest value (kg/m^2)	21.9	2.5		
Subtype				
Restricting	23	(60%)		
Binge-purge	15	(40%)		
Psychiatric comorbidities				
MDD	32	(84%)		
GAD	1	(3%)		
Ongoing medications				
Yes	27	(71%)		
Antidepressants	23	(60%)		
Antipsychotics	23	(60%)		
Mood stabilizers	2	(5.3%)		

Continuous variables are presented as mean ± standard deviation (SD). Categorical variables are presented as frequencies. Age of onset, illness duration, BMI lowest and highest values apply only to the AN group. Some patients were taking multiple medications concurrently. *AN* anorexia nervosa, *BMI* body mass index, *CTRL* control, *GAD* generalized anxiety disorder, *MDD* major depressive disorder

Controls had to be aged over 18, self-report no ED history, and have a BMI in the normal range according to World Health Organization [46]. An ad-hoc survey collected socio-demographics (e.g., age, sex at birth, education, occupation), anamnestic and anthropometrics data (i.e., weight, height). Severe psychiatric comorbidities (neurodevelopmental disorders, disorders in the schizophrenia spectrum, bipolar disorders, neurocognitive disorders, substance use disorder), as well as medications (e.g., orosoluble tablets) or known diseases affecting the oral cavity and/or the gastrointestinal system, or the immune system overall were considered exclusion criteria for both groups.

Participants and legal representatives for minors were duly informed about study aims, procedures, anonymisation, and data handling. Informed consent was obtained prior any procedure. The study was approved by the Ethical Committee of "Regione Calabria, sezione Area Centro" (identifier: 395/18.11.2021) and participants were treated in accordance with the Declaration of Helsinki [47].

After screening for eligibility, 38 individuals were enrolled as cases and 40 as controls (see Table 1 for demographic and clinical details).

Saliva sample collection and nucleic acid extraction

Saliva samples were collected from participants in the morning and immediately stored at – 20 °C. Prior to collection, participants were instructed to refrain from eating, drinking, smoking, or brushing their teeth for at least 1 h.

Genomic DNA was extracted from saliva samples via salting-out method adapted from Garbieri et al. [48]. Total exosomal RNA was isolated using the Total Exosome Isolation from Saliva protocol (Invitrogen, Carlsbad, CA, USA) followed by Total Exosome RNA and Protein Isolation protocol (Invitrogen, Carlsbad, CA, USA).

The concentration and purity of DNA was assessed using NanoSNAP spectrophotometer (Spex, Metuchen, NJ, USA). A260/280 ratios near 1.8 were considered acceptable.

DNA methylation and SNP analysis by pyrosequencing

The methylation status of human *CNR1* and *FAAH* genes was assessed by pyrosequencing of bisulfite-converted DNA. A total of 500 ng of genomic DNA from each sample was subjected to bisulfite conversion using the EZ DNA methylation-Gold kit (Zymo Research, Orange, CA, USA). Bisulfite-treated DNA was amplified using the PyroMark PCR Kit (Qiagen, Hilden, Germany) with a biotinylated primer, following the manufacturer's recommendations.

Pyrosequencing was carried out on a PyroMark Q48 Autoprep using PyroMark Q48 Advanced Reagents (Qiagen, Hilden, Germany), according to the manufacturer's recommendations. Specific PyroMark CpG assays (Qiagen, Hilden, Germany) were used to analyze the regulatory regions of *FAAH* and *CNR1* genes, targeting 4 and 5 CpG sites, respectively. Detailed information on the sequences and assays is reported in Table S1 and Figure S1 (Supporting information).

CpG methylation was quantified using PyroMark Q48 Autoprep and expressed as mC/(mC + C), where mC represents methylated cytosines and C unmethylated cytosines. Results were expressed as the methylation percentage at each individual CpG site as well as the average methylation level across all analyzed sites.

Genotyping of the *CNR1* rs1049353 and *FAAH* rs324420 single nucleotide polymorphisms (SNPs) was performed by pyrosequencing using 100 ng of genomic DNA. Five AN samples were excluded due to insufficient initial sample concentration. PCR amplification was carried out using the PyroMark PCR Kit (Qiagen, Hilden, Germany) with a biotinylated primer, according to the manufacturer's instructions. Custom PyroMark assays specifically designed for the *CNR1* rs1049353 and *FAAH* rs324420 SNPs were used for the analysis. Detailed information on the sequences and assay conditions is provided in Table S2.

cDNA synthesis and MiRNA analysis

miRNAs targeting *CNR1* and *FAAH* were identified via three databases: miRDB (https://mirdb.org/mirdb/index.html) [49], miRWalk (http://mirwalk.umm.uni-heidelberg.de/) [50], and TargetScan (https://www.targetscan.org/vert_80/) [51]. In miRDB and TargetScan, we considered miRNAs with a score greater than 80, while for miRWalk, we included miRNAs that specifically target the 3' UTR. For *CNR1*, miRNAs appearing in all 3 databases were selected; for *FAAH*, those found in at least two, given the lower number of matches. This yielded 39 miRNAs for *CNR1* and 42 for *FAAH*.

Further screening via Human microRNA Disease Database (HMDD, http://www.cuilab.cn/hmdd) [52] and PubMed identified miRNAs linked to psychiatric disorders, mental health conditions, and metabolic disorders. Based on these findings, four miRNAs (miR-16-2-5p, miR-342-3p, miR-23b-3p and miR-212-3p) for *CNR1* gene and five (miR-4505, miR-664b-5p, miR-1275, miR-4270 and miR-1249-5p) for *FAAH* genes were selected for further analysis.

miRNA expression was assessed by quantitative PCR (qPCR) after reverse transcription using miRCURY LNA RT kit (Qiagen, Hilden, Germany). qPCR reaction was performed using sensiFAST SYBR ™ Green. Detailed information on primer sequences is provided in Table S3.

The level of microRNA was normalized using hsa-miR-16-5p as endogenous reference. Reactions were performed in duplicates on a QIAquant 96 5 plex Real-Time PCR System (Qiagen, Hilden, Germany). The $2^{-\Delta Ct}$ method was used to evaluate the relative expression of selected microRNAs using mean log2 fold change values of selected microRNAs and controls.

Transcription factors binding site analysis

To identify transcription factors (TFs) which binding might be affected by the differentially methylated CpG sites regions of *CNR1* and *FAAH* genes, we performed motif scanning of the JASPAR2024 database [53] using the JASPAR2024 package (DOI: https://doi.org/10.18129/B9.bioc.JASPAR2024) in R. Specifically, we queried our sequences of interest against the curated position frequency matrices (PFMs) of TF available in JASPAR and selected TFs whose consensus motifs aligned with our regions with a minimum score of 80%.

To refine biologically relevant candidates, we applied a multi-step filtering strategy focused on CpG sensitivity. We first selected TFs whose consensus motifs included CpG dinucleotides (i.e., "CG") within the core binding site. We then restricted the results to TFs whose predicted binding sites occurred precisely at CpG sites identified in our methylation analysis of *FAAH* and *CNR1*, with a conservation score ≥ 0.8 in the position probability matrix, PPM.

Statistical analysis

Sample size calculations were performed using G*Power 3.1.9.7, assuming a medium effect size (Cohen's d = 0.5), α = 0.05, and power = 0.80, yielding a minimum required sample size of 32 per group. Our sample sizes exceed this threshold.

Statistical data analysis was performed using Prism® 11 (GraphPad Software, San Diego, CA) and R studio 4.4.2 (Posit, Boston, MA, USA). Results are expressed as mean ± SD, frequencies or percentages. Mann–Whitney test assessed methylation and miRNA expression; Holm-Sidak correction was applied for multiple comparisons. Genotype distributions were analyzed via chi-square test. Spearman's coefficient was used for correlations. Significance was set at $p < 0.05$.

Results

DNA methylation analysis

CNR1 gene showed significant hypermethylation in AN compared to CTRL (average methylation: 8.61 ± 3.87% vs. 5.19 ± 1.393%, $p < 0.0001$), at all five CpG sites examined ($p < 0.05$ for all positions) (Fig. 1a). Position-specific mean methylation levels (± SD) for controls vs. AN were: Position 1 (2.046 ± 0.769% vs. 3.72 ± 4.36%), Position 2 (8.83 ± 2.76% vs. 16.55 ± 9.96%), Position 3

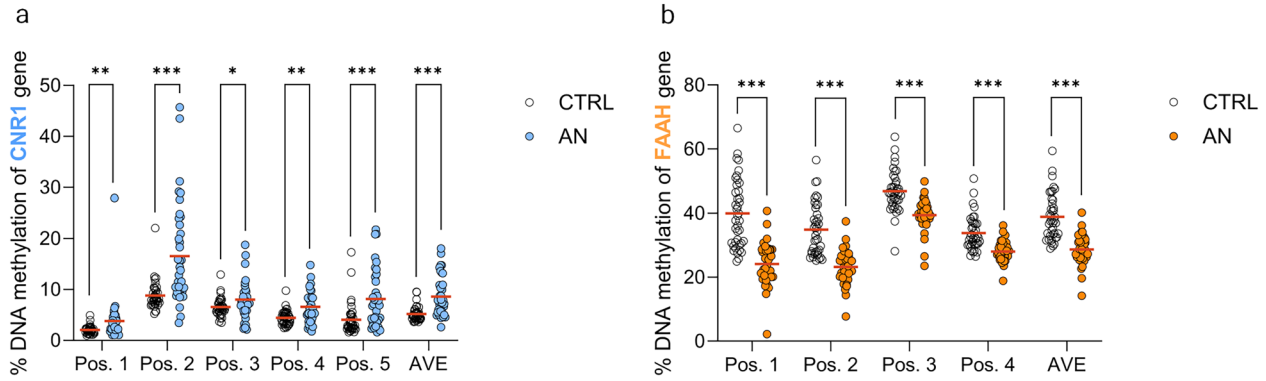

Fig. 1 DNA methylation at **a** *CNR1* and **b** *FAAH* gene promoters. DNA methylation percentages are shown for the individual CpG sites and their AVE in CTRL (open circles), and AN (blue (*CNR1*) and orange (*FAAH*) circles) groups. Red horizontal lines indicate group medians. Statistical significance is indicated by asterisks (Mann–Whitney test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). *AN* anorexia nervosa, *AVE* average, *CNR1* cannabinoid receptor 1, *CTRL* control, *FAAH* fatty acid amide hydrolase

(6.57 ± 1.712% vs. 8.01 ± 3. 88%), Position 4 (4.44 ± 1.41% vs. 6.60 ± 3.27%), and Position 5 (4.06 ± 3.07% vs. 8.16 ± 5.53%).

In contrast to the hypermethylation observed in *CNR1*, the *FAAH* gene showed significant hypomethylation in AN compared to controls across all CpG sites ($p < 0.0001$ for all positions, average methylation: 28.76 ± 4.66% vs. 38.88 ± 7.12%) (Fig. 1b). Position-specific mean methylation levels (± SD) for controls vs. AN were: Position 1 (39.94 ± 10.50% vs. 24.15 ± 6.75%), Position 2 (34.89 ± 8.04% vs. 23.21 ± 5.28%), Position 3 (46.87 ± 6.30% vs. 39.45 ± 4.76%), and Position 4 (33.81 ± 5.30% vs. 28.05 ± 3.30%).

Correlation analysis revealed significant associations between methylation levels at CpG sites within the *CNR1* and *FAAH* genes. Notably, methylation at *CNR1* CpG Position 2 showed a strong and highly significant negative correlation with methylation at all *FAAH* CpG sites ($p < 0.05$) (Fig. 2), indicating that higher methylation levels in *CNR1* correspond to lower methylation levels in *FAAH* gene. Additionally, the mean *CNR1* methylation level was also negatively correlated with all *FAAH* CpG sites ($p < 0.05$), except for CpG site 3, further supporting the presence of a coordinated and site-specific epigenetic interaction between these two key components of the endocannabinoid system. Moreover, *FAAH* CpG site 1 was significantly correlated with *CNR1* CpG Positions 4 and 5 ($p < 0.05$), reinforcing the idea of specific cross-regulatory methylation patterns between distinct loci within the two genes.

Genetic analysis

The allelic distribution of *CNR1* SNP rs1049353 did not differ significantly between AN patients and controls ($p = 0.356$). The observed genotype frequencies were as follows: CTRL-G = 29, G/A = 11; AN—G = 27, G/A = 6.

Similarly, analysis of the *FAAH* SNP rs324420 revealed no statistically significant differences in genotype distribution between groups ($p = 0.680$). Genotype frequencies were: CTRL-C = 26, A/C = 13, A = 1; AN—C = 25, A/C = 6, A = 2.

Genotype-stratification DNA methylation analysis showed that among G/G carriers of rs1049353, individuals with AN had significantly higher methylation at all CpG sites except CpG site 3 compared to controls ($p < 0.05$, Fig. 3a). No significant differences were found in G/A carriers (Fig. 3b), suggesting a possible modulatory role of the A allele on nearby CpG methylation.

For rs324420, C/C carriers with AN exhibited significant lower methylation across all CpG sites than controls ($p < 0.001$, Fig. 3c), while C/A carriers also showed reduced methylation in AN patients compared to controls ($p < 0.01$, Fig. 3d).

MiRNA expression analysis

Exosomal miRNA profiling from salivary samples revealed distinct expression patterns of miRNAs predicted to target key components of ECS. Nine candidate miRNAs, selected for their potential regulation of *CNR1* and *FAAH* genes, were subsequently analyzed for their potential associations with psychiatric disorders, mental health conditions, and metabolic diseases.

Individual miRNA measurements showing delayed reference miRNA amplification were excluded from analysis to maintain data quality, accounting for the missing values observed in the expression dataset.

Out of the nine selected miRNAs, only three targeting *CNR1* (miR-342-3p, miR-23b-3p and miR-212-3p) and two targeting *FAAH* genes (miR-4505 and miR-1275) showed significant differential expression, indicating functional relevance. Among the remaining miRNAs, miR-1249-5p did not yield any detectable expression levels in our samples and was therefore excluded from

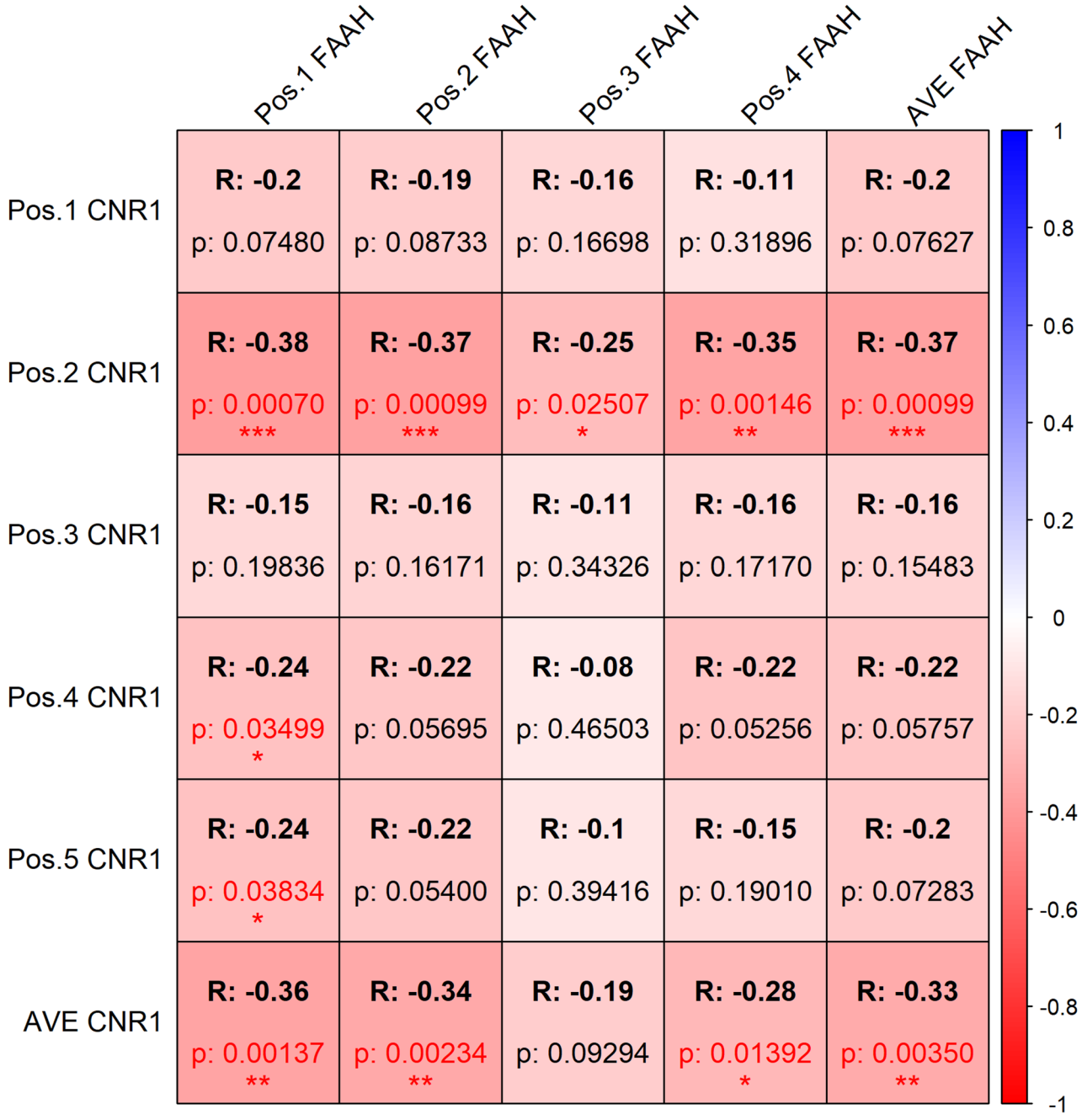

Fig. 2 *CNR1-FAAH* methylation correlation heatmap in all study subjects. Correlation matrix showing Spearman correlation coefficients (R) and *p*-values between *CNR1* and *FAAH* gene methylation levels at multiple positions. Color scale represents correlation strength; asterisks indicate significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). *CNR1* cannabinoid receptor 1, *FAAH* fatty acid amide hydrolase

further analysis, while the others did not display significant expression differences between experimental groups.

For miRNAs without significant differential expression, the mean $\log_2$ fold change ($\log_2$FC) values in CTRL vs. AN were as follows: – 3.67e–9 vs. 0.116 for miR-16-2-5p; – 6.071e–7 vs. 0.011 for miR-664b-5p and – 4.41e–7 vs. 0.062 for miR-4270.

miR-342-3p and miR-23b-3p, which target the *CNR1* mRNA, were significantly upregulated in AN patients compared to controls ($\log_2$FC = 0.29 vs. – 9.09e–6, $p = 0.0024$ for miR-342-3p; $\log_2$FC = 0.44 vs. 5.556e–6, $p < 0.0001$ for miR-23b-3p) (Fig. 4a and b).

In contrast, miR-212-3p showed the opposite trend. When comparing the two groups, a significant downregulation was observed in the AN group ($\log_2$FC = – 0.30 vs. – 3.12e–6, $p < 0.047$) (Fig. 4c).

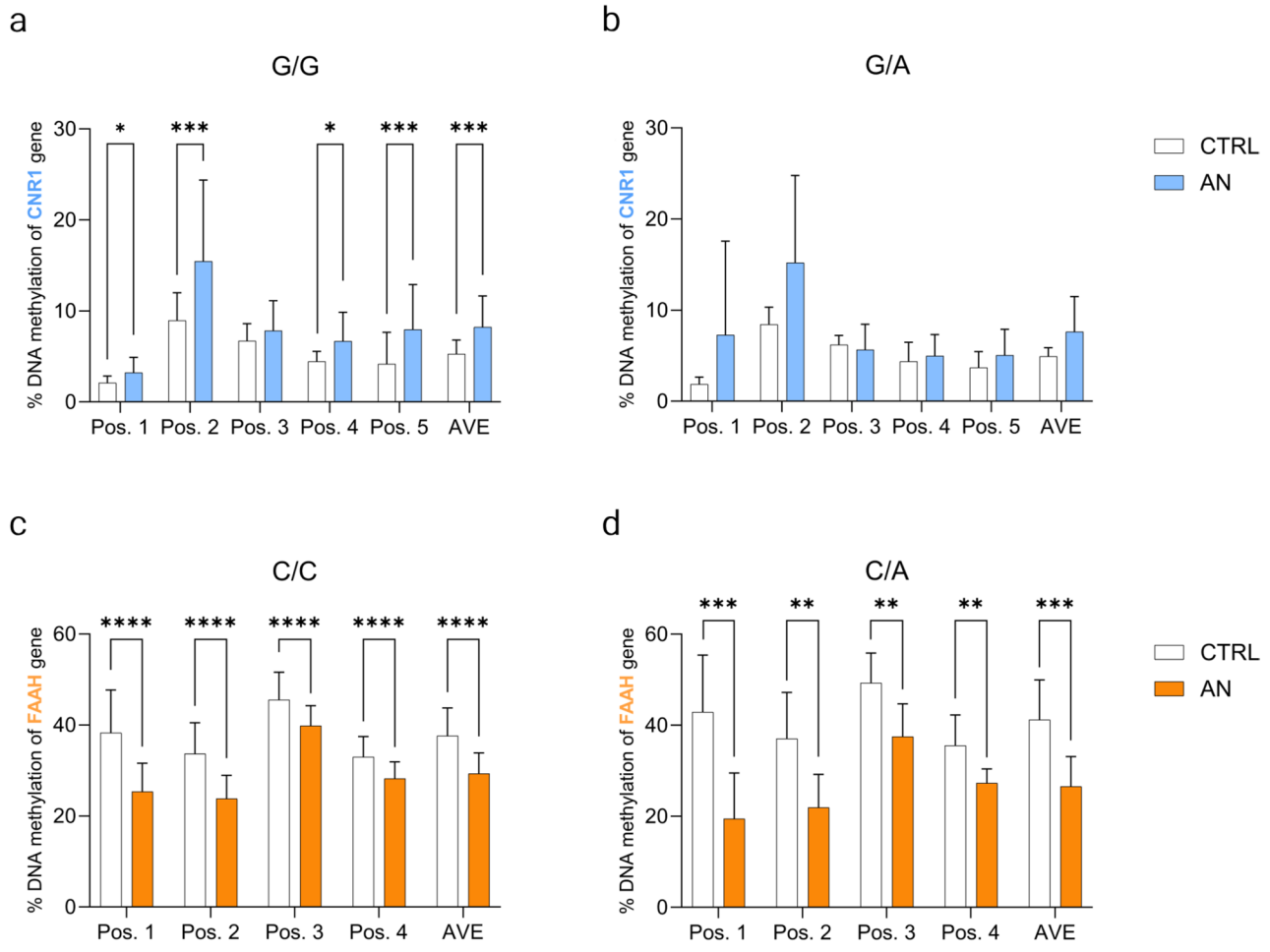

Fig. 3 **a**, **b** *CNR1* methylation by rs1049353 genotype in CTRL and AN patients. Mean *CNR1* methylation levels (± SD) at five CpG sites and their AVE, comparing CTRL and AN groups within rs1049353 G/G (**a**) and G/A (**b**) genotype carriers. Asterisks indicate statistical significance (Mann–Whitney test, *$p < 0.05$, ***$p < 0.001$). **c**, **d** *FAAH* methylation by rs324420 genotype in CTRL and AN patients. Mean *FAAH* methylation levels (± SD) at four CpG sites and their AVE, comparing CTRL and AN groups within s324420 C/C (**c**) and C/A (**d**) genotype carriers. Asterisks indicate statistical significance (Mann–Whitney test, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). *AN* anorexia nervosa, *AVE* average, *CNR1* cannabinoid receptor 1, *CTRL* control, *FAAH* fatty acid amide hydrolase

Among the five selected miRNAs predicted to target *FAAH*, only miR-4505 and miR-1275 were found to be differentially expressed. In both cases, AN patients showed increased expression levels compared to the total control group ($log_2FC = 0.85$ vs. – 6.06e–7, $p < 0.0001$ for miR-4505 and $log_2FC = 1.04$ vs. 3.23e–7, $p < 0.0001$ for miR-1275) (Fig. 4d and e).

Correlation between DNA methylation and MiRNA expression

We observed variable correlations between *CNR1* methylation and miR-342-3p expression, with Spearman's r ranging from 0.09 to 0.27 and non-significant p-values ($p > 0.1$), except for CpG site 5, which showed a significant positive correlation ($r = 0.27$, $p = 0.03$). A similar pattern emerged for miR-23b-3p, with r values ranging from 0.12 to 0.39 and significant positive correlations at CpG sites 2, 4 and 5 and in the average methylation level ($p < 0.05$), suggesting that increased methylation is associated with higher miRNA expression. In contrast, miR-212-3p showed weak negative correlations with *CNR1* methylation (r ranging from – 0.22 to – 0.04), none statistically significant ($p > 0.07$), indicating a possible inverse relationship that requires further investigation (Fig. 5a).

The correlation between *FAAH* methylation and its targeting miR-4505 and miR-1275 expression was consistently negative (r = – 0.42 to – 0.52) and statistically significant ($p < 0.001$) across all sites, suggesting that increased *FAAH* methylation is associated with reduced miRNA expression (Fig. 5b).

TF binding site (TFBS) analysis

This analysis identified a total of three TFs with potential binding sites overlapping CpG dinucleotides, two for *CNR1* gene—BARHL1 and ZIC5—and one for *FAAH* gene—GMC1. These candidates showed a positional

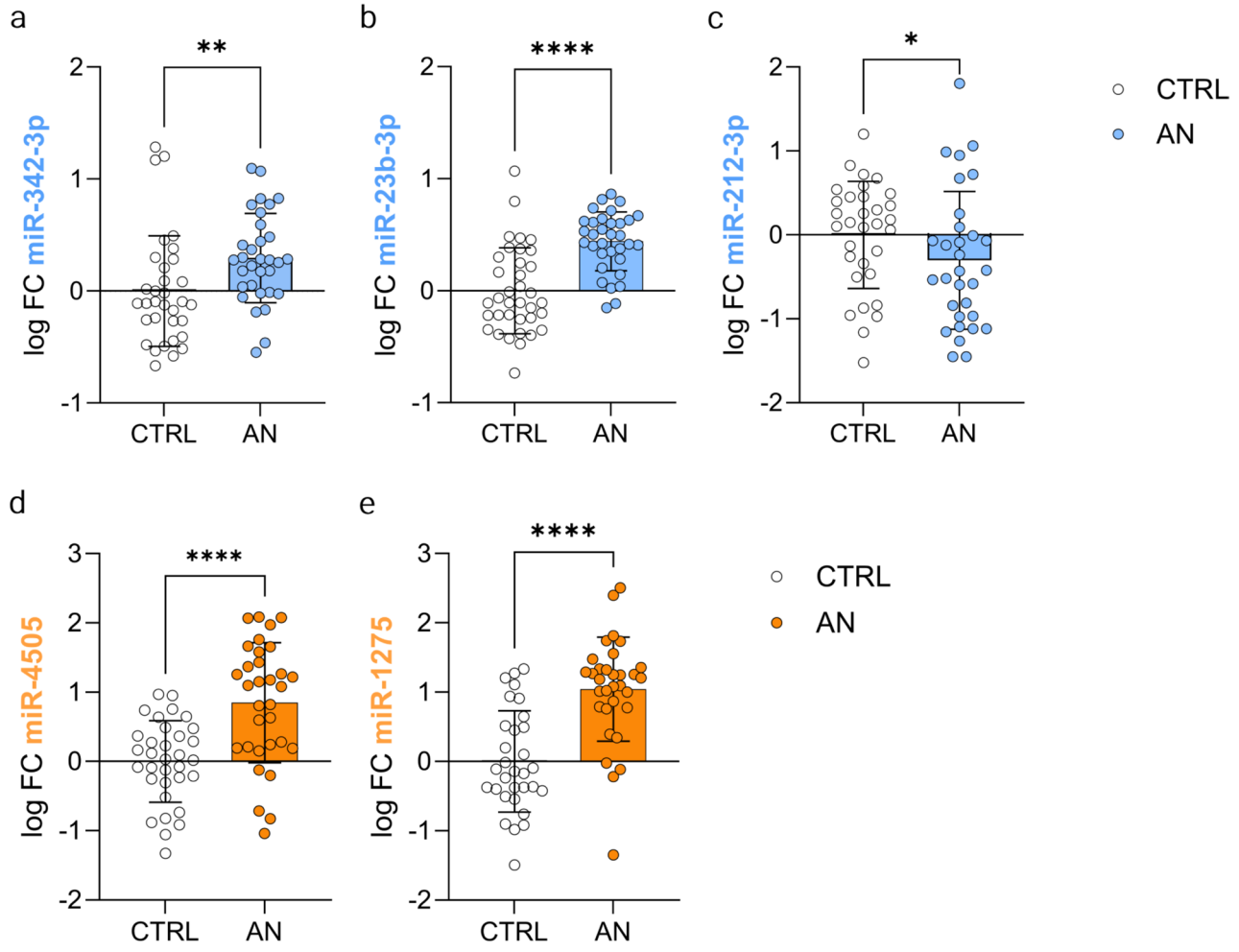

Fig. 4 *CNR1 and FAAH* targeting miRNA expression in CTRL and AN patients. Log2 fold change values for *miR-342-3p* (**a**), *miR-23b-3p* (**b**), *miR-212-3p* (**c**) (binding *CNR1*) and miR-4505 (**d**), miR-1275 (**e**) (binding *FAAH*). Data are presented as individual values with box plots showing distribution. Asterisks indicate statistical significance (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$). *AN* Anorexia Nervosa, *CNR1* Cannabinoid Receptor 1, *CTRL* control, *FAAH* fatty acid amide hydrolase

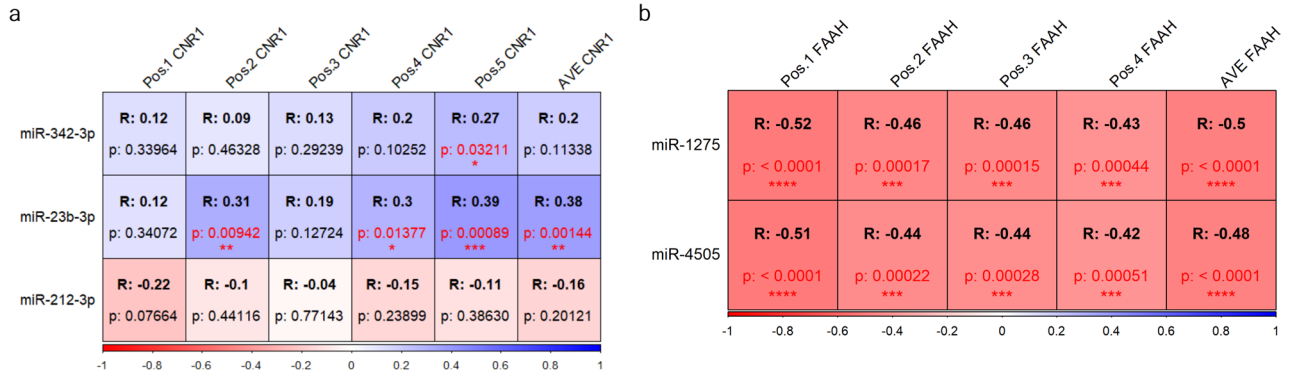

Fig. 5 **a** Correlation heatmap between *CNR1*-targeting miRNAs and *CNR1* methylation levels. Spearman correlation coefficients (R) and p-values are shown for each pairwise comparison. Blue indicates positive correlations; red indicates negative correlations. Asterisks denote statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). **b** Correlation heatmap between *FAAH*-targeting miRNAs and *FAAH* methylation levels. Spearman correlation coefficients (R) and p-values are shown. Red indicates negative correlations. Asterisks denote statistical significance (***$p < 0.001$, ****$p < 0.0001$). *CNR1* cannabinoid receptor 1, *FAAH* fatty acid amide hydrolase

compatibility with the CpG sites characterized in our methylation analysis (detailed information on the sequence analyzed is provided in Table S1). These TFs represent the most compelling candidates for methylation-dependent regulation at the CpG sites analyzed in this study. Detailed results on TFBS analysis are reported in Table S4.

Subgroup analyses

To examine whether our findings were influenced by clinical characteristics, we performed stratification analyses based on BMI, illness duration, medication use, and presence of purging behaviors. No significant differences were observed between AN patients and controls across any of these subgroups (all $p > 0.05$), indicating that our results are robust across different clinical presentations (Table S5).

Discussion

The present study aimed to provide evidence for the involvement of the ECS in AN pathophysiology and highlight *CNR1* and *FAAH* as potential biomarkers for this challenging psychiatric disorder.

To address existing knowledge gaps, we conducted an integrated analysis of DNA methylation, genetic variants, TFs and exosomal miRNA profiles in AN patients compared to healthy controls.

DNA methylation analysis revealed distinct patterns in *CNR1* and *FAAH* genes. AN patients showed significant hypermethylation across all CpG sites in the *CNR1* promoter, likely reducing CB1 receptor expression, as demonstrated in previous studies where *Cnr1* promoter methylation negatively correlated with the CB receptor expression levels [41]. In contrast, *FAAH* promoter region was consistently hypomethylated in AN group. Based on established epigenetic principles, hypomethylation typically is associated with increased gene expression [54], which, in this case, potentially enhance anandamide degradation.

These opposite methylation patterns result in convergent functional impairment: reduced CB1 receptor expression (via *CNR1* hypermethylation) and increased ligand degradation (via *FAAH* hypomethylation), indicating overall reduction in endocannabinoid tone. This dual-target approach provides mechanistic insight into how the biological system systematically compromises ECS function by simultaneously targeting receptor availability and endocannabinoid degradation.

Targeting single components might allow compensatory responses maintaining residual ECS function. However, simultaneous dysregulation of both components creates effective pathway-wide impairment. This suggests AN involves sophisticated regulatory orchestration rather than random molecular disruptions.

This mechanistic framework helps contextualize the complex and often contradictory findings regarding ECS function in AN. Prior evidence on CB1 receptor and AEA/2-AG levels in AN, have been limited and inconsistent so far [20, 43, 55–57]. Some studies suggest elevated AEA and CB1 receptor upregulation as adaptive responses to promote hunger and energy balance [20, 31, 32]. Gérard and colleagues explained the increased CB1 receptor availability in the insular, frontal, and temporal brain regions of AN patients as a functional compensatory response to the underactive ECS [56], partially supported by PET imaging in ABA rats [29]. Others associate receptor downregulation with greater ED severity and impulsivity [20, 55]. Lastly, some studies report ECS normalization after weight restoration [29], while others show persistent dysregulation [31, 33]. These discrepancies may reflect complex CB1 receptor and ligand regulation in eating disorders, where the epigenetic up- or downregulation could be explained by ED timing or severity, or could eventually coexist according to tissue-specific differences or specific functional connectivity circuits [27].

Having identified coordinated methylation changes, we next examined the potential functional consequences of these epigenetic alterations. Since TFs play a pivotal role in regulating gene expression [58] and DNA methylation at CpG sites can alter TF binding affinity [59, 60], we analyzed the differentially methylated *CNR1* and *FAAH* genes regions and identified three predicted TFBS: BarH like homeobox 1 (BARHL1), glial cells missing transcription factor 1 (GCM1) and Zic family member 5 (ZIC5). ZIC5 is involved in central nervous system development [61], while BARHL1 has been reported as methylation-sensitive, exhibiting reduced DNA binding affinity when CpG dinucleotides carry 5-methylcytosine [60].

Hypermethylation in the *CNR1* promoter may block TF binding and repress transcription, while *FAAH* hypomethylation may enhance TF recruitment and gene activation [62]. This suggests reduced CB1 receptor availability and increased endocannabinoid degradation. While our results are correlative, they provide a mechanistic framework that warrants further validation through functional assays.

Our findings also suggest that genetic variation may influence these epigenetic patterns, adding another layer to the coordinated dysregulation. The observed correlation between genetic variants and methylation patterns, with carriers of the *CNR1* rs1049353 risk allele showing higher methylation levels at specific CpG sites, exemplifies the complex interplay between genetics and epigenetics in multifactorial disorders [63]. Genetic studies have identified specific polymorphisms in both *CNR1* and *FAAH*, such as rs1049353 in *CNR1* and rs324420 in *FAAH*, which have been linked to altered ECS function

and an increased predisposition to eating disorders [43]. These polymorphisms may influence mRNA stability, receptor activity, or enzyme efficiency, ultimately affecting ECS signaling in AN. This suggests that genetic predisposition may influence epigenetic responses to environmental factors, creating personalized vulnerability profiles.

To complete our multi-layered analysis of ECS regulation, we investigated miRNA-mediated post-transcriptional control. The exosomal miRNA profiling yielded particularly interesting results that align with emerging literature on post-transcriptional regulation in psychiatric disorders [64–66]. To our knowledge, no prior miRNA sequencing or array studies have been conducted specifically in AN, highlighting the novelty of these findings.

We identified miR-342-3p and miR-23b-3p—both targeting *CNR1* mRNA—as significantly upregulated in AN, while miR-212-3p showed a notable decrease. The miR-342-3p and miR-23b-3p could contribute to the downregulation of CB1 receptor signaling, potentially explaining some of the characteristic symptoms of AN such as hypophagia, anhedonia, and heightened anxiety. Conversely, the reduction in miR-212-3p, a miRNA previously linked to neuroplasticity and stress resilience [67], may reflect a vulnerability to chronic stress and emotional dysregulation in AN, reflecting a loss of regulatory input that normally supports *CNR1* expression, suggesting that both increased inhibitory and decreased supportive miRNA signals converge to suppress the endocannabinoid system in anorexia nervosa.

Regarding *FAAH*-targeting miRNAs, miR-4505 and miR-1275 were upregulated, typically leading to reduced *FAAH* expression, contrasting with observed hypomethylation, which would typically be associated with increased gene expression. This finding presented an apparent paradox but suggests a complex regulatory network where the increased expression of these miRNAs expression may represent a compensatory mechanism attempting to counteract the effects of *FAAH* promoter hypomethylation.

Thus, ECS dysregulation seems to involve competing regulatory forces—primary epigenetic drivers versus secondary miRNA responses—suggesting a dynamic equilibrium rather than unidirectional pathway suppression.

To further explore this dynamic regulatory relationship, we also investigated the relationship between miRNA expression and DNA methylation. At specific *CNR1* CpG sites, increased methylation correlated with higher miR-342-3p and miR-23b-3p expression, suggesting a coordinated regulatory mechanism where epigenetic modifications may influence miRNA expression [68], potentially contributing to the fine-tuning of gene expression in AN patients. However, this was not consistent across all sites, indicating that methylation's impact on miRNA regulation might be site-specific or modulated by additional factors. Interestingly, miR-212-3p exhibited a weak inverse trend with methylation, though not statistically significant. This divergent pattern hints at the complexity of epigenetic and post-transcriptional interactions, suggesting that different miRNAs might respond distinctly to methylation changes, reflecting diverse regulatory roles or feedback mechanisms. Further research is necessary to elucidate these dynamics and their functional consequences in the pathophysiology of AN.

The FAAH system exhibited an opposite regulatory pattern, where the inverse correlation between DNA methylation and miRNA expression adds another layer of complexity. While hypomethylation would typically predict increased gene expression, the concurrent reduction in its targeting miRNAs could represent a compensatory response aimed at maintaining homeostasis. Such opposing regulatory influences might reflect the biological system's attempt to balance FAAH activity through multiple levels of control, highlighting the intricate crosstalk between epigenetic and post-transcriptional mechanisms. This apparent paradox aligns with observations in other complex disorders, where competing regulatory pathways often coexist, underscoring the need for integrated approaches to fully understand gene regulation in psychiatric conditions [69].

These results not only point to a previously unexplored epigenetic modulation of the ECS in AN, but also suggest that specific miRNAs could serve as biomarkers or therapeutic targets in the future management of the disorder [70].

One key finding of our study is the potential of integrated biomarker panels combining methylation status, genetic variants, and miRNA profiles. Such a multi-dimensional approach might provide superior diagnostic accuracy compared to individual markers alone, aligning with the comprehensive biomarker approach proposed for complex psychiatric disorders [71, 72].

Using salivary samples could provide practical advantages for biomarker development in AN. Saliva collection is non-invasive, stress-free, and well-accepted by patients with psychiatric disorders [73], potentially allowing for more frequent monitoring of disease status and treatment response in future clinical applications. This could be particularly relevant in AN, where patients often exhibit high levels of medical distrust and resistance to invasive procedures. Moreover, emerging research suggests that salivary DNA methylation patterns may reflect certain central processes [74], indicating potential for psychiatric biomarker development. However, significant additional research and validation studies are needed before such approaches could be considered for clinical implementation.

Our findings have potential therapeutic implications beyond biomarker development. Studies on ECS dysregulation already suggested that targeting this system might offer novel treatment approaches for AN. Cannabinoid receptor agonists have been showed to stimulate appetite and reduce anxiety-like behaviors in AN models [75], and dronabinol, a synthetic cannabinoid, has yielded promising results in severe cases [76]. Moreover, the identified ECS genetic variants, given their associations with AN susceptibility [42, 43], may potentially predict individual treatment response. Such genotype-guided approaches align with precision psychiatry principles advocated for ED [77]. However, despite these promising observations, the absence of curative ECS-based therapies for AN highlights the need for treatments that address the coordinated dysregulation we identified rather than isolated molecular targets. In fact, given the bidirectional epigenetic changes we observed, therapeutic strategies may be more effective when designed to modulate the entire dysregulated pathway rather than single molecular targets. A strength of our study is the consistency of findings across AN subtypes. Despite differences in clinical presentation and behaviors (including presence or absence of purging), both AN-restrictive and AN-binge/purge patients exhibited similar patterns of ECS dysregulation. This suggests that our salivary biomarkers reflect core AN pathophysiology rather than being confounded by subtype-specific factors such as purging behaviors that might alter salivary composition.

Limitations

While these insights are promising, limitations must be acknowledged. The cross-sectional nature of our primary analysis limits conclusions about causality—whether ECS alterations drive AN or result from malnutrition and weight loss is unclear, as noted in biomarker research [78]. Future longitudinal studies tracking patients across different illness phases would help clarify whether the observed epigenetic alterations represent state- or trait-markers of AN. Furthermore, while saliva offers practical advantages, the link between peripheral and central epigenetic markers requires further investigation [79]. Moreover, several AN-specific factors may influence salivary sample quality and composition. For instance, purging behaviors can alter salivary pH and composition or medications taken by some patients may affect saliva production and characteristics. However, the consistency of findings across AN subtype and across multiple molecular measures (DNA methylation, miRNAs), as well as the significant correlations between them, suggest that our results reflect biological signals rather than technical artifacts related to sample quality. Future studies should include systematic assessment of salivary pH, flow rate, and composition parameters to better understand their potential confounding effects. Another limitation is the absence of direct *CNR1* and *FAAH* mRNA expression measurements. Even if both genes are expressed in salivary leukocytes, we prioritized exosomal miRNA analysis due to the greater technical challenges and lower reproducibility associated with cellular mRNA extraction from saliva, where RNase activity and variable sample quality can compromise mRNA integrity.

Conclusions

In conclusion, our findings establish bidirectional epigenetic dysregulation as a fundamental mechanism underlying ECS dysfunction in AN, while also supporting the potential of *CNR1* and *FAAH* as biomarkers. The integrated analysis of DNA methylation, genetic variants, transcription factors, and exosomal miRNA profiles provides a comprehensive approach to understanding ECS dysregulation in AN and developing clinically useful biomarkers. This mechanistic framework transforms biomarker identification from descriptive observation to functional understanding, revealing how coordinated molecular changes systematically impair appetite-regulating pathways.

Our work demonstrates that multi-modal molecular analysis can generate genuine mechanistic insights that inform therapeutic strategy development. Future research should focus on validating these markers in larger, longitudinal cohorts, establishing normative ranges, and developing standardized assays for clinical implementation. Additionally, translating the coordinated dysregulation concept into targeted therapeutic interventions represents a critical next step toward mechanistically-informed precision medicine for AN.

This work represents an important step toward objective biological markers for AN, potentially improving diagnosis, treatment monitoring, and personalized intervention strategies for this challenging disorder.

Understanding the relationship between peripheral and central epigenetic signatures is essential before any clinical application can be considered, and our findings should be viewed as a preliminary step toward clarifying the potential utility of salivary biomarkers in AN research.

Abbreviations

2-AG	2-Arachidonoylglycerol
AEA	Anandamide
AN	Anorexia nervosa
AVE	Average
BARHL1	BarH like homeobox 1
CB1/CNR1	Cannabinoid receptor 1
CTRL	Controls
ECS	Endocannabinoid system
ED	Eating disorders
FAAH	Fatty acid amide hydrolase
GCM1	Glial cells missing transcription factor 1
miRNA	microRNA

PFMs	Position frequency matrices
SNP	Single nucleotide polymorphisms
TFs	Transcription factors
TFBS	Transcription factor binding sites
ZIC5	Zic family member 5

Supplementary Information

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Supplementary Material 1

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Author contributions

CD conceived and designed the research; FG, FM, MP, CSG and MR performed the research and acquired the data; FG and AS analysed and interpreted the data; CC supervised the project. All authors were involved in drafting and revising the manuscript.

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Data availability

The datasets and code generated and/or analysed during the current study are available in the Zenodo repository, [https://doi.org/10.5281/zenodo.16965204](https:/doi.org/10.5281/zenodo.16965204).

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of "Regione Calabria, sezione Area Centro" (identifier: 395/18.11.2021) and participants were treated in accordance with the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study and from legal guardians of participants who were minors.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Fichter MM, Quadflieg N, Crosby RD, Koch S. Long-term outcome of anorexia nervosa: results from a large clinical longitudinal study. Int J Eat Disord. 2017;50(9):1018–30.
2. Arcelus J, Mitchell AJ, Wales J, Nielsen S. Mortality rates in patients with anorexia nervosa and other eating disorders: A Meta-analysis of 36 studies. Arch Gen Psychiatry. 2011;68(7):724–31.
3. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. American Psychiatric Publishing; 2013.
4. Galmiche M, Déchelotte P, Lambert G, Tavolacci MP. Prevalence of eating disorders over the 2000–2018 period: a systematic literature review. Am J Clin Nutr. 2019;109(5):1402–13.
5. Watson HJ, Yilmaz Z, Thornton LM, Hübel C, Coleman JRI, Gaspar HA, et al. Genome-wide association study identifies eight risk loci and implicates metabo-psychiatric origins for anorexia nervosa. Nat Genet. 2019;51(8):1207–14.
6. Keski-Rahkonen A, Mustelin L. Epidemiology of eating disorders in Europe: prevalence, incidence, comorbidity, course, consequences, and risk factors. Curr Opin Psychiatry. 2016;29(6):340.
7. Paolacci S, Kiani AK, Manara E, Beccari T, Ceccarini MR, Stuppia L, et al. Genetic contributions to the etiology of anorexia nervosa: new perspectives in molecular diagnosis and treatment. Molec Gen Gen Med. 2020;8(7):e1244.
8. Zipfel S, Giel KE, Bulik CM, Hay P, Schmidt U. Anorexia nervosa: aetiology, assessment, and treatment. Lancet Psychiatry. 2015;2(12):1099–111.
9. Clarke TK, Weiss ARD, Berrettini WH. The genetics of anorexia nervosa. Clin Pharmacol Ther. 2012;91(2):181–8.
10. Higgins A. The neurobiology of anorexia nervosa. In: Anorexia and bulimia nervosa. IntechOpen; 2018. https://www.intechopen.com/chapters/64858. Accessed 4 Feb 2025.
11. Kaye WH, Frank GK, Bailer UF, Henry SE. Neurobiology of anorexia nervosa: clinical implications of alterations of the function of serotonin and other neuronal systems. Int J Eat Disord. 2005;37(S1):S15–9.
12. Pjetri E, Schmidt U, Kas MJ, Campbell IC. Epigenetics and eating disorders. Curr Opin Clin Nutr Metabolic Care. 2012;15(4):330.
13. Kaye WH, Klump KL, Frank GKW, Strober M. Anorexia and bulimia nervosa. Annu Rev Med. 2000;51:299–313.
14. Starzomska M, Tadeusiewicz R. Pitfalls in anorexia nervosa research: the risk of artifacts linked to denial of illness and methods of preventing them. Psychiatr Danub. 2016;28(3):202–10.
15. Couturier JL, Lock J. Denial and minimization in adolescents with anorexia nervosa. Int J Eat Disord. 2006;39(3):212–6.
16. Malcolm A, Phillipou A. Current directions in biomarkers and endophenotypes for anorexia nervosa: A scoping review. J Psychiatr Res. 2021;137:303–10.
17. Dalton B, Campbell IC, Chung R, Breen G, Schmidt U, Himmerich H. Inflammatory markers in anorexia nervosa: an exploratory study. Nutrients. 2018;10(11):1573.
18. Lopez RB, Cruz-Vespa I. The brain bases of regulation of eating behaviors: the role of reward, executive control, and valuation processes, and new paths to propel the field forward. Curr Opin Behav Sci. 2022;48:101214.
19. Berner LA, Brown TA, Lavender JM, Lopez E, Wierenga CE, Kaye WH. Neuroendocrinology of reward in anorexia nervosa and bulimia nervosa: beyond leptin and Ghrelin. Mol Cell Endocrinol. 2019;497:110320.
20. Frieling H, Albrecht H, Jedtberg S, Gozner A, Lenz B, Wilhelm J, et al. Elevated cannabinoid 1 receptor mRNA is linked to eating disorder related behavior and attitudes in females with eating disorders. Psychoneuroendocrinology. 2009;34(4):620–4.
21. Lu HC, Mackie K. Review of the endocannabinoid system. Biol Psychiatry: Cogn Neurosci Neuroimaging. 2021;6(6):607–15.
22. Maccarrone M, Gasperi V, Catani MV, Diep TA, Dainese E, Hansen HS, et al. The endocannabinoid system and its relevance for nutrition. Annu Rev Nutr. 2010;30:423–40.
23. Di Marzo V, Matias I. Endocannabinoid control of food intake and energy balance. Nat Neurosci. 2005;8(5):585–9.

24. Kurtov M, Rubinić I, Likić R. The endocannabinoid system in appetite regulation and treatment of obesity. Pharmacol Res Perspect. 2024;12(5):e70009.
25. Marco EM, García-Gutiérrez MS, Bermúdez-Silva FJ, Moreira FA, Guimarães F, Manzanares J, et al. Endocannabinoid system and psychiatry: in search of a Neurobiological basis for detrimental and potential therapeutic effects. Front Behav Neurosci. 2011;5:63.
26. Titova OE, Hjorth OC, Schiöth HB, Brooks SJ. Anorexia nervosa is linked to reduced brain structure in reward and somatosensory regions: a meta-analysis of VBM studies. BMC Psychiatry. 2013;13:110.
27. Miranda-Olivos R, Baenas I, Steward T, Granero R, Pastor A, Sánchez I, et al. Exploring the influence of Circulating endocannabinoids and nucleus accumbens functional connectivity on anorexia nervosa severity. Mol Psychiatry. 2023;28(11):4793–800.
28. Monteleone P, Maj M. Dysfunctions of leptin, ghrelin, BDNF and endocannabinoids in eating disorders: beyond the homeostatic control of food intake. Psychoneuroendocrinology. 2013;38(3):312–30.
29. Casteels C, Gérard N, van Kuyck K, Pottel L, Nuttin B, Bormans G, et al. Small animal PET imaging of the type 1 cannabinoid receptor in a rodent model for anorexia nervosa. Eur J Nucl Med Mol Imaging. 2014;41(2):308–21.
30. Monteleone P, Piscitelli F, Scognamiglio P, Monteleone AM, Canestrelli B, Di Marzo V, et al. Hedonic eating is associated with increased peripheral levels of Ghrelin and the endocannabinoid 2-Arachidonoyl-Glycerol in healthy humans: A pilot study. J Clin Endocrinol Metabolism. 2012;97(6):E917–24.
31. Tam FI, Steding J, Steinhäuser JL, Ritschel F, Gao W, Weidner K, et al. Hair endocannabinoid concentrations in individuals with acute and weight-recovered anorexia nervosa. Prog Neuropsychopharmacol Biol Psychiatry. 2021;107:110243.
32. Monteleone P, Matias I, Martiadis V, De Petrocellis L, Maj M, Di Marzo V. Blood levels of the endocannabinoid Anandamide are increased in anorexia nervosa and in Binge-Eating Disorder, but not in bulimia nervosa. Neuropsychopharmacol. 2005;30(6):1216–21.
33. Piccolo M, Claussen MC, Bluemel S, Schumacher S, Cronin A, Fried M, et al. Altered Circulating endocannabinoids in anorexia nervosa during acute and weight-restored phases: A pilot study. Eur Eat Disorders Rev. 2020;28(1):46–54.
34. Collu R, Scherma M, Piscitelli F, Giunti E, Satta V, Castelli MP, et al. Impaired brain endocannabinoid tone in the activity-based model of anorexia nervosa. Int J Eat Disord. 2019;52(11):1251–62.
35. Perera BPU, Silvestre F. Environ Epigenomes Epigenomes. 2023;7(3):21.
36. Riggs AD, Porter T. Overview of epigenetic mechanisms. Epigenetic mechanisms of gene regulation. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press; 1996. pp. 29–45.
37. Lande-Diner L, Zhang J, Ben-Porath I, Amariglio N, Keshet I, Hecht M, et al. Role of DNA methylation in stable gene repression. J Biol Chem. 2007;282(16):12194–200.
38. Rask-Andersen M, Olszewski PK, Levine AS, Schiöth HB. Molecular mechanisms underlying anorexia nervosa: focus on human gene association studies and systems controlling food intake. Brain Res Rev. 2010;62(2):147–64.
39. Steiger H, Thaler L. Eating disorders, gene-environment interactions and the epigenome: roles of stress exposures and nutritional status. Physiol Behav. 2016;162:181–5.
40. D'Addario C, Di Francesco A, Pucci M, Finazzi Agrò A, Maccarrone M. Epigenetic mechanisms and endocannabinoid signalling. FEBS J. 2013;280(9):1905–17.
41. D'Addario C, Zaplatic E, Giunti E, Pucci M, Di Micioni MV, Scherma M, et al. Epigenetic regulation of the cannabinoid receptor CB1 in an activity-based rat model of anorexia nervosa. Int J Eat Disord. 2020;53(5):702–16.
42. Ando T, Tamura N, Mera T, Morita C, Takei M, Nakamoto C, et al. Association of the c.385C > A (p.Pro129Thr) polymorphism of the fatty acid amide hydrolase gene with anorexia nervosa in the Japanese population. Mol Genet Genomic Med. 2014;2(4):313–8.
43. Monteleone P, Bifulco M, Di Filippo C, Gazzerro P, Canestrelli B, Monteleone F, et al. Association of CNR1 and FAAH endocannabinoid gene polymorphisms with anorexia nervosa and bulimia nervosa: evidence for synergistic effects. Genes Brain Behav. 2009;8(7):728–32.
44. First MB, Williams JBW, Karg RS, Spitzer RL. Structured clinical interview for DMS-5 disorders—clinician version. American Psychiatric Association; 2016.
45. Calugi S, Ricca V, Castellini G, Lo Sauro C, Ruocco A, Chignola E, et al. The eating disorder examination: reliability and validity of the Italian version. Eat Weight Disord. 2015;20(4):505–11.
46. WHO Consultation on Obesity. (1999: Geneva S, Organization WH. Obesity : preventing and managing the global epidemic : report of a WHO consultation. World Health Organization; 2000. https://iris.who.int/handle/10665/42330. Accessed 15 Jul 2025.
47. World Medical Association. World medical association declaration of helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191–4.
48. Garbieri TF, Brozoski DT, Dionísio TJ, Santos CF, Neves LT das. Human DNA extraction from whole saliva that was fresh or stored for 3, 6 or 12 months using five different protocols. J Appl Oral Sci. 2017;25:147–58.
49. Chen Y, Wang X. MiRDB: an online database for prediction of functional MicroRNA targets. Nucleic Acids Res. 2020;48(D1):D127–31.
50. Sticht C, De La Torre C, Parveen A, Gretz N. miRWalk: An online resource for prediction of microRNA binding sites. PLoS One. 2018;13(10):e0206239.
51. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. eLife. 2015;4:e05005.
52. Cui C, Zhong B, Fan R, Cui Q. HMDD v4.0: a database for experimentally supported human microRNA-disease associations. Nucleic Acids Res. 2024;52(D1):D1327–32.
53. Rauluseviciute I, Riudavets-Puig R, Blanc-Mathieu R, Castro-Mondragon JA, Ferenc K, Kumar V, et al. JASPAR 2024: 20th anniversary of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 2024;52(D1):D174–82.
54. Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacol. 2013;38(1):23–38.
55. Schroeder M, Eberlein C, de Zwaan M, Kornhuber J, Bleich S, Frieling H. Lower levels of cannabinoid 1 receptor mRNA in female eating disorder patients: association with wrist cutting as impulsive self-injurious behavior. Psychoneuroendocrinology. 2012;37(12):2032–6.
56. Gérard N, Pieters G, Goffin K, Bormans G, Van Laere K. Brain type 1 cannabinoid receptor availability in patients with anorexia and bulimia nervosa. Biol Psychiatry. 2011;70(8):777–84.
57. Müller TD, Reichwald K, Brönner G, Kirschner J, Nguyen TT, Scherag A, et al. Lack of association of genetic variants in genes of the endocannabinoid system with anorexia nervosa. Child Adolesc Psychiatry Ment Health. 2008;2:33.
58. Islam Z, Ali AM, Naik A, Eldaw M, Decock J, Kolatkar PR. Transcription factors: the fulcrum between cell development and carcinogenesis. Front Oncol. 2021. https://doi.org/10.3389/fonc.2021.681377/full.
59. Sabatucci A, Berchet V, Bellia F, Maccarrone M, Dainese E, D'Addario C, et al. A new methodological approach for in vitro determination of the role of DNA methylation on transcription factor binding using AlphaScreen® analysis: focus on CREB1 binding at hBDNF promoter IV. J Neurosci Methods. 2020;341:108720.
60. Yin Y, Morgunova E, Jolma A, Kaasinen E, Sahu B, Khund-Sayeed S, et al. Impact of cytosine methylation on DNA binding specificities of human transcription factors. Science. 2017;356(6337):eaaj2239.
61. Aruga J. The role of Zic genes in neural development. Mol Cell Neurosci. 2004;26(2):205–21.
62. Domcke S, Bardet AF, Adrian Ginno P, Hartl D, Burger L, Schübeler D. Competition between DNA methylation and transcription factors determines binding of NRF1. Nature. 2015;528(7583):575–9.
63. Fries GR. Genetics and epigenetics as tools to inform the pathophysiology of neuropsychiatric disorders. Braz J Psychiatry. 2019;41(1):5–6.
64. Lim M, Carollo A, Neoh MJY, Esposito G. Mapping MiRNA research in schizophrenia: A scientometric review. Int J Mol Sci. 2022;24(1):436.
65. Li LD, Naveed M, Du ZW, Ding H, Gu K, Wei LL, et al. Abnormal expression profile of plasma-derived Exosomal MicroRNAs in patients with treatment-resistant depression. Hum Genomics. 2021;15(1):55.
66. Fries GR, Zhang W, Benevenuto D, Quevedo J. MicroRNAs in major depressive disorder. In: Guest PC, editor. Reviews on biomarker studies in psychiatric and neurodegenerative disorders. Cham: Springer International Publishing; 2019. pp. 175–90. https://doi.org/10.1007/978-3-030-05542-4_9.
67. Aten S, Page CE, Kalidindi A, Wheaton K, Niraula A, Godbout JP, et al. miR-132/212 is induced by stress and its dysregulation triggers anxiety-related behavior. Neuropharmacology. 2019;144:256–70.
68. Chhabra R. MiRNA and methylation: a multifaceted liaison. ChemBioChem. 2015;16(2):195–203.
69. Sathyanarayanan A, Mueller TT, Ali Moni M, Schueler K, Baune BT, Lio P, et al. Multi-omics data integration methods and their applications in psychiatric disorders. Eur Neuropsychopharmacol. 2023;69:26–46.
70. Roy B, Yoshino Y, Allen L, Prall K, Schell G, Dwivedi Y. Exploiting Circulating MiRNAs as biomarkers in psychiatric disorders. Mol Diagn Ther. 2020;24(3):279–98.

71. Glannon W. Biomarkers in psychiatric disorders. Camb Q Healthc Ethics. 2022;31(4):444–52.
72. Liang J, Li ZW, Yue CT, Hu Z, Cheng H, Liu ZX, et al. Multi-modal optimization to identify personalized biomarkers for disease prediction of individual patients with cancer. Brief Bioinform. 2022;23(5):bbac254.
73. Woźniak M, Paluszkiewicz C, Kwiatek WM. Saliva as a non-invasive material for early diagnosis. Acta Biochim Pol. 2019;66(4):383–8.
74. Langie SAS, Moisse M, Declerck K, Koppen G, Godderis L, Vanden Berghe W, et al. Salivary DNA methylation profiling: aspects to consider for biomarker identification. Basic Clin Pharmacol Toxicol. 2017;121(S3):93–101. S3.
75. Scherma M, Satta V, Collu R, Boi MF, Usai P, Fratta W, et al. Cannabinoid CB1/CB2 receptor agonists attenuate hyperactivity and body weight loss in a rat model of activity-based anorexia. Br J Pharmacol. 2017;174(16):2682–95.
76. Andries A, Frystyk J, Flyvbjerg A, Støving RK. Dronabinol in severe, enduring anorexia nervosa: A randomized controlled trial. Int J Eat Disord. 2014;47(1):18–23.
77. Kan C, Cardi V, Stahl D, Treasure J. Precision psychiatry—What it means for eating disorders? Eur Eat Disorders Rev. 2019;27(1):3–7.
78. Käver L, Hinney A, Rajcsanyi LS, Maier HB, Frieling H, Steiger H, et al. Epigenetic alterations in patients with anorexia nervosa—a systematic review. Mol Psychiatry. 2024;29(12):3900–14.
79. Yang H, Sun W, Li J, Zhang X. Epigenetics factors in schizophrenia: future directions for etiologic and therapeutic study approaches. Ann Gen Psychiatry. 2025;24(1):21.

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