# Epigenetics in eating disorders: a systematic review

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## Abstract

Eating disorders are complex heritable conditions influenced by both genetic and environmental factors. Given the progress of genomic discovery in anorexia nervosa, with the identification of the first genome-wide significant locus, as well as animated discussion of epigenetic mechanisms in linking environmental factors with disease onset, our goal was to conduct a systematic review of the current body of evidence on epigenetic factors in eating disorders to inform future directions in this area. Following PRISMA guidelines, two independent authors conducted a search within PubMed and Web of Science and identified 18 journal articles and conference abstracts addressing anorexia nervosa (n = 13), bulimia nervosa (n = 6), and binge-eating disorder (n = 1), published between January 2003 and October 2017. We reviewed all articles and included a critical discussion of field-specific methodological considerations. The majority of epigenetic analyses of eating disorders investigated methylation at candidate genes (n = 13), focusing on anorexia and bulimia nervosa in very small samples with considerable sample overlap across published studies. Three studies used microarray-based technologies to examine DNA methylation across the genome of anorexia nervosa and binge-eating disorder patients. Overall, results were inconclusive and were primarily exploratory in nature. The field of epigenetics in eating disorders remains in its infancy. We encourage the scientific community to apply methodologically sound approaches

using genome-wide designs including epigenome-wide association studies (EWAS), to increase sample sizes, and to broaden the focus to include all eating disorder types.

# 1 Introduction

Eating disorders are serious illnesses associated with significantly reduced health-related quality of life.[1,2] Our current understanding of their etiology is piecemeal and the evidence base for their treatment, especially anorexia nervosa (AN) in adults, is inadequate.[3] Over the past two decades, family, twin, and adoption studies have robustly shown that eating disorders reflect the pattern of complex trait inheritance being influenced by both genetic and environmental factors. Twin-based heritabilities for AN range from 48% to 74%, for bulimia nervosa (BN) from 55% to 62%, and for binge-eating disorder (BED) from 39% to 45%.[4] A genome-wide association study of AN has yielded the first genome-wide significant locus on chromosome 12—a chromosomal region previously associated with autoimmune diseases including type 1 diabetes.[5] AN, furthermore, shows significant genetic correlations with various psychiatric, personality, and metabolic phenotypes, including schizophrenia, neuroticism, glucose metabolism, and lipid metabolism. This panel of findings has encouraged a reconceptualization of AN as both a metabolic and psychiatric disorder.[6] At the same time, epigenetic mechanisms have garnered much interest, offering an added layer of gene regulatory information, which could link external and internal environmental stimuli as well as non-coding genetic variation with transcriptional consequences, altering downstream phenotypes.[4,7–9] Together with enhanced understanding of the genetic variants underlying heritable disease risk in eating disorders, epigenetics has the potential to aid in disentangling the molecular genetic pathways that contribute to the development and progression of the illnesses.

## 1.1 Epigenetics

In the context of this review, epigenetics refers to various biochemical mechanisms giving rise to changes in gene regulation, which are either heritable or characterized by long-term stability.[10] Biologically, epigenetic mechanisms can be categorized into three groups: DNA modifications, histone modifications, and non-coding RNA (for details, see Figure 1). DNA modifications are chemical modifications that bind to the DNA itself. Histone proteins constitute the cores around which DNA is wrapped in the cell nucleus. They can exert an effect on gene regulation by altering the accessibility of DNA sequences.[10] Finally, non-coding RNAs—expressed transcripts which do not code for proteins—have widespread effects on gene regulation via mechanisms including post-transcriptional silencing[11,12] or chromatin remodeling.[13]

DNA methylation is the most widely studied epigenetic mechanism in the context of complex traits thus far and disease-associated methylomic dysregulation has been reported for a number of psychiatric disorders, including schizophrenia,[14,15] Alzheimer's disease, [16,17] and autism spectrum disorder.[18,19] In addition to DNA methylation, its oxidized derivatives constitute further DNA modifications, with DNA hydroxymethylation generating increasing interest in the context of neuropsychiatric disease, due to its enrichment in the human brain.[20,21] While historically defined as occurring independent of the DNA

sequence, recent work has provided evidence for widespread effects of genetic variants on epigenetic states. In particular, methylation quantitative trait loci (mQTLs) are increasingly being characterized: single nucleotide polymorphisms (SNPs) that exert influence on the methylation state of a CpG site, usually in close vicinity to the SNP.[22,23]

Unlike the genome sequence, epigenetic marks are dynamic and can vary across cell- and tissue-types, age and development, and are subject to environmental stimuli including medication and stress (Figure 2). Perhaps most strikingly, this has been shown for tobacco smoking, which was found to have considerable effects on DNA methylation across several genomic regions.[24] Similarly, epigenetic profiles are highly correlated with chronological age and an accurate predictor of age has been derived based on the DNA methylation profiles of only around 300 CpG sites.[25] In this sense they are more accurately characterized as intermediate biological phenotypes and are susceptible to confounding and other problems faced in traditional observational studies. This phenotypic feature of epigenetic profiles means that sources of variation or confounding need to be taken into account in the experimental design and statistical analyses.[26,27] For example, if all individuals in the control group are older than the affected individuals, an epigenome-wide association study (EWAS) may detect epigenetic differences between the two groups related to ageing, rather than differences associated with disease status.

Several characteristics of eating disorders support investigation into the potential contribution of epigenetic factors, including sex differences (i.e., females are ~8 times more likely to suffer from AN or BN than males),[28] periods of increased risk of onset (i.e., particularly in adolescence and young adulthood),[29] and reported discordance between monozygotic twins.[30,31] Eating disorders are associated with early life stress[32] and emerging evidence links early life stress with epigenetic profiles.[33] Empirical evidence confirming this association in humans is limited due to the low availability of brain tissue and the scarcity of large longitudinal studies that collect information on early traumatic experiences and biological samples enabling epigenetic analysis.[34] The largest study of early-life adversity and DNA methylation in blood published to date identified no significant differential methylation.[35] These characteristics suggest that the interaction of genetic risk factors and environmental stressors, can contribute to the onset of eating disorders and make them an excellent target for the examination of epigenetic effects on appetite regulation and eating behavior.

New technical advances in genetic and epigenetic research, including array-based genome-wide analysis methods, have led to rapid accumulation of evidence in the field of psychiatric epigenetics and could serve to expedite understanding of the biology of eating disorders and to identify more efficient treatment options.[36] Therefore, we performed a systematic review including a critical appraisal of the recent body of evidence of epigenetic research in eating disorders to reflect on past research and its limitations and offer guidance for future investigations.

## 2 Method

### 2.1 Search strategy

Our systematic literature review was conducted according to PRISMA guidelines.37 We conducted an exhaustive literature search from 16.10.2017 until the 30.10.2017 using the electronic databases PubMed and Web of Science with a time limitation starting with articles published after 01.01.2003 marking the first published paper on epigenetics of eating disorders. We used following key search terms including (anorexia OR bulimia OR "binge-eating disorder" OR "eating disorder") AND (epigenetics OR methylation OR histone OR "non-coding RNA"). The search was repeated by the co-primary author to avoid selection bias. Furthermore, we screened the references of published articles and reviews. Our search results including the selection process are presented in Figure 3 according to PRISMA guidelines.

### 2.2 Selection criteria

Our inclusion criteria were as follows:

a.  Studies investigating humans only

b.  Any age group

c.  Clinical diagnoses of AN, BN, or BED according to Diagnostic and Statistical Manual of Mental Disorders (DSM) versions IV or 5 and their revisions38 or International Classification of Diseases (ICD)39

d.  Investigation of any type of epigenetic mechanism: methylation, histone modification, non-coding RNAs

e.  Published after 01.01.2003 (date that the first article on epigenetics of eating disorders appears in the literature)

f.  Study includes a control group or comparison group

g.  Publications in any language

### 2.3 Data extraction

We extracted following information from every identified study:

a.  Author

b.  Publication year

c.  Sample including gender and age

d.  Follow-up period

e.  Diagnostic criteria

f.  Participant screening and exclusion criteria

g.  Number of cases (AN, BN, BED)

h.  Number of controls

> **i.**  Matching of cases and controls
>
> **j.**  Outcome variables (Genome-wide methylation level, candidate genes, number of CpG sites)
>
> **k.**  Covariates
>
> **l.**  Tissue
>
> **m.**  Correction for multiple comparison
>
> **n.**  Laboratory methods
>
> **o.**  Limitations

### Quality of evidence assessment (GRADE criteria)

We used the GRADE criteria to assess the quality of evidence of each outcome in our review against eight criteria, including risk of bias, indirectness, inconsistency, imprecision, and publication bias.40 The quality is graded high, moderate, low, or very low and reflects the degree of confidence in the reviewed effects. We assessed the quality of evidence for the three outcomes based on their study design: global methylation level, all candidate genes together, and EWAS associations.

## 3   Results

A total of 178 papers were identified by our search terms. We excluded 67 studies because they did not cover eating disorders, six did not investigate humans, 19 were reviews, one was a book chapter, and three did not examine epigenetic mechanisms. This resulted in 16 published studies and two conference abstracts on epigenetics that met our predetermined inclusion criteria (Figure 3). One full-text article was a duplicate of a conference abstract, resulting in 17 studies. To our knowledge these represent all published studies and conference abstracts investigating epigenetics in eating disorders that were available at the close of our search in October, 2017. We contacted authors of conference abstracts for additional information on their studies (Table 1).

### 3.1   Recent body of evidence

To date, 17 studies on the epigenetics of eating disorders have been published of which four investigated global DNA methylation levels, 13 investigated candidate genes, and three used microarray-based technologies to profile DNA methylation across the human genome. One study design was longitudinal, but within this study one time point was selected and analysed cross-sectionally.41 All other studies were purely cross-sectional in design and analysis. Studies primarily investigated young adult females and focused exclusively on DNA methylation and some also investigated expression levels, but did not investigate other epigenetic mechanisms, such as histone modifications or non-coding RNAs (Table 1). The studies show extensive sample overlap as four studies are part of the homocysteine and DNA methylation in eating disorders (HEaD) study,42–45 two studies recruited inpatients at the Universitätsmedizin Charité Berlin, Germany,46,47 and four studies recruited at the Douglas Institute Eating Disorders Program in Montreal, Canada.48–51 Most studies investigated

surrogate tissues with regard to their biological hypotheses, including whole blood, lymphocytes, or buccal cells instead of brain or metabolic tissue, and did not correct for heterogeneity of these tissues. Ten studies discussed the limitations of using surrogate tissues in their articles, two mentioned the issue, and four did not elaborate on this limitation (Table 1).

### 3.2   Global DNA methylation levels

Four studies investigated global DNA methylation differences in eating disorders. All studies primarily focused on AN,[45,51–53] with one study also investigating BN.[45] Two studies reported global hypomethylation in individuals with AN,[45,53] one study reported global hypermethylation in AN,[51] and one reported no difference in global DNA methylation levels between AN cases and controls.[52] Patients suffering from BN showed no difference in their global DNA methylation levels compared with controls.[45] Overall the quality of the studies was very low with inconsistent findings of opposite effects (Supplementary Table 1).

### 3.3   Candidate gene studies

Candidate gene studies are hypothesis-driven and investigate DNA methylation in the vicinity of selected genes. These candidate genes are selected based on prior knowledge, for example, following differences in protein levels measured in clinical studies assessing patients with AN or BN. Overall, 13 studies have been published profiling DNA methylation in candidate gene regions in the context of eating disorders, 11 of which focus on AN and BN. These eleven studies investigated genes relating to synaptic transmission,[45] endoplasmatic reticulum stress response,[45] growth hormone signaling,[52] fluid balance,[42] the cannabinoid system,[43] dopamine transmission,[44,50,54] stress response,[46,48] appetite regulation,[46,49,54] serotonin transmission,[54] and oxytocin.[55] One methylomic study of candidate genes in BED has been reported.[56,57] The study primarily investigated promoter methylation of *SLC1A2*, a gene involved in glutamate clearance, in bipolar disorder (BD). The authors found decreased DNA methylation in BD patients who also suffered from BED, compared to those who were only affected by BD. However, their sample of patients reporting binge-eating behavior seemed to comprise BN and BED cases, rendering the interpretation of the results ambiguous.[56,57] All candidate gene studies of eating disorders are described in detail in Table 1. The overall quality of the studies was very low with extremely small sample sizes never exceeding ~120 participants (Supplementary Table 1).

### 3.4   Epigenome-wide association studies (EWAS)

Three EWAS investigated genome-wide DNA methylation profiles in AN using the Illumina Infinium® HumanMethylation450 BeadChip. No genome-wide studies of DNA methylation have been published on other eating disorders. Booij et al.[51] reported 14 differentially methylated CpG sites comparing 29 AN patients with 15 normal-weight controls. These 14 hypermethylated CpG sites were annotated to 11 genes (*PRDM16, HDAC4, TNXB, FTSJD2, PXDNL, DLGAP2, FAM83A, NR1H3, DDX10, ARHGAP1, PIWIL1*).[51] Kesselmeier et. al[30] reported 51 differentially methylated CpG sites when comparing 22 AN cases with 24 lean individuals and 81 CpG sites when comparing AN cases with 30 individuals from a general population sample. They also showed that 54 of the 81 sites exhibited directionally consistent differential DNA methylation differences in a comparison

of twins discordant for AN assessed by a binomial sign test (Table 2). Although the authors report a replication of hypermethylation previously reported at a CpG site annotated to *TNXB*,30,51 the significance level for this replication was only suggestive. In this study, controls recruited from the population were on average significantly older than the AN patients potentially confounding the results as methylation patterns are age-dependent.25,30 In a conference presentation, Ramoz et al.41 conducted the only longitudinal investigation of 36 acutely ill AN patients of whom half remitted after one year. However, the statistical analysis performed was cross-sectional. No significant differences in DNA methylation emerged between remitted AN patients and those patients who were still ill after a follow-up period of one year. However, the study did not include a control group.41 Two of the three EWAS were followed up by pathway analyses (Table 2).41,51

## 4 Discussion

The current research on epigenetics in eating disorders is limited and not yet sufficiently mature to draw sound conclusions with most of the reviewed studies being of very low quality. To date, epigenetic research in eating disorders has, to our knowledge, focused exclusively on DNA methylation, using three different approaches to investigate disease-associated methylomic variation. First, early DNA methylation studies measured global methylation levels in eating disorder cases comparing them with methylation levels in healthy controls. Second, DNA methylation at selected candidate genes has been assessed. Third, genome-wide approaches are applied in the investigation of epigenetic alterations in epigenome-wide association studies (EWAS). In general, studies were cross-sectional and primarily focused on females. Most studies were conducted on surrogate tissue and presented varying degrees of acknowledgement and discussion of the limitations of using surrogate tissues in epigenetic epidemiology.

Overall, global methylation study results were inconclusive and inconsistent and did not reveal a clear and replicable global DNA methylation pattern in either AN or BN. All four studies were small, with the largest study profiling 32 AN cases and 24 BN cases, substantially limiting the power to detect effects. More generally, global levels of DNA methylation may not be of much relevance to epigenetic epidemiology, as they fail to provide information on region-specific DNA methylation, and lack the specificity to associate the dysregulation of biological pathways with the occurrence of a disease.58 Even within the framework of global DNA methylation studies, the methods employed in these four studies limit the examination of DNA methylation to either promoter regions (for the approaches based on methylation sensitive restriction enzymes) or LINE1 elements,52 overlooking other parts of the genome.

Across candidate gene studies, no clear differentially methylated candidate genes for AN, for BN, or for BED were robustly identified. Most candidate regions were only profiled once, and results of repeatedly measured genes involved in dopamine signaling did not replicate across studies,44,50,54 showing no clear eating disorder-associated methylomic variation across the selected candidate genes. In addition to non-replication, these studies were limited by small sample sizes rendering them imprecise: most of the study populations included on average only 30 cases with two studies including 64 cases.49,50 Furthermore

subjects occasionally comprised a mixture of acutely ill and recovered patients[55] or a mixture of different eating disorders[56,57] introducing heterogeneity. This is particularly concerning as dietary changes, weight changes, and accompanied alterations of hormonal levels during the recovery process can have a major effect on epigenetic profiles in individuals with eating disorders. Further possible confounders are discussed below. In epigenetics, as in genetics, a general drawback of a candidate gene studies is their hypothesis-driven design. Specific genes are selected for investigation based on prior knowledge, narrowing the investigation to only a very limited part of a large system and failing to attend to the majority of other genomic regions.

In general, hypothesis-free approaches that explore the whole genome are the gold standard in genetic research. Genome-wide approaches are applied in the investigation of common genetic variation (i.e., single nucleotide polymorphisms, SNPs) in genome-wide association studies (GWAS) as well as in the examination of epigenetic alterations in epigenome-wide association studies (EWAS). EWAS examining CpG sites at a genome-wide level have identified multiple AN-associated differentially methylated sites, replicating a differentially methylated position at *TNXB* in one independent study.[51] However, the hypermethylation at this CpG site annotated to *TNXB* only reached suggestive significance in the replication attempt,[30] failing to survive stringent correction for multiple comparisons. A false positive finding, therefore, cannot be ruled out.

Statistically, EWAS share similarities with GWAS in that site-specific associations with a phenotype across a large number of genomic loci are conducted. These statistical properties limited the findings in EWAS investigating eating disorders: First, the reported samples never included more than 29 cases of AN which is are far too small to robustly detect patterns of differential methylation at a genome-wide scale, i.e., when conducting over 450,000 statistical tests,[59] leading to imprecise estimates of the effects. Second, multiple testing correction was not always performed stringently, e.g., when "suggestive" significant results were reported or examined sites were filtered before or after analysis based on methylation variability.

As such, many of the EWAS included above labeled as pilot studies by the authors, provide motivation for further investigation, and are a springboard to launch full-scale projects with larger sample sizes and careful study design, data collection, and analysis. Future studies will also require replication in independent samples and should adhere to stringent methodological criteria, including multiple test correction, no subjective filtering of CpG sites, and controlling for confounding factors.

## 4.1 Future directions

**Sample size**—One of the primary goals of future epigenetic investigations of eating disorders should be to increase sample sizes by international collaborations to improve the power to detect effects, even when effect sizes are small. Recent epigenetic studies of other psychiatric disorders and environmental exposures have examined epigenetic differences in samples comprising thousands of participants and notably, replicated successes have been documented for a number of exposures and diseases including tobacco smoking,[24] C-reactive protein levels in serum blood,[60] and Alzheimer's disease.[16,17] While several large

consortium efforts have led to advances in characterizing baseline human tissue epigenomes, 10,61,62 this is rarely extended to the realm of epigenetic epidemiology in complex diseases. Unique challenges do exist in conducting large-scale collaborative epigenetic studies. Combining raw data (i.e., mega-analytic approaches) in epigenetics is problematic because technical variation in the data stemming from different laboratories and procedures (i.e., batch effects) has substantial impact on overall epigenomic profiles and can be insufficiently controlled for by post-hoc statistical or computational approaches.63,64 Nonetheless, approaches in which each site generates a sufficiently large sample under nearly identical conditions that can later be meta-analyzed are feasible.27 Alternatively, consistent sampling at different study sites including careful preanalytic sample collection and processing followed by analysis in a central laboratory could prevent many of the aforementioned technical issues. However, this approach is only feasible if all study sites meticulously follow the same protocol regarding tissue sampling, sample handling, and phenotyping of participants to control for possible confounders across study sites. This kind of pooling approach tends to be complicated by challenges associated with sample storage, transportation, and loss.

**Statistical methods—**As with any genome-wide investigation, the large number of tests performed requires special considerations for statistical analysis. Most importantly, it is essential to correct for the number of tests performed. The latest generation of DNA methylation arrays can simultaneously quantify epigenetic profiles at up to 850.000 CpG sites. An EWAS then tests for associations between a phenotype of interest and DNA methylation at each of these sites. Each of the 850.000 tests has a small probability of reporting a false positive association (usually 5%). In order to keep the probability of making any false positive discovery below this probability threshold, the individual P value thresholds for each test need to be adjusted, resulting in a genome- or array-wide significance threshold. This correction for multiple testing can be achieved by common methods such as Bonferroni correction (dividing the P value threshold by the number of tests conducted) or a false discovery rate correction.65,66

**Tissue specificity—**Given the prominent role epigenetic mechanisms play in cellular differentiation, genome-wide epigenetic profiles tend to differ substantially between different tissues and cell-types, matching the differences in function (e.g., fat storage in adipose tissue vs. synaptic transmission performed by neurons). Different cellular functions require particular sets of proteins acting in concert (i.e., pathways) and epigenetic mechanisms control which genes are active in which cell-type and tissue-context. Interestingly, epigenetic profiles can distinguish functionally different brain regions67 and cell-types.68,69 Is disease-associated epigenetic dysregulation tissue-specific? For example, are epigenetic correlates of psychiatric diseases restricted to the brain? In some disorders, including AN, it is less straightforward to pinpoint the affected tissue of interest. AN is characterized by both psychiatric and metabolic features.6 Therefore, one would ideally investigate epigenetic profiles in both brain tissue and metabolic tissues (e.g., adipose tissue, pancreas, liver, stomach, and the intestine). The investigation of brain in particular, however, poses considerable challenges and is typically only possible in postmortem samples, which introduces additional complications for epigenetic studies (e.g., time of death, cause of

death, etc.). Nonetheless, carefully designed, ethical discussions of organ donations with patients and families are worthy of consideration.

**Surrogate tissues**—Although investigation of epigenetic profiles in the disease-affected tissue is the gold standard in epigenetic studies, it is also valuable to examine epigenetics in surrogate peripheral tissues, including whole blood, epithelial cells, and saliva. First, while epigenetic profiles are highly tissue-specific and profiles observed in peripheral tissues are not generally representative of epigenetic variation in brain, specific genomic regions manifest high levels of epigenetic covariation.[67,70,71] For example, an existing online platform allows for the profiling of DNA methylation covariation between whole blood and multiple brain regions (http://epigenetics.essex.ac.uk/bloodbrain/). Second, while results emerging from epigenetic studies from peripheral samples might not necessarily reflect the epigenetic changes in disorder-relevant tissues, they can still be used as potential biomarkers, and are collected more readily and less invasively than the affected tissue itself. Importantly, when analyzing whole blood epigenetic profiles, the blood cell-type composition also needs to be assessed. Blood is a heterogeneous organ comprised of distinct cell types, fulfilling specific tasks, such as oxygen transport, immune function, and nutrient distribution. Because blood composition in patients suffering from eating disorders differs from controls,[30] it is imperative to control for these differences in cell-type composition. If unaccounted for, epigenetic differences identified in an association scan could be related purely to differences in cellular composition rather than epigenetic dysregulation directly linked to disease etiology and progression. Where blood cell counts are not available, validated estimators of subcell proportions based on large reference panels can be used; i.e. cell-type proportions can accurately be estimated using microarray-based DNA methylation data. This also applies to other cell-types, and estimators based on DNA methylation array data have been previously reported for whole brain, buccal swabs, and saliva samples. These estimators require array-wide DNA methylation profiles and are therefore not applicable in candidate gene studies.[72]

**Genome-wide integrated epigenetic studies**—Many of the studies reviewed here use targeted sequencing approaches, which only allow the investigation of DNA methylation in limited genomic regions and ignore information from the rest of the genome. This may increase the chances for false positive reports via a publication bias of positive findings. Genome-wide technologies are less prone to this phenomenon and allow for the verification of previously reported differentially methylated sites. While only whole genome bisulfite sequencing allows full coverage of the entire genome, array-based approaches like Illumina's EPIC array, allow widespread coverage of CpG sites in most genomic regions and can be a more cost-effective solution.

All epigenetic studies of eating disorders published to date focused on DNA methylation only. A host of other sources of regulatory variation including other DNA modifications, histone modifications, and non-coding RNAs should also be investigated. Furthermore, to better interpret the role of epigenetic modifications in disease, it is important to understand their interactions with the genetic sequence itself. Integrated analyses incorporating genotypic, epigenetic, transcriptomic, and detailed environmental data are beginning to

emerge, elucidating the role of disease-associated epigenetic dysregulation in specific genetic and environmental contexts. Increasingly detailed maps of genetic and (multi-)epigenetic profiles in health and disease will be essential to improve our understanding of the molecular biological pathways implicated in complex disease.

### 4.2   Eating disorder-specific considerations

In addition to these general recommendations for improving epigenetic research in disease epidemiology, there are also a number of important eating disorder-specific complexities to be considered.

**Eating disorder-specific confounders—**Because epigenetic modifications are dynamic and can be altered by environmental influences, epigenetic association studies are subject to a wide range of confounders. Confounding in EWAS is comparable to classic observational epidemiological studies and ideally these confounders are addressed in the study design in such a way that they can be controlled for in the statistical analyses. For example, age, sex, diet, micronutrients, medication, dietary supplements, hormones, smoking, and alcohol consumption can interact with an individual's epigenetic profile, obfuscating EWAS analyses (Figure 2).

A large body of evidence confirms that diet composition can have an effect on an individual's epigenetic profile.[73–77] Eating and compensatory behaviors can include binge eating and purging behaviors, abuse of diet medication, laxatives, and diuretics altering fluid balance. It is important to record their typical use, as well as the frequencies and recency of use and, ideally, obtaining blood levels of diet medications when possible. Both indicated and off-label prescribed antidepressants, anticonvulsants, and atypical antipsychotics are used to control accompanying symptoms observed in patients suffering from eating disorders.[78] Dosage and intake should be included in the analysis, ideally, blood levels should be measured, and statistical analyses corrected for. This strategy should also be followed for supplements, such as vitamins and micronutrients, and hormones as patients with eating disorders often show hormonal alterations, such as high cortisol and low sex hormones.[79,80] These types of hormones are direct ligands to so-called promoters, enhancers, and silencers and, therefore, influence gene expression and protein levels directly. However, if substantial between group differences exist between cases and controls, disease and weight-associated epigenetic variation will remain convoluted. One option in addressing this issue is using a matched weight control group, potentially in addition to normal weight controls, in order to tease apart epigenetic correlates of eating disorder versus altered weight phenotypes. However, this approach may be limited because constitutionally thin individuals rarely have a BMI as low as patients suffering from AN. As environmental toxins, smoking and alcohol can have a profound impact on the epigenome. For example, prevalence differences in smoking between cases and controls have been shown to confound the association between DNA methylation and schizophrenia.[14]

As with every observational study design, the causal attribution of epigenetic associations in eating disorders is extremely difficult. The epigenetic dysregulation could potentially have causally contributed to the disease or have arisen as a consequence of the disease, its

symptoms or even treatments, such as medication;81 or, in a third scenario, there could be a third factor driving both the disease and the epigenetic alteration, which have no direct link between one another. One important approach to addressing causality is to consider temporal factors.26,27 A variety of chronologically variable factors should be taken into account, such as age of disorder onset, duration of illness, onset of menstrual disturbances, and duration of amenorrhea (in women). Longitudinal sampling and within-subject comparisons can help differentiate between sequelae of starvation or overeating and epigenetic factors that contribute to the liability to develop an eating disorder. In addition to this, methods using genetic variants as instrumental variables can improve causal inference. In epigenetic epidemiology, Mendelian Randomization is of particular importance, exploiting genetic influences on DNA methylation (mQTLs) to understand whether phenotypic associations of DNA methylation are indeed causal.82

## 5   Conclusion

Epigenetic research in eating disorders is still in its infancy, but initial results from pilot studies encourage further and larger-scale investigation. Much like progress in genomics, international collaborations are required to amass adequately powered sample sizes to draw credible conclusions from epigenetic investigations. Even more importantly, careful study design is of vital importance in epigenetics and can aid in avoiding potential pitfalls. Robust, replicable results from carefully designed studies have the potential to uncover the molecular biological processes involved in disease onset and progression, they may help characterize gene regulatory effects of non-coding genetic variation, and, hopefully, give indications into disease-relevant biological pathways which could be addressed by therapeutic interventions. Clearly a considerable amount of functional work is required in follow-up of epigenetic association studies to better understand the gene regulatory, cellular, and organismal outcomes of epigenetic variation and derive potential translational implications and therapeutic avenues. Even non-functional disease-associated epigenetic variation from peripheral tissue sources could, however, have useful implications as biomarkers for risk and prognosis assessment and for use in early diagnosis.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

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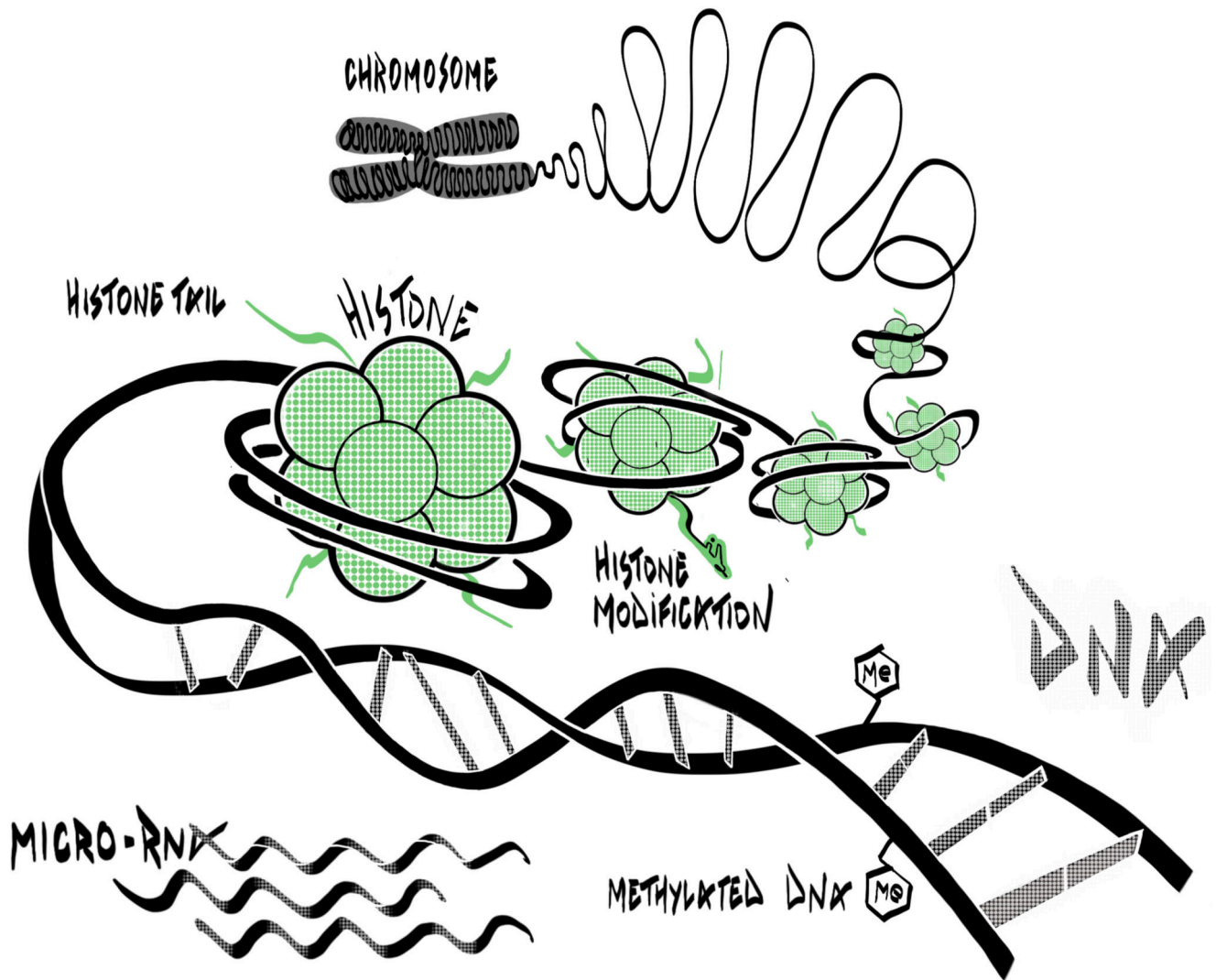
**Figure 1.**
The **epigenetic profile** of a human cell comprises several epigenetic mechanisms: a) **DNA methylation** is the most prominent and prevalent DNA modification characterized by an addition of of a methyl-group to cytosine in the context of cytosine-guanine dinucleotides (i.e., CpG sites). b) Histone proteins compact chromosomal DNA in the nucleus of the cell and regulate gene expression and **histone modifications** are chemical modifications to the N-terminal histone tails, which extend out of the nucleosome complex (i.e., the transcription apparatus). An increasing number of modifications to amino acids in the histone tails are being identified, including methylation, acetylation, and phosphorylation. These modifications are characterized by tissue specificity and are highly correlated with different transcriptional chromatin states. c) **Non-coding RNAs** are expressed transcripts that do not code for proteins. They affect gene regulation by binding to transcripts and inhibiting their translation to proteins (i.e., post-transcriptional silencing) or by guiding the positioning of nucleosomes along the genome and thereby altering DNA accessibility. Designed by Vinícius Gaio, London, UK.

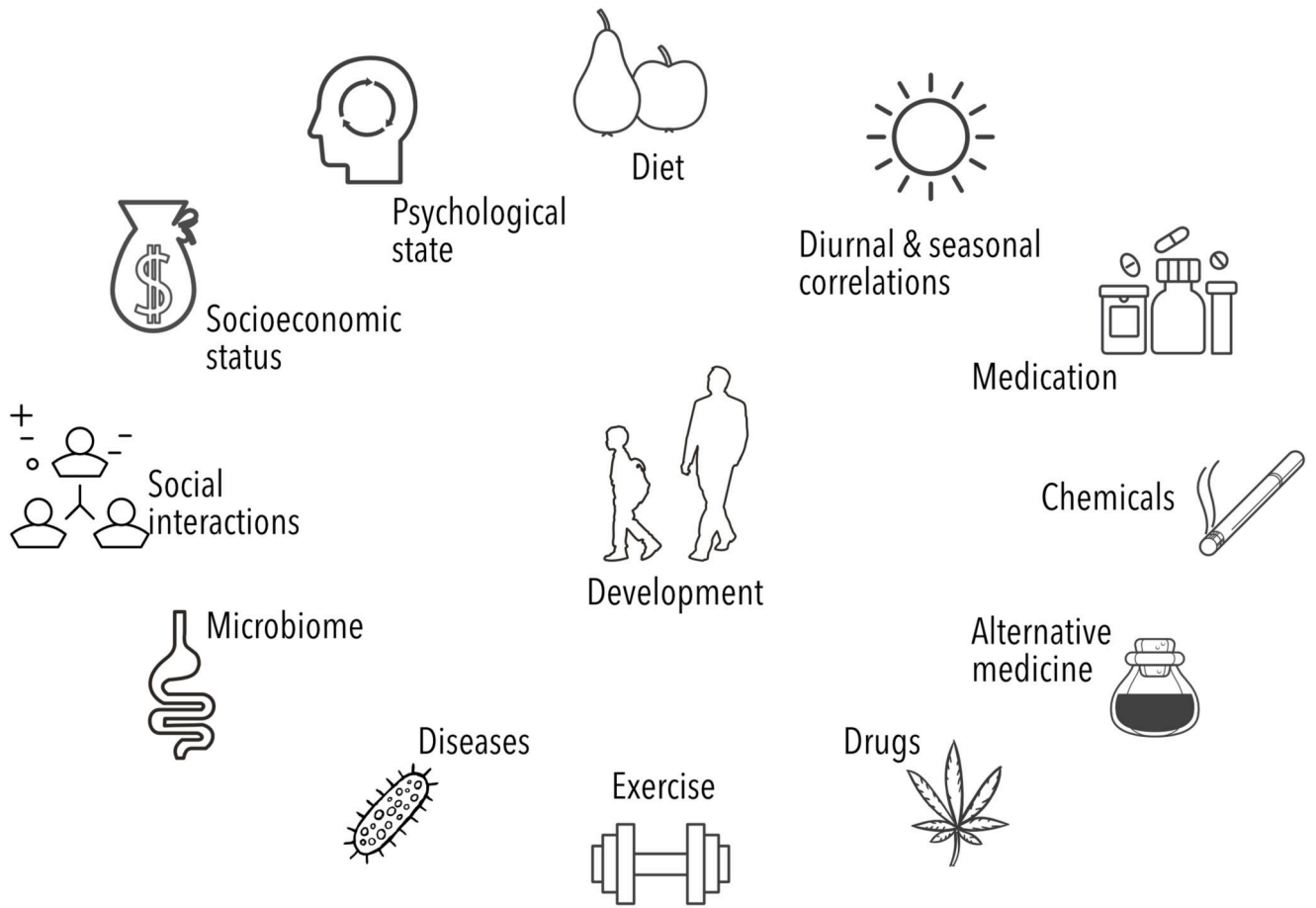**Figure 2.**
Factors and environmental confounders influencing epigenetic profiles. The assessment of these factors should be included in the design of a study investigating epigenetic profiles.
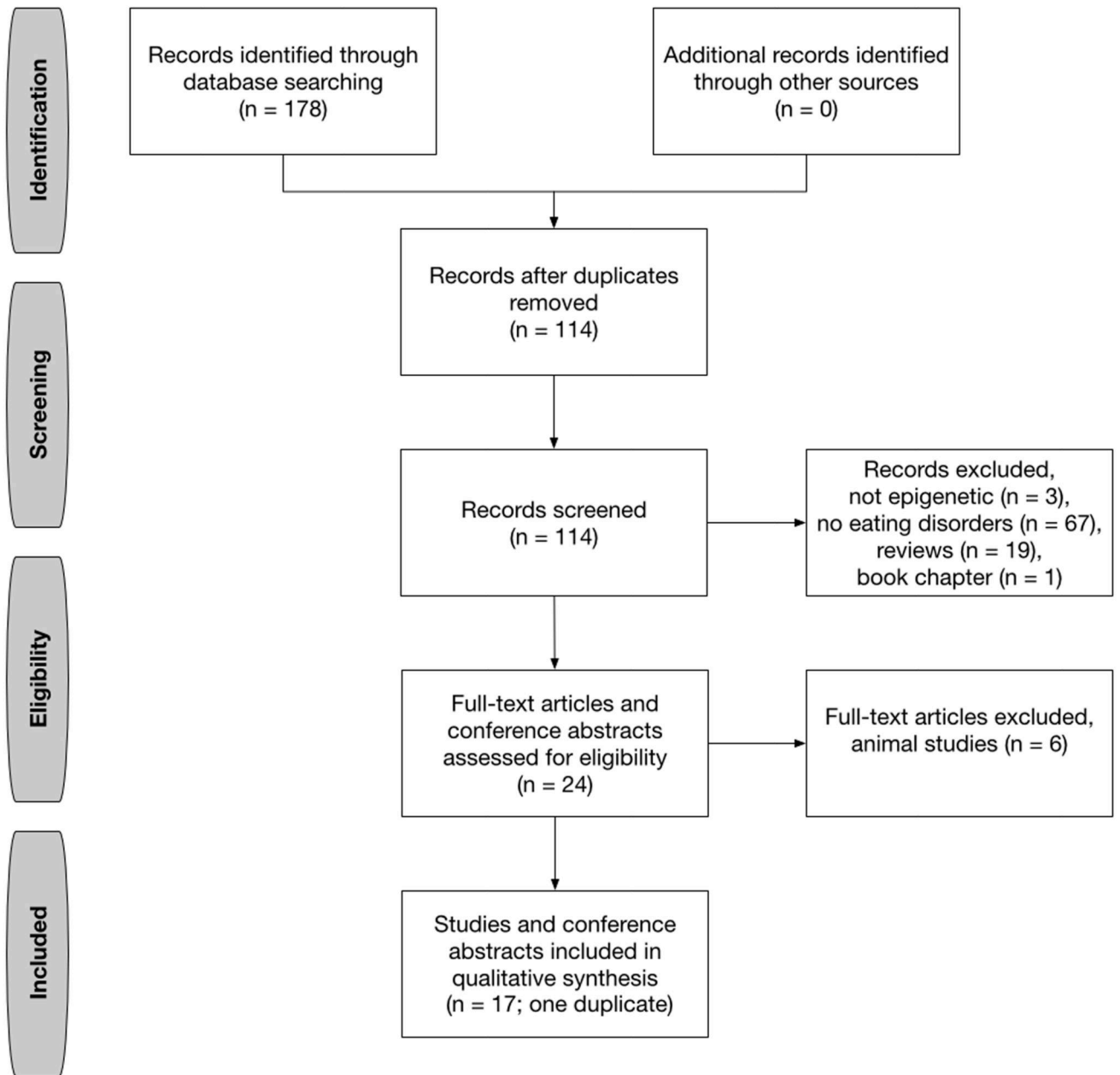
**Figure 3.**
PRISMA flow diagram of study selection

**Table 1**

Overview of included studies. Gene names are those used in the articles and additionally standardized gene names according to www.genenames.org. All studies were cross-sectional in their statistical analysis.

| Author (Year) | Sample N | Female | Age (SD) [years] | Diagnoses | Medicated | Tissue/cell type - Correction - Suitability - Limitation | Methylation Results: Global methylation | AN | BN | BED | Assessed confounds and exclusion criteria | Biological hypothesis (PMID) → **mechanistic or potential biomarker study** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Frieling (2007) | AN: 22 / BN: 24 / CO: 30 | 100% / 100% / 100% | 26.5 (10.3) / 25.8 (7.7) / 22.0 (4.8) | DSM-IV | n.a. | Whole blood - uncorrected - proxy tissue - not mentioned | | → | ↔ | | **Confounds:** Homocysteine **Exclusion:** None | Elevated homocysteine blood levels in 18 AN cases (PMID: 15937640) → mechanistic hypothesis of homocysteinemia altering methylation levels |
| Saffrey (2014) | AN: 10 / CO: 10 | 100% / 100% | 21.5 (10.2) / 23.1 (9.5) | n.a. | n.a. | Buccal cells - uncorrected - proxy tissue - discussed | | ↔ | | | **Confounds:** None **Exclusion:** None | No hypothesis, only prior epigenetic studies |
| Tremolizzo (2014) | AN: 32 / CO: 13 | 100% / 100% | 15.5 (1.4) / 16.3 (1.3) | DSM-IV-TR | 53.2% | Whole blood - uncorrected - proxy tissue - not mentioned | | → | | | **Confounds:** Leptin, homocysteine, vitamin B12, folate, cortisol, testosterone, progesterone, estradiol, dehydroepiandrosterone, CDI, STAI-Y S & T, EDI-3 **Exclusion:** All: hematological or hepatic disorders, cancer, recent infections or surgery, <14 and >18 years, alcohol abuse; CO: neurological or psychiatric disorder, medication | No hypothesis, only prior epigenetic studies → biomarker study for early diagnosis |
| Booij (2015) | AN: 29 / CO: 15 | 100% / 100% | 22.7 (5.9) / 24.2 (5.8) | DSM-5 | ~70% | Lymphocytes - not necessary - proxy tissue - mentioned | | ← | | | **Confounds:** None **Exclusion:** None | No hypothesis, only prior epigenetic studies |
| Frieling (2007) | AN: 22 / BN: 24 / CO: 30 | 100% / 100% / 100% | 26.5 (10.3) / 25.8 (7.7) / 22.0 (4.8) | DSM-IV | n.a. | Whole blood - uncorrected - proxy tissue - not mentioned | SNCA / HERP | ← / ↔ | ↕ / ↕ | | **Confounds:** Homocysteine **Exclusion:** None | Elevated homocysteine blood levels in 18 AN cases (PMID: 15937640) → mechanistic hypothesis of homocysteinemia altering methylation levels |
| Frieling (2008) | AN: 22 / BN: 22 / CO: 30 | 100% / 100% / 100% | 22 (18–51) / 23 (18–42) / 21 (19–43) | DSM-IV | n.a. | Whole blood - uncorrected - proxy tissue - partially discussed | ANP / Vasopressin | ↕ / ↕ | ← / ↕ | | **Confounds:** Purging, binge eating, age, BMI, duration of illness, electrolytes, hematocrit, creatinine **Exclusion:** None | Elevated vasopressin in 10 AN cases (PMID: 10960163) → mechanistic hypothesis of the dysregulation of fluid homeostasis |
| Frieling (2009) | AN: 20 / BN: 23 / CO: 26 | 100% / 100% / 100% | 26.4 (10.6) / 25.9 (7.9) / 21.3 (2.2) | DSM-IV | n.a. | Whole blood - uncorrected - proxy tissue - not mentioned | CNR1/CB1 / CB2 | ↔ / n.a. | | | **Confounds:** None **Exclusion:** None | Elevated endocannabinoids in 10 AN cases (PMID: 15841111) |

| Author (Year) | Sample | | | Diagnoses | Medicated | Tissue/cell type - Correction - Suitability - Limitation | Methylation Results | | | | Assessed confounds and exclusion criteria | Biological hypothesis (PMID) → mechanistic or potential biomarker study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Female | Age (SD) [years] | | | | Global methylation | AN | BN | BED | | |
| | | | | | | | | | | | | → mechanistic hypothesis of altered endocannabinoid signaling |
| Frieling (2010) | AN: 22<br>BN: 24<br>CO: 30 | 100%<br>100%<br>100% | 26.5 (10.3)<br>25.8 (7.7)<br>22.0 (4.5) | DSM-IV | n.a. | Whole blood<br>- uncorrected<br>- proxy tissue<br>- discussed | SLC6A3/DAT<br>DRD2<br>DRD4 | ←<br>←<br>↕ | ←<br>↕<br>↕ | | **Confounds:** None<br>**Exclusion:**<br>All: high coffee consumption, alcohol abuse, medication, endocrinological conditions, other diseases (i.e., thromboembolic, diabetes mellitus, cardiovascular diseases)<br>CO: any medical or psychiatric condition | Six recovered AN cases showed lower levels of homovanillic acid in CSF (PMID: 10481833)<br>Increased binding of [11C]-raclopride at cerebral D2/D3 receptors in 10 recovered AN patients (PMID: 15992780)<br>→ mechanistic hypothesis of disturbed dopaminergic signaling |
| Ehrlich (2010) | AN: 31<br>AN-Rec: 30<br>CO: 30 | 100%<br>100%<br>100% | 16.4 (1.3)<br>19.3 (3.0)<br>16.4 (1.5) | DSM-IV | 0% | Whole blood<br>- uncorrected<br>- proxy tissue<br>- discussed | POMC | ↕ | | | **Confounds:** Age, duration of illness, BMI, leptin, cortisol, EDI-2<br>**Exclusion:**<br>All: IQ less than 85, current inflammatory, neurological or metabolic illness, chronic bowel diseases, cancer, anemia, pregnancy, breastfeeding, treatment with cortisone.<br>AN-Rec: BMI <18.5 (if older than 18 years) or a BMI <10th BMI percentile last 3 months prior to study, binged, purged or engaged in significant restrictive eating patterns<br>CO: psychiatric illness, organic brain syndrome, schizophrenia, substance dependence, bipolar illness, bulimia nervosa, binge-eating disorder | Reduced POMC-related peptides in CSF of 10 AN cases (PMID: 282304)<br>→ mechanistic hypothesis of altered central nervous appetite regulation |
| Ehrlich (2012) | AN: 40<br>AN-Rec: 21<br>CO: 54 | 100%<br>100%<br>100% | 17.88 (3.2)<br>19.25 (3.7)<br>17.10 (2.3) | DSM-IV | n.a. | Whole blood<br>- uncorrected<br>- proxy tissue<br>- discussed | POMC | ↕<br>↕ | | | **Confounds:** Age, duration of illness, leptin, BMI, smoking<br>**Exclusion:**<br>All: IQ <85, current inflammatory, neurological or metabolic illness, chronic bowel diseases, cancer, anemia, pregnancy, breastfeeding, treatment with cortisone, and use of psychotropic medications within the past 6 months<br>Patients: organic brain syndrome, schizophrenia, substance dependence, bipolar disorder, bulimia nervosa, binge-eating disorder | Follow-up study to Ehrlich (2010) to correct for possible confounds |
| Pjetri (2013) | AN: 45<br>CO: 45 | n.a.<br>n.a. | 16–60<br>n.a. | DSM-IV-R | n.a. | Whole blood<br>- uncorrected<br>- proxy tissue<br>- discussed | DRD2<br>LEP<br>BDNF<br>SLC6A4 | ↕<br>↕<br>↕<br>↕ | | | **Confounds:** BMI | No hypothesis, only prior epigenetic studies and candidate gene relevance to neurotransmitter and body weight regulation systems |
| Steiger (2013) | BN: 64<br>CO: 32 | 100%<br>100% | 26.1 (6.6)<br>23.7 (5.7) | DSM-IV-TR | 52.4% | Whole blood<br>- uncorrected<br>- proxy tissue<br>- discussed | GR | | ↕ | | **Confounds:** Borderline personality disorder, suicidality, childhood abuse, binge eating and vomiting frequency, BMI, medication | Less suppression in a dexamethasone suppression test of 60 women affected by bulimia spectrum disorder (PMID: 22575215) |

| Author (Year) | Sample N | Female | Age (SD) [years] | Diagnoses | Medicated | Tissue/cell type - Correction - Suitability - Limitation | Methylation Results — Global methylation | AN | BN | BED | Assessed confounds and exclusion criteria | Biological hypothesis (PMID) → mechanistic or potential biomarker study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | → mechanistic hypothesis of glucocorticoid system alterations |
| Groleau (2014) | BN: 52 CO: 19 | 100% 100% | 24.7 (5.7) 23.7 (4.6) | DSM-IV-TR | 67.3% | Whole blood - uncorrected - proxy tissue - discussed | DRD2 | | ↔ | | **Confounds:** Borderline personality disorder, physical and sexual childhood abuse, bingeing and vomiting frequency, BMI, medication | No hypothesis, only prior epigenetic studies |
| Kim (2014) | AN: 15 CO: 36 | 100% 100% | 24.7 (10.7) 22.1 (2.2) | DSM-IV | 0% | Buccal cells - uncorrected - proxy tissue - discussed | OXTR | ↑ | | | **Confounds:** Age, BMI, EDE-Q global score, AQ total score, BDI, STAI-S, STAI-T **Exclusion:** All: smoking, non-heterosexual, parous, medications, including contraceptives, history of psychiatric illness, <18 years old Patients: active substance use disorder, psychotic disorder (schizophrenia, schizoaffective, psychosis not otherwise specified), autism, Asperger's syndrome | Derangement of the oxytocin system in AN cases (PMID: 24115458) → mechanistic hypothesis of oxytocin system alterations |
| Saffrey (2014) | AN: 10 CO: 10 | 100% 100% | 21.5 (10.16) 23.1 (9.45) | n.a. | n.a. | Buccal cells - uncorrected - proxy tissue - discussed | IGF2 | ↔ | | | **Confounds:** None **Exclusion:** None | No hypothesis, only prior epigenetic studies |
| Thaler (2014) | BN: 64 CO: 32 | 100% 100% | 26.1 (6.6) 23.7 (5.7) | DSM-5 | 52.4% | Lymphocytes - not necessary - proxy tissue - discussed | BDNF | | ↑ | | **Confounds:** Borderline personality disorder, physical or sexual childhood abuse **Exclusion:** Controls: Childhood abuse | Decreased serum BDNF in 22 AN cases (PMID: 15385700) Decreased serum BDNF in 18 BN cases and 12 AN cases (PMID: 12915293) → mechanistic hypothesis that alterations in BDNF methylation are related to early-life adversities |
| Veldic (2017) Jia (2017) | BED/BN: 30 CO: 32 | 76.7% 56.3% | 45.2 (14.8) 37.4 (13.4) | DSM-IV-TR | 60% | Blood - uncorrected - proxy tissue - not mentioned | SLC1A2 | | ↑ | → | **Confounds:** BMI, age, sex, cumulative illness rating scale, rapid cycling, increased severity, mixed episodes, cycle acceleration, psychosis, anxiety disorder, early onset (<20 years), atypical antipsychotics, lithium, valproate, and lamotrigine, current alcohol use ( 2x per month), current smoker status, and lifetime history of smoking ( 100 cigarettes) | In a sample of 68 bipolar disorder patients comorbid BED was prevalent after controlling for obesity (PMID: 23742827) Antagonism of the glutamatergic system decreased food intake in baboons (PMID: 18573641) → mechanistic hypothesis of glutamatergic neurotransmission influencing food intake |

| Author (Year) | Sample N | Sex | Age (SD/Q1, Q3) [years] | Diagnoses | Medicated | Tissue/cell type - Correction - Suitability - Limitation | Methylation Results — EWAS | AN | BN | BED | Assessed confounds and exclusion criteria | Biological hypothesis (PMID) → mechanistic or potential biomarker study |
|---|---|---|---|---|---|---|---|---|---|---|---|---|

| Author (Year) | Sample | | | | Medicated | Tissue/cell type - Correction - Suitability - Limitation | Methylation Results | | | | Assessed confounds and exclusion criteria | Biological hypothesis (PMID) → mechanistic or potential biomarker study |
| | N | Female | Age (SD) [years] | Diagnoses | | | Global methylation | AN | BN | BED | | |
| Booij (2015) | AN: 29 CO: 15 | 100% 100% | 22.7 (5.9) 24.2 (5.8) | DSM-5 | 73% | Lymphocytes - not necessary - proxy tissue - uncritically discussed | | 14 CpG | | | **Confounds:** None | No hypothesis, only prior epigenetic studies |
| Kesselmeier (2016) | AN: 47 CT: 47 CO: 100 | 100% 100% 100% | 16 (14, 17) 22 (21, 23) 60 (54, 69) | DSM-IV | n.a. | Whole blood - FastLMM-EWASher corrected (PMID: 24464286) and RefFree-EWAS corrected (PMID: 24451622) - proxy tissue - mentioned | AN vs CT AN vs CO | 51 CpG 81 CpG | | | **Confounds:** None **Exclusion:** <u>CT:</u> somatic disorders, 10 cigarettes per day, AN, BN, cognitive restraint (Three-Factor Eating Questionnaire), weight history (higher weight than same-aged individuals at ages 10, 15 and/or 18) <u>CO:</u> none | No hypothesis, only prior epigenetic studies |
| Ramoz (2017) | AN: 18 AN-Rec: 18 | 100% 100% | n.a. | n.a. | n.a. | n.a. | n.s. | ↔ | | | **Confounds:** n.a. **Exclusion:** n.a. | n.a. |

↑=increased methylation level, ↔=unchanged methylation level, ↓=decreased methylation level, AN=anorexia nervosa, AN-Rec=recovered from anorexia nervosa, ANP/NPPA=natriuretic peptide A, AQ=Autism Spectrum Quotient, BDI=Beck Depression Inventory, BDNF=brain derived neurotrophic factor, BED=binge-eating disorder, BMI=body mass index, BN=bulimia nervosa, CDI=Children's Depression Inventory, CNR1/CB1=cannabinoid receptor 1, CNR2/CB2= cannabinoid receptor 2, CO=controls, CpG=CpG sites, CSF=cerebrospinal fluid, CT=constitutionally thin, DAT/SLC6A3=dopamine transporter, DRD2=dopamine receptor D2, DRD4=dopamine receptor D4, DSM=Diagnostic and Statistical Manual of Mental Disorders, EDE-Q=Eating Disorder Examination Questionnaire, EDI=Eating Disorders Inventory, EWAS=epigenome-wide association study, GR/NR3C1=glucocorticoid receptor, HERP/HERPUD1=homocysteine inducible ER protein with ubiquitin like domain 1, IGF2=insulin like growth factor 2, IQ=intelligence quotient, LEP=leptin, n.a.=not available, N=number, NR3C1/GR=nuclear receptor subfamily 3 group C member 1, n.s. = not significant, OXTR=oxytocin receptor, PMID=PubMed-Indexed for MEDLINE, POMC=proopiomelanocortin, Q1=quartile 1, Q3=quartile 3, SD=standard deviation, SLC1A2=solute carrier family 1 member 2, SLC6A3/DAT=solute carrier family 6 member 3, SLC6A4=solute carrier family 6 member 4, SNCA=synuclein alpha, STAI=Spielberger State and the Trait Anxiety Inventory

**Table 2**

Epigenome-wide association study (EWAS) follow-on investigations

| Author (Year) | Multiple testing correction | Variability filters | Follow-up on hits | Validation on different platform | Function of identified sites |
|---|---|---|---|---|---|
| Booij (2015) | Bonferroni & False Discovery Rate (FDR) | Probes needed to have a standard deviation of at least 0.05 (5% deviation in methylation) | Pathway analysis (Ingenuity Pathway Analysis) | No | Histone acetylation and RNA modification, cholesterol storage and lipid transport, and dopamine and glutamate signaling |
| Kesselmeier (2016) | None | Average beta value across all samples between 0.1 and 0.9 | Validation of direction of effect in monozygotic twins discordant for AN | No | n.a. |
| Ramoz (2017) | n.a. | n.a. | Pathway analysis | n.a. | n.a. |

n.a.=not available, RNA=ribonucleic acid