EMPIRICAL ARTICLE

DNA Methylation in Individuals with Anorexia Nervosa and in Matched Normal-Eater Controls: A Genome-Wide Study

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ABSTRACT

Objective: Evidence associates anorexia nervosa (AN) with epigenetic alterations that could contribute to illness risk or entrenchment. We investigated the extent to which AN is associated with a distinct methylation profile compared to that seen in normal-eater women.

Method: Genome-wide methylation profiles, obtained using DNA from whole blood, were determined in 29 women currently ill with AN (10 with AN-restrictive type, 19 with AN-binge/purge type) and 15 normal-weight, normal-eater control women, using 450 K Illumina bead arrays.

Results: Regardless of type, AN patients showed higher and less-variable global methylation patterns than controls. False Discovery Rate corrected comparisons identified 14 probes that were hypermethylated in women with AN relative to levels obtained in normal-eater controls, representing genes thought to be associated with histone acetylation, RNA modification, cholesterol storage and lipid transport, and dopamine and glutamate signaling. Age of onset was significantly associated with differential methylation in gene pathways involved in development of the brain and spinal cord, while chronicity of illness was significantly linked to differential methylation in pathways involved with synaptogenesis, neurocognitive deficits, anxiety, altered social functioning, and bowel, kidney, liver and immune function.

Discussion: Although pre-existing differences cannot be ruled out, our findings are consistent with the idea of secondary alterations in methylation at genomic regions pertaining to social-emotional impairments and physical sequelae that are commonly seen in AN patients. Further investigation is needed to establish the clinical relevance of the affected genes in AN, and, importantly, reversibility of effects observed with nutritional rehabilitation and treatment. © 2015 Wiley Periodicals, Inc.

Keywords: DNA methylation; anorexia nervosa; body mass index; eating disorders; epigenetics; brain development

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Introduction

Anorexia nervosa (AN) is believed to have significant biological underpinnings, to implicate altered brain function, to be significantly heritable, but also to be strongly influenced by environmental factors.[1–3] Often quite damaging to the medical, psychological, and social adjustments of those affected, AN displays marked potential for chronicity.[2] To date, core causal and maintaining factors of this disorder remain poorly understood.

Various forms of evidence suggest that AN implicates the environmental activation of genetic

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susceptibilities in vulnerable individuals, with some studies pointing to detrimental effects of stressful family environments,[4] others to environments that encourage excessive caloric restraint.[5,6] Epigenetic mechanisms are believed to provide a physical substrate for gene-environment interactions that link environmental stressors to mental-disorder phenotypes[7]—among them, eating disorders.[1,3] A main epigenetic mechanism (which we selected to study here) is DNA methylation. Among studies that explore methylation of candidate genes in AN, one reported hypermethylation of the alpha-synuclein gene (linked to sensitivity to dietary folate).[8] Another indicated hypermethylation of the volume-regulating atrial natriuretic peptide gene, implicated in anxiety, depression and stress responses;[9] yet another one linked weight loss to altered methylation and expression of the propiomelanocortin (POMC) gene, implicated in hunger, satiety and energy homeostasis.[10] A recent study indicated hypermethylation in the oxytocin receptor (OXT) gene in AN, associating the level of methylation with body mass index (BMI).[11] Finally, a study in a mixed sample of AN and bulimia nervosa (BN) patients showed increased methylation of Dopamine (DA)-system genes.[12] To date, genome-wide studies on methylation in AN are rare, and provide inconsistent findings. A very small ($N = 10$) genome-wide methylation study observed no differences in methylation between AN patients and controls,[13] whereas two studies, one involving 22 women with AN (subtype unspecified),[8] and another involving $N = 32$ with AN, restricting type,[14] found lower global methylation in peripheral blood tissues. The methods used in the latter three studies did not allow for the identification of specific functional gene pathways. Taken together, findings suggest that eating disorders are associated with alterations in DNA methylation that could influence a wide variety of systems implicated in behavioral/affective regulation, sensitivity to nutritional insufficiencies, and body-weight maintenance.

The present study examined the extent to which AN is associated with a distinct methylation "signature" in peripheral lymphocytes, expressed in the form of genome-wide DNA methylation alterations and probe-wise differences in methylation levels. Given the multi-faceted etiology of AN, and widespread sequelae of the disorder on the mental and physical status, we expected to observe alterations in DNA methylation patterns across a wide variety of genes, including those associated with behavioral phenotypes that characterize AN (e.g., anxious, depressive, or impulsive tendencies), and the functioning of diverse organ systems-especially among very severely ill individuals (e.g., those with particularly long duration of illness, or especially low BMI). We also investigated the association between GW methylation and AN type (AN-Restrictive type vs. AN-Binging/Purging type (AN-R vs. AN-BP).

Method

Participants

Eating-disordered (ED) participants were recruited from the patient cohort at the Douglas Institute Eating Disorders Program in Montreal, Canada, and included 30 women meeting full DSM-5 criteria for AN,[15] all with BMI < 17.5. Of these, 10 showed AN-Restrictive type, and 20 AN-Binging/Purging type-16 of whom binged and purged, and 4 of whom only purged (through vomiting). Diagnoses were based on Eating Disorders Examination (EDE) interviews[16] or (in five cases for whom the EDE interview was not obtained) clinical interviews complemented with EDE-Q questionnaire results.[17] All diagnoses were confirmed by senior clinicians (HS or MI), having many years of experience with EDs. We used retrospective EDE interviews to estimate age of illness onset (available in $n = 25$), using first estimated occurrence of threshold-level symptoms as our criterion. Limiting recruitment to unmedicated individuals was impractical (and undesirable on grounds of representativeness), and we therefore included some patients ($n = 21$) who had been on a stable medication regimen for at least 2 weeks prior testing.

Normal-eater (NE) participants were selected from a pre-existing sample ($N = 79$), developed during a large-scale, institutional ethics board approved study on the relevance of genetic factors and childhood abuse to eating and comorbid-psychiatric symptoms. Recruited through public and university/college-based announcements, eating- and other psychiatric disorders were ruled out in these individuals using EDE interviews[16] or questionnaires[17] and Structured Clinical Interviews for DSM-IV Axis I Disorders (SCID),[18] respectively. Selecting from this pool of participants who compared closely to clinical participants for age, we derived a sample of 15 individuals who were free of any past or present psychiatric disorders. None of the NE women were using psychoactive medications.

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees (Douglas Institute, McGill University) on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Clinical Measures

Eating-disorder diagnoses and symptoms were assessed using the Eating Disorders Examination interview[16] and/or the Eating Disorders Examination

Questionnaire (EDE-Q).[17] The EDE is the current "Gold Standard" interview for assessing anorexic and bulimic symptoms, with Cronbach alphas ranging from 0.67 to 0.90. Derived from the EDE, the EDE-Q uses 41 self-report questions to assess presence and severity of criterion eating-disorder symptoms, along the dimensions Restraint, Weight Concerns, Shape Concerns and Eating Concerns. These four EDE-Q subscales have shown excellent internal consistency (alphas ranging from .78 to .93) and 2-week test–retest reliability (ranging from .81 to .92).[17] In addition, EDE-Q indices correspond well with those obtained using the EDE.[19] We also computed participants' BMI (kg m^{-2}).

We used additional questionnaires to characterize our sample according to prominent concurrent internalizing and externalizing tendencies that typify individuals with AN: The Center for Epidemiological Studies-Depression (CES-D) scale,[20] a 20-item questionnaire with Cronbach alpha of .90 assessing depression; and the Barratt Impulsiveness Scale (BIS:[21] a 30-item Likert scale showing good internal consistency (Alpha = 0.83) and reliability for measurement of impulsivity.

DNA Methylation Analyses

DNA samples, obtained using lymphocytes from whole blood, were amplified by polymerase chain reaction according to standardized protocol. To assess genome wide methylation, we made use of the available Illumina Infinium HumanMethylation450 BeadChip Kit, which covers more than 480,000 methylation sites per sample, including 96% of CpG islands as well as additional coverage in island shores and surrounding regions, again at single-nucleotide resolution. Analysis was conducted at the Genome Quebec Innovation Centre. The manual protocol supplied by Illumina was followed for all steps except for Single Base Extension and Staining, which was conducted using the automated protocol. Briefly, the isolated DNA was first checked for quality with picogreen, and bisulfite converted using the Zymo EZ-96 DNA Methylation-Gold Kit. Samples were transferred to BCD and then MSA4 plates, and neutralized before overnight amplification. MSA4 plates were fragmented, precipitated, and re-suspended before hybridization and transfer to Multi BeadChips. The Multi BeadChips then underwent washing, single-base extension, and staining, before imaging using the HiScan array scanner.

Raw data were processed with the minfi tools from http://bioconductor.org. Values were normalized and background corrected, and β values (representing the proportion of methylated samples with a number ranging from 0 to 1) were calculated as the primary index of methylation. The CpGs were assigned chromosomal positions, and associated with genes, and CpG islands/shores, using the manifest files provided by Illumina (http://support.illumina.com/downloads/humanmethylation450_15017482_v1-2_product_files.ilmn). Data were then exported as comma separated variables.

Data Reduction and Statistical Analysis

Groups were compared on descriptive and clinical variables using chi-squared or one-way ANOVAs, as appropriate, using SPSS 20. All methylation analyses were conducted using Matlab (http://mathworks.com, version 13a). The initial dataset consisted of 482,421 probes with data from 45 participants. Because all participants were female, data from probes located on the Y chromosome were removed (416 probes), and because the primary question of interest concerned epigenetic regulation of biologically relevant gene products, only CpGs associated with known, protein-coding genes were included in the dataset (117,778 probes). An additional constraint, that probes needed to have a standard deviation of at least 0.05 (5% deviation in methylation) was added, so as to reduce any tendency for random technical variations with no functional significance to be mistaken for significant results. Thus, we included only those probes that could plausibly yield functionally relevant group differences, or that contained a broad-enough range of data for meaningful regression. The final dataset had 24,662 probes (5.1% of the original probes). The dataset included 1549 not a number (NaN) values (0.14% of all values), which were replaced by the mean methylation of all other participants at that CpG.

The Matlab statistics toolbox was used for the group and correlational analyses. Group comparisons of epigenome-wide methylation were made using t-tests (*ttest2* function) and one-way ANOVA (*anova1* function) with Bonferroni corrected post hoc tests (*multcompare* function) as appropriate. Group comparisons at individual probes were conducted using *t* tests, and significance was assessed after controlling for the false discovery rate using the *mafdr* function. For analyses involving many thousands of multiple comparisons the false discovery rate is often considered a preferable method of controlling for type I errors, rather than using traditional post-hoc adjustments such as the Bonferroni correction. As the number of multiple comparisons becomes large, the Bonferroni procedure becomes increasingly conservative, as it controls for the possibility of allowing even one false positive result. The false discovery rate approach attempts to keep the rate of false positives below a given threshold. The percentage of expected false positives is expressed using Q values, which are interpreted much like p values, but the meaning of $Q = 0.05$ is that at this threshold of significance 5 of every 100 findings are likely to be false positives. In exploratory analyses that yield long lists of effects, allowing a certain number of false

TABLE 1. Characteristics of the included sample [mean (SD) if applicable]

Variable Mean (SD)	AN, Restricting Type (AN-R; $n = 10$)	AN, Binging/Purging Type (AN-BP; $n = 19$)	Normal Eaters ($n = 15$)
Age (years)	21.5 (6.4); range: 18–38	23.4 (5.6); range: 18–40	24.2 (5.8); range: 19–40
BMI	14.9 (1.8)[a]	15.9 (1.1)[a]	21.9 (2.1)
EDEQ-total	4.3 (0.8)[a]	4.7 (1.2)[a]	0.90 (0.3)
Age of onset (years)	18.4 (6.6); range: 13–32	16.6 (5.6); range: 5–29	n/a; n/a
Cumulative illness duration (months)	54.9 (30); range: 12–84	83.3(87.8); range: 12–336	n/a; n/a
BIS total	55.5 (7.3)	68.3 (10.7)[b,c]	59.0 (8.1)
CES-D	26.4 (16.8)[a]	34.45 (13.0)[a]	6.0 (4.0)
% Psycho-active medication (yes/no)[d]	70/30	74/26	n/a
% Antidepressants	50%	37%	n/a
% Antipsychotics	20%	16%	n/a
% Anxiolytics	10%	21%	n/a

n/a = not applicable; BMI = body mass index; EDEQ = eating disorder examination-questionnaire, BIS = behavioral inhibition scale; CES-D = center for epidemiological studies-depression [a] vs. controls: $p \leq .001$ [b] $.002 \leq p \leq .007$. [c] vs. restrictive, $p \leq .01$; [d]Sometimes more than one medication type. Missing data: EDEQ: (1 AN-R, 2 AN-BP); BIS (1 AN-R, 2 AN-BP); CES-D (3 AN-R, 2 AN-BP).

positives may be preferable to erroneously rejecting potentially interesting findings. Data clustering was conducted using the *clustergram* function. Linear associations between predictor variables and methylation within the AN sample was assessed with robust regression using the *robustfit* function with a bisquare weight function. Robust regression was considered more appropriate than simple regression as not all probes conformed to assumptions of data normality, and spurious associations were detected due to high leverage datapoints—the robust procedure is less sensitive to these influences.

Pathway Analysis

Probes found to significantly differentiate the groups or that were associated with diagnosis, age of onset, indices of severity of illness at an FDR adjusted threshold of $Q = 0.05$ were subjected to pathway analysis. The gene names associated with each significant probe were entered into Ingenuity's Interactive Pathway Analysis tool (https://analysis.ingenuity.com/pa/). This tool was used with default parameters, and associated the lists of affected genes with (1) gene networks, (2) canonical pathways, and (3) known diseases and biological functions. Associations with p values smaller than .0005 were reported here.

Results

Data-screening/Quality Control

Examination of the quality control report generated by Minfi did not suggest any unusual samples, however, examination of mean methylation, and clustergrams of inter-subject correlations of methylation at all probes suggested that one participant (AN-BP type) was an outlier. Analyses were conducted both with and without this individual, and effects observed were not substantively altered. Analyses reported below are those in which data for the participant in question were excluded.

Descriptive Data

Demographic and clinical characteristics of individuals, accepted in the final sample are presented in **Table 1**. As expected, AN participants differed from NE participants on variables reflecting BMI, global eating-symptom severity and associated psychopathological traits. AN-restrictive and AN-binge/purge groups compared on most dimensions but, predictably in keeping with classical "restricter"/"binger" differences, AN-BP participants displayed more-pronounced impulsivity than did AN-R participants.

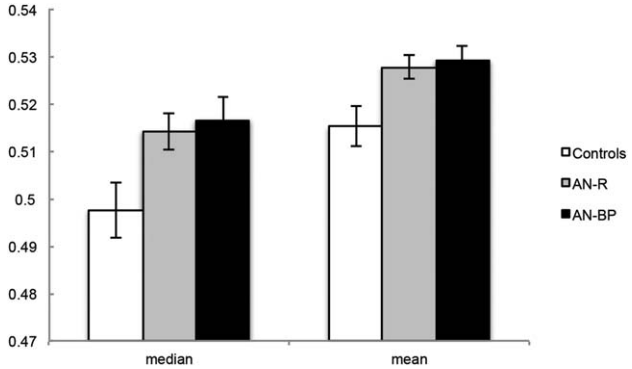
Group Differences in Global Methylation

We evaluated group effects on global methylation in the reduced dataset of 24,622 probes (**Fig. 1**) and found differences among means ($F_{2,41} = 4.91$, $p = .01$), medians ($F_{2,41} = 4.01$, $p = .02$), and standard deviations ($F_{2,41} = 4.38$, $p = .019$). Post-hoc tests showed the AN-R and AN-BP groups to both have higher mean values (AN-R vs. control: $p = .029$; AN-BP vs. control: $p = .005$) and median values (AN-R vs. control: $p = .052$; AN-BP vs. control: $p = .01$), and lower standard deviations than controls (AN-R vs. control: $p = .026$; AN-BP vs. control: $p = .01$). The means, medians and standard deviations did not differ between the AN subtypes (all p values $> .77$).

Group Differences at Individual CpGs

As the AN-R and the AN-BP subgroups appeared to have highly similar overall methylation levels, we collapsed these groups into one AN group for purposes of probe-wise comparisons of methylation levels. After controlling for the false discovery rate, we identified 14 probes on which significant

FIGURE 1. Global methylation levels (across 24,622 probes) for controls, Anorexia nervosa—restrictive and anorexia nervosa—binging purging subtype groups. Values are expressed as median (±SE) and means (±SE).



between-group differences were obtained (**Table 2**). These included two CpGs associated with the NR1H3 gene, and three associated with the PXDNL gene. These genes have been implicated with histone acetylation and RNA modification, cholesterol storage and lipid transport, and dopamine and glutamate signalling. Comparing AN participants who were or who were not medicated at the time of testing, we found differential methylation on only one probe, at cg09097916 on chromosome 5 ($p = 2.750\text{e-}06$, $Q = 0.037$). This probe is associated with the PDCD6 gene, involved in programmed cell death. Pathway analyses did not identify methylation differences in specific functional gene pathways related to AN.

Associations Between Individual CpGs and Age of Onset and Illness Duration

Age of illness onset correlated significantly with 12 probes (see Supporting Information Table S1). Seven of the 12 probes were associated with the gene GDF7, a growth factor involved in brain and spinal cord development, as well as SP6, involved in hair growth and development/maintenance of skin and teeth. We also observed associations between cumulative duration of illness and methylation levels at 142 probes (Supporting Information Table S2), including (but not limited to) genes related to immune function, behavior, liver function and metabolism. Current BMI was not significantly associated with DNA methylation levels.

Pathway Analyses of Hypervariable Genes. We found that age of illness onset was associated with gene pathways primarily involved in brain development and morphology. Duration of illness was associated with gene pathways associated with anxiety and social functioning and physical complications of AN, including bowel dysfunction, immune function and liver damage (see **Table 3**). Notably, among the probes that achieved significance, we found genes acting upon OXT and the 5-HT_{2A} receptor. The functioning of Oxytocin and the 5-HT_{2A} receptor has previously been quite strongly implicated in AN.[22] We will address this point further in the discussion.

Discussion

The present study is among the first to document altered DNA methylation in people with active AN (as compared to healthy normal-eater individuals), and is the first to compare "restricter" and "binger/purger" AN types on methylation indices. Although we observed no marked "restricter/binger" differences as to methylation levels, we did observe widespread differences as to methylation status in AN when compared to that in NE participants—in a variety of specific genes associated with histone acetylation and RNA modification, cholesterol storage and lipid transport, dopamine and glutamate signaling, and metabolism and weight loss. The latter findings suggest that methylation alterations have an interpretable functional significance related to the specific pathophysiology (physiological and psychological) of AN.

More importantly, several of our findings were indicative of epigenetically relevant effects of prolonged illness exposure. First, we found early age of disorder onset to be associated with more pronounced alterations of methylation levels in genes related to brain and spinal cord development, as well as physical characteristics affecting appearance of skin, teeth, and hair. Second, we found chronicity of illness to be associated with more marked alteration of methylation levels in genes and gene pathways involved in the regulation of anxiety and social functioning, synaptogenesis, brain morphology and spine development, neurocognitive function, immunity, and the functioning of peripheral organs (including liver, kidney, and bowel). The pattern of results obtained provides physical evidence of mechanisms that could underlie pervasive (and detrimental) consequences of AN upon bodily systems that are critical for nervous system functioning, psychological status and physical health. In other words, our findings suggest the presence of altered methylation levels in specific genes and gene pathways that could plausibly underlie specific developmental, psychological and medical consequences of AN. Furthermore, our results suggest that any such

TABLE 2. Results of *t* test comparing AN and normal-eater groups at each probe

P Value	*Q* Value	CpG Site	Chromosome	Gene Symbol	Mean Beta AN-Control
3.70e-05	0.0408	cg10493186	1	PRDM16	0.0739
4.57e-05	0.0455	cg01114124	2	HDAC4	0.0625
5.28e-05	0.0489	cg03556669	6	TNXB	0.0823
1.66e-05	0.0269	cg14422240	6	FTSJD2	0.0928
1.07e-05	0.0253	cg00269553	8	PXDNL	0.0783
4.85e-06	0.0253	cg20073313	8	PXDNL	0.1119
1.87e-05	0.0269	cg03371609	8	PXDNL	0.0682
3.78e-05	0.0408	cg04058153	8	DLGAP2	0.0648
1.07e-05	0.0253	cg05645982	8	FAM83A	0.0724
6.73e-06	0.0253	cg03732020	11	NR1H3	0.0653
4.70e-07	0.0061	cg09548275	11	NR1H3	0.0731
1.43e-05	0.0264	cg04514998	11	DDX10	0.0673
3.30e-05	0.0408	cg27652459	11	ARHGAP1	0.0669
1.17e-05	0.0253	cg13900773	12	PIWIL1	0.1306

effects may be especially pronounced in individuals who are chronically ill and/or who have had an early disorder onset.

Various studies in AN associate longer duration of illness with more-malignant consequences—including more severe eating-specific and comorbid psychopathological symptoms, poorer response to treatment, and increased mortality.[23–25] Various aspects of our findings suggest that more-prolonged illness, or the correlated feature of early illness onset ($r = -.52$, $p = .01$), may be associated with differential DNA methylation.

Illness chronicity was also associated with methylation in gene pathways relevant to some aspects of a behavioral phenotype characterized by social inhibition and anxiety. Findings implicating the OXT gene are compatible with known social and attachment deficits in AN,[26] findings from longitudinal studies showing poorer social functioning in those with a persistent ED,[27] and with findings of a candidate methylation gene study showing greater methylation levels in the OXT gene in AN patients.[11] Likewise, our finding of chronicity-linked alterations in methylation of the 5-HT_{2A} receptor gene is consistent with a well-established role in AN of the serotonin system[28] and of 5-HT_{2A} in particular (see Ref. [22). Notably, alterations in the functioning of the 5-HT system would be consistent with the widely demonstrated role of anxiety in the etiology and maintenance of AN.[29] We acknowledge, however, that our design renders it impossible to fully differentiate developmental effects owing to an early illness onset from disorder sequelae attributable to a long illness exposure.

Our data provided evidence of effects of early age of onset in the form of an association between early age of onset and greater methylation of GDF7 and WASF2. This association is of particular interest since these genes appear to have an important role in brain development. Although the functional role during the postnatal period of the genes in question is largely unknown, in the adult brain, GDF7 is primarily expressed in the motor cortex.[30] Given the overlap in distribution pattern with DA innervation, it has been suggested that this gene may play a role in maintaining the region-specific innervation of dopaminergic terminals[30]—and altered dopaminergic activity has been postulated to be a central factor in AN.[31] Other studies have shown that GDF7 is implicated in the embryonic development of spinal cord sensory neurons.[32] An additional association was found for WASF2, which is involved in the embryonic development of brain ventricles,[33] as well as in a variety of (postnatal) basic physiological cellular processes, such as T-cell receptor signalling.[34] Taken together, these findings may indicate that AN interferes with normal development, and point to a physical basis for the diverse cognitive, emotional, immunological, and behavioral sequelae that commonly co-occur in chronic AN. However, given the demonstrated role for the WASF2 and GDF7 gene for brain and spine development during the embryonic stage, yet, it is tempting to speculate that some specific alterations in these genes during pregnancy might put people at increased risk for AN.

Should molecular changes constitute the physical substrates for many medical sequelae in AN, then an intriguing possibility arises: As many medical complications of AN are reversible with weight restoration, it is of interest to establish whether remission of anorexic symptoms coincides with normalization (or resetting) of methylation levels, coincident with refeeding. Current work in our group is oriented toward exploring this question.

Our observation of epigenome-wide *hyper*methylation in women with AN is compatible with findings from several previous studies that have documented hypermethylation in candidate genes in eating-disorder patients.[11,35–37] Nonetheless, one

TABLE 3. Results of the gene-pathway ingenuity analyses of age of onset and disease chronicity

p value	Function	Gene symbol
Age of onset of first illness		
$p = 7.65e-03$	Brain development	GDF7,WASF2
Cumulative illness duration		
$p = 3.18e-04$	Synaptogenesis	GHSR, KALRN, NRXN1, OXT, PCDHB2, TNC
$p = 4.34e-04$	Anxiety	HTR2A, IDS, KALRN, OXT, SHANK2, TNC
$p = 4.69e-04$	Social behavior	KALRN, NRXNI, OXT, SHANK2
$p = 2.48e-05$	Contraction of the hindgut	GHSR, OXT
$p = 8.37e-04$	Antigen presentation pathway	HLA-DRBI, HLA-F, HLA-G
$p = 2.28e-04$	Abnormal morphology of the thymus medulla	IKZF1, ITPKB, TESPA1
$p = 8.71e-06$	(List) Hepatic fibrosis	CC2D2A, COL1A1, COL4A2, LTBP3, TLBP4, TNC
$p = 5.48e-04$	Dupuytren's contracture	ADAMTS6, COL1A1, COL4A2, COL5A3

genome-wide DNA methylation study in AN shows no global alterations in DNA methylation patterns compared to controls,[13] and two others report global *hypo*methylation in AN patients.[8, 14] We note that the patient sample in the Saffrey et al. 2014 study[13] was exceedingly small, and that Tremolizzo et al.[14] included only adolescent AN patients with the restrictive type, in whom methylation alterations, due to relatively shorter illness duration, may not have had time to accumulate. Moreover, all three studies used methylation assays that covered a much smaller portion of the genomic DNA than did the assay applied in our study.

Our study detected changes in DNA methylation in brain related genes using DNA derived from peripheral lymphocytes. DNA methylation patterns are involved in cell type specific gene regulations and therefore the common wisdom is that DNA methylation in peripheral lymphocytes should not occur in brain-related genes. However, the finding that methylation in brain-related genes measured in peripheral DNA is consistent with findings from many other studies suggesting association of peripheral DNA methylation profiles with brain related phenotypes. For example, we have shown that methylation of the 5-HT transporter in peripheral T cells is associated with in vivo measures of brain 5-HT synthesis.[38] Similarly we have shown overlap between DNA methylation profiles associated with maternal separation in rhesus monkeys in both the prefrontal cortex and T cells.[39] Likewise, in an eating-disordered context, using peripheral DNA, we have observed altered methylation levels in locations corresponding to such neuro-regulatory genes as DRD2, BDNF, and the glucocorticoid receptor (NR3C1), with the degree of alteration corresponding to level of manifest psychopathology.[35–37] More generally, epigenetic marks (especially those acquired during early development) show strong cross-tissue (blood-brain) correspondences, suggesting that changes in DNA methylation and gene expression in peripheral cells may reflect a peripheral component of the body-wide response to environmental signals that also alter DNA methylation in the central nervous system,[40] with putative consequences for further brain (developmental) processes.[41]

Strengths and Limitations

Although our sample size was modest, it was comparable to, or larger than, those of other methylation studies in AN patients, especially those that provide genome-wide analyses. Furthermore, our phenotypes were well characterized, and accounted for potential "restricter"/"binge-purger" differences. However, it is important to note that, as is the case for other methylation studies in AN patients, our design was cross-sectional. Consequently, we do not know whether the observed methylation differences are related to pre-existing characteristics that predispose individuals to AN, or are consequences of a prolonged course of AN. Although our findings often linked methylation differences to genes associated with state-like physical complications related to AN, and are in this sense most consistent with an interpretation couched in terms of disorder sequelae, longitudinal studies are needed to further disentangle cause and effect. As a proportion of our AN participants were on medication, we cannot fully rule out the possibility that some of the methylation differences between AN patients and controls we observed may be attributable to medication effects. Several studies demonstrate that pharmacological agents of the type that are frequently used as adjuncts in eating-disorder treatment--such as antidepressants[42–44]—affect methylation and other epigenetic marks. Nonetheless, observed associations between methylation patterns and AN diagnosis and chronicity, in the absence of any association between methylation and personality traits, argues in favor of a relatively specific link to the AN diagnosis per se.

Notwithstanding these limitations, the present study is among the first to show that AN is

associated with altered methylation patterns across the epigenome, and in specific sets of genes believed to be associated with anxiety, altered social functioning, and physical eating-disorder sequelae—and especially those that occur in the more chronically ill. Such findings imply that epigenetic marks may provide a useful correlate of possible eating-disorder sequelae that affect both the mental and physical status. If so, we speculate that such indices may serve as useful markers of disorder entrenchment and recovery. Further research is required to establish the clinical relevance of the observed effects, its diagnostic potential, and, importantly, to establish causality and putative reversibility of the effects in question.

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