One-carbon metabolism nutrients and DNA methylation in individuals with Anorexia Nervosa

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Abstract

Background: Anorexia Nervosa (AN) is a serious psychiatric illness, characterized by a phobia of weight gain resulting in low weight or malnutrition. DNA methylation, an epigenetic regulator of gene expression, is reportedly altered in AN at genes that influence diverse physiological and brain functions. DNA methylation relies on nutrients in the one-carbon metabolism pathway to donate methyl groups for the methylation of DNA. As DNA methylation is directly responsive to nutritional factors, nutrition-linked epigenetic alterations constitute a plausible etiological factor in AN.

Methods: We investigated the association between one-carbon metabolism nutrients and epigenome-wide DNA methylation levels in individuals with AN. We compared plasma levels of one-carbon metabolism nutrients (folate, B12, betaine, choline, methionine, DMG) across 53 individuals with active AN, 40 individuals remitted from AN, and 36 non-eating disordered individuals. We also compared epigenome-wide methylation patterns in a subset of individuals from each diagnostic group. Using general linear models, we then assessed whether or not the relationship between levels of one-carbon metabolism nutrients and DNA methylation differed across active AN, remitted, and non-eating disordered groups.

Results: Group comparisons on nutrient levels indicated that individuals with active AN exhibited elevated levels of B12 compared to non-eating disordered individuals and possibly, elevations of betaine levels compared to non-eating disordered and remitted individuals. Group comparisons of methylation levels indicated that, compared to non-eating disordered and remitted individuals, individuals with AN exhibited altered methylation levels (at 8 and 10 sites, respectively; $Q<0.01$), that corresponded to genes relevant to physiological processes such as cell-growth and metabolism of lipids and glucose. Finally, preliminary results indicated that

individuals with AN exhibited a stronger association than did non-eating disordered individuals between levels of some one-carbon metabolism nutrients (i.e., B12 and methionine) and methylation at several probes—many of which corresponded to genes relevant to psychiatric, physiological, and immunological functioning. Compared to remitted individuals, individuals with AN also exhibited an altered association between B12 and methylation levels, with the effects at several probes pointing to a stronger association in individuals with AN.

Conclusions: Findings from the present thesis indicate that individuals with AN may exhibit unexpected elevations in plasma levels of one-carbon metabolism nutrients, as well as an altered correspondence between one-carbon metabolism nutrients and DNA methylation. Such anomalies in people with active AN may be attributable to nutritional adaptations that develop as a consequence of illness. Investigations into the nutritional epigenomics in AN may contribute to more precise etiological models of the disorder and, eventually, to novel nutritional or pharmacological treatments.

Résumé Vulgarisé

Contexte: L'anorexie nerveuse (AN) est une maladie psychiatrique grave caractérisée par une phobie de la prise de poids entraînant un faible poids ou une malnutrition. La méthylation de l'ADN, un régulateur épigénétique de l'expression des gènes, serait altérée au niveau des gènes qui influencent diverses fonctions physiologiques et cérébrales dans l'AN. La méthylation de l'ADN s'appuie sur les nutriments contenus dans la voie du métabolisme à un carbone pour donner des groupements méthyle pour la méthylation de l'ADN. Comme la méthylation de l'ADN est associé aux facteurs nutritionnels, les altérations épigénétiques liées à la nutrition constituent un facteur étiologique plausible chez l'AN.

Méthodes: Nous avons étudié l'association entre les nutriments du métabolisme à un carbone et les niveaux de méthylation de l'ADN à l'échelle de l'épigénome des personnes atteintes d'AN. Nous avons comparé les taux plasmatiques de nutriments du métabolisme monocarboné (folate, B12, bétaïne, choline, méthionine, DMG) chez 53 personnes ayant présentement un diagnostic d'AN, 40 personnes remis d'une AN et 36 personnes n'ayant jamais souffert de troubles de la conduite alimentaire (TCA). Nous avons également comparé les modèles de méthylation à l'échelle de l'épigénome dans un sous-ensemble d'individus appartenant à chaque groupe diagnostique. En utilisant des modèles linéaires généraux, nous avons ensuite évalué si la relation entre les niveaux de nutriments du métabolisme à un carbone et la méthylation de l'ADN était différente entre les groupes.

Résultats: Les comparaisons de groupe sur les niveaux de nutriments ont indiqué que les individus avec une AN présentaient des niveaux élevés de B12 par rapport aux individus qui ne souffraient pas de troubles alimentaires, et possiblement, des niveaux de bétaïne élevés comparés aux individus n'ayant jamais eu de TCA et aux individus remis d'une AN. Les comparaisons de

groupe sur les niveaux de méthylation ont montré que, comparés aux individus n'ayant jamais eu de TCA et ceux remis d'une AN, les individus avec une AN présentaient des niveaux altérés de méthylation (à 8 et 10 sites, respectivement; Q <0,01), ce qui correspond à des gènes pertinents pour les processus physiologiques, tels que la croissance de la cellule et le métabolisme des lipides et du glucose. Enfin, les résultats préliminaires indiquaient que les individus avec une AN présentaient une association plus forte que les individus n'ayant jamais eu de TCA entre les niveaux de certains nutriments du métabolisme monocarboné (B12 et méthionine) et la méthylation à plusieurs sondes, dont plusieurs correspondaient à des gènes impliqués dans le fonctionnement psychiatrique, physiologique et immunologique. Comparés aux individus remis d'une AN, les individus avec une AN présentaient également une association altérée entre les niveaux de B12 et de méthylation, et pour plusieurs sondes, l'effet de cette association était plus importantes pour les individus avec une AN.

Conclusions: Les conclusions de la présente thèse indiquent que les personnes atteintes d'AN peuvent présenter des élévations inattendues des taux plasmatiques de nutriments du métabolisme monocarboné, ainsi qu'une correspondance modifiée entre les nutriments du métabolisme monocarboné et la méthylation de l'ADN. De telles anomalies chez les personnes atteintes d'AN pourraient être attribuées à des adaptations nutritionnelles résultant de la maladie. Les recherches sur l'épigénomique nutritionnelle dans le traitement de l'anorexie nerveuse pourraient contribuer à l'élaboration de modèles étiologiques plus précis du trouble et, éventuellement, à la mise au point de nouveaux traitements nutritionnels ou pharmacologiques.

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Contribution of Authors

I, Jessica Burdo, developed the research hypotheses, helped collect and enter participant data, conducted statistical analyses, and wrote the manuscript of this thesis. Howard Steiger helped develop the research hypotheses and fine-tune my interpretation of the data. He also read several drafts of my thesis and provided instrumental feedback. Linda Booij provided guidance on statistical design and analysis, and reviewed various drafts of my thesis. Howard Steiger and Linda Booij designed the overall investigation and are co-principal investigators on the study grant. Lea Thaler reviewed statistical analyses and interpretations. Esther Kahan oversaw data entry and management. Esther Kahan, Emilie Fletcher, Charlotte Corran, and Audrey Mariamo helped conduct participant interviews, and Xing Dai performed participant blood-draws. Adam Torkaman-Zehi (Rhida Joober lab) extracted DNA and prepared DNA methylation plates, which were analyzed at the Genome Quebec Innovation Centre. With my participation, Kevin McGregor conducted the analyses using R. Evan Nitschmann, Luis Agellon and Linda Wykes supervised the biochemical assays.

Anorexia Nervosa (AN) can severely disrupt the medical, psychological, and social well-being of individuals affected by the disorder. Even with evidence-based treatment, many individuals with AN do not show substantial improvement (Steiger, 2017). Investigations into the etiological factors in AN may pave the way to development of novel treatments with more favourable outcomes. One neurobiological factor that may provide insight into the etiology of AN is DNA methylation. As DNA methylation is a nutritionally-responsive mechanism, it may constitute a meaningful target of investigation in individuals with AN – given that they are, by definition, a malnourished population.

Phenomenology of Anorexia Nervosa

Anorexia Nervosa (AN), is a psychiatric illness characterized by low weight and weight-gain phobia. Lifetime prevalence estimates range from 0.9% to 2.2% for females – generally 10-times greater than the corresponding estimates for males (Hudson, Hiripi, Pope, & Kessler, 2007; Keel, 2017). Individuals with AN struggle to maintain normal weight—as defined by low measures of body mass index (BMI; American Psychiatric Association, 2013). Difficulty maintaining normal weight stems from a phobic avoidance of weight gain that motivates such behaviours as restricting food intake, purging (e.g., vomiting, laxative misuse) and compulsive exercise. There are two subtypes of AN: restricting type (AN-R) and binge eating/purging type (AN-B/P). Individuals with AN-R and AN-B/P both restrict their food intake, however, individuals with AN-BP also engage in regular binging and/or purging (American Psychiatric Association, 2013).

In addition to eating-specific disturbances, individuals with AN may experience other challenging symptoms. For instance, affect and self-worth may be disproportionately dependent on shape or weight (Gordon, Holm-Denoma, Douglas, Crosby, & Wonderlich, 2017).

Individuals with AN may also exhibit concurrent psychiatric conditions (e.g., mood and anxiety disorders; Halmi, 2017; Hudson et al., 2007; Kaye, Bulik, Thornton, Barbarich, & Masters, 2004), and experience difficulties with affect regulation and social behaviour (Ruscitti, Rufino, Goodwin, & Wagner, 2016; Tasca & Balfour, 2014).

Unfortunately, AN can have devastating consequences for those affected. AN has the highest mortality rate of any psychiatric illness, estimated to be about 5% per decade of illness. One in five AN-related deaths is due to suicide (Arcelus, Mitchell, Wales, & Nielsen, 2011). AN is also associated with various medical complications (Strober, Freeman, & Morrell, 1997). Almost every organ-system is adversely affected by AN-induced malnutrition including the heart, lungs, gastrointestinal tract, bones, and skin (Mehler, 2017). AN may also impede on social functioning (Patel, Tchanturia, & Harrison, 2016). Studies suggest that individuals with AN have social networks that are smaller (Doris, Westwood, Mandy, & Tchanturia, 2014) and of worse quality (Tiller et al., 1997) than those of individuals without an eating disorder. Despite the severe psychiatric, medical, and social consequences of AN, treatment outcomes are poor; only half of patients achieves remission, while 20% maintain threshold-level illnesses (Keel, 2017; Steinhausen & Jensen, 2015). It is hoped that a better understanding of AN etiology will improve treatment outcomes in individuals affected by the disorder.

Etiology of Anorexia Nervosa

AN is understood to have multiple determinants including genetic factors (which influence the regulation of emotions, personality traits, susceptibility to reward, metabolism, appetite and more), environmental stressors during perinatal, developmental and later-life periods, and social encouragement of weight-control and caloric restriction. Consistent with a multidimensional etiological concept, genetic and environmental factors have both been

implicated in causality (Hübel, Marzi, Breen, & Bulik, 2018). It is postulated that environmental experiences may "activate" genetic susceptibilities to AN (Steiger & Thaler, 2016). The preceding concept will be explored in more detail below.

Genetic factors. Molecular studies have attempted to identify so called genetic risk variants (Shih & Woodside, 2016). For example, candidate-gene studies have examined genes that may be relevant to anorexic phenotypes according to pre-existing theory (Pinheiro, Root, & Bulik, 2009). Differences in allelic variation between people with and without AN have been observed in genes regulating serotonin (implicated in for example mood and appetite regulation), and dopamine (implicated in for example reward, affect, and feeding behaviour). For instance, several meta-analyses have reported that the S-allele of the of the serotonin-transporter-linked polymorphic region (5-HTTLPR), may be associated with an increased risk of AN (e.g., Calati, Ronchi, Bellini, & Serretti, 2011; Gorwood, 2004; Lee & Lin, 2010). Other studies have reported an association between polymorphisms in the serotonin 1D receptor gene (*HTR1D*) and AN (Bergen et al., 2003; Brown et al., 2007). With respect to dopamine-related genes, reports suggest that AN may associated with polymorphisms of the dopamine receptor D2 gene (*DRD2*; Bergen et al., 2005), as well as the D4 receptor gene (*DRD4;* Bachner-Melman et al., 2007). Candidate gene studies have also investigated the potential role of genes implicated in hunger and energy metabolism in AN, such as those that encode the agouti-related peptide (*AGRP*) and ghrelin *(GHRL*). However, findings to this effect are inconclusive (Rask-Andersen, Olszewski, Levine, & Schiöth, 2010).

Linkage studies, which analyze the transmission of a disorder within affected families, have implicated multiple genomic regions in the development of AN. One study reported that a locus on chromosome 1 was associated with the restricting type of AN (Grice et al., 2002). A

related study identified additional loci on chromosomes 1, 2 and 13 (Devlin et al., 2002). Two regions on chromosome 1 that were significantly associated with AN include genes that encode serotonin and cannabinoid receptors, respectively involved in appetite regulation, and food intake (Chen et al., 2013; Ishiguro et al., 2010).

Notwithstanding the insights provided by candidate gene and linkage studies, genome-wide association studies (GWASs) are the preferred method for genetic investigations (Tam et al., 2019). As the name implies, a GWAS surveys the entire genome for significant variations as opposed to specific, pre-selected genes (Bulik, Slof-Op't Landt, van Furth, & Sullivan, 2007). To date, two published GWASs on AN have yielded significant results. The first study (Duncan et al., 2017) included almost 3 500 individuals with AN and spanned over 10 million single-nucleotide polymorphisms (SNPs). Findings reported a significant association between AN and a locus on chromosome 12, which was previously linked to type-1 diabetes and rheumatoid arthritis (both auto-immune conditions; Barrett et al., 2009; Okada et al., 2014). The authors also reported significant genetic correlations between AN and psychiatric traits (e.g., neuroticism and schizophrenia), as well as metabolic indices (e.g., cholesterol, glucose, and body-mass index). Findings were recently extended by a second GWAS (Watson et al., 2019) that included almost 17, 000 individuals with AN and over 55, 000 healthy controls. The authors identified eight significant genetic loci and reported associations between AN and psychiatric traits (e.g. depression), metabolic indices (e.g. fat mass), and indices of physical activity. Findings from these GWASs corroborate the notion that the etiology of AN implicates genes associated with psychiatric and metabolic functioning.

Although findings from the studies reviewed seem promising, our knowledge of genetic contributions to AN remains limited. Watson et al. (2019) , for example, utilized precise

statistical methods and a substantial sample size in their GWAS yet their estimate of SNP-based heritability (11-17%) accounts for only a proportion of the heritability estimates of AN in twin studies (i.e., 74% at their upper limit; Yilmaz, Hardaway, & Bulik, 2015). More studies are needed to elucidate the remaining genetic variance in AN.

Environmental factors. In addition to genetic risk factors, environmental exposures throughout the lifespan have been implicated in the development of AN. For instance, maternal stress during pregnancy is associated with an increased risk of AN in offspring (St-Hilaire et al., 2015). Perinatal exposures such as premature birth, and low gestational weight may also increase the risk of developing AN later in life (Cnattingius, Hultman, Dahl, & Sparén, 1999; Favaro, Tenconi, & Santonastaso, 2006). A variety of environmental experiences during childhood and adolescence are likewise associated with AN-risk. For example, studies suggest that experiencing familial conflict or high parental expectations may increase one's risk of developing AN (Karwautz et al., 2001; Pike et al., 2008). Childhood trauma such as physical and sexual abuse is also believed to be a risk factor (Treasure, Claudino, & Zucker, 2010).

Environmental factors that promote dietary restriction and the desire to lose weight, may be especially associated with the risk of developing AN. In particular, exposure to, and the internalization of the thin ideal is often cited as strong risk factor of AN (Keel & Forney, 2013; Striegel-Moore & Bulik, 2007). Media content may promote unattainable standards of beauty, such as extreme thinness. Peer groups that judge and ridicule members' weights may introduce additional pressure to be thin (Fairweather-Schmidt & Wade, 2017; Stice, Maxfield, & Wells, 2003; Striegel-Moore & Bulik, 2007). The thin ideal may thus decrease feelings of satisfaction towards one's body and increase the desire to change it (Stice, Spangler, & Agras, 2001). As a

result, an individual may decide to start dieting, which often precipitates the onset of AN (Steiger & Thaler, 2016)

Genetic and environmental interaction effects. In keeping with a gene x environment interaction concept, twin studies suggest that genetic effects account for 48% to 74% of the variance in risk of AN, while the remaining variance is attributable to environmental exposures (Yilmaz et al., 2015). Of note, twin studies underscore the role of environmental encouragements towards a thin appearance, suggesting that eating disorder risk is increased when genetically predisposed individuals engage in extreme caloric restraint (Racine, Burt, Iacono, McGue, & Klump, 2011). Likewise, candidate-gene studies that explore gene-environment interaction (GxE) effects also suggest that the risk associated with environmental exposures may depend on an individual's genotype. The number of studies that have investigated GxE effects in individuals with AN is substantially lower than in individuals with other psychiatric disorders, including other eating disorders such as Bulimia Nervosa. Yet, one study investigated whether or not environmental risk factors interact with a polymorphism of the serotonin transporter gene (Karwautz et al., 2011). Findings suggested that experiencing problematic parenting styles was associated with the risk of developing AN, especially in individuals with the S-allele of the 5-HTTLPR polymorphism. Another study in mice reported that the interaction between a polymorphism of the *BDNF* gene and two environmental exposures (social isolation and caloric restraint) increased the likelihood of behaviours that were analogous to those seen in AN—running to exhaustion on a treadmill in favor of eating (Madra & Zeltser, 2016).

Epigenetic Regulation

Fairly recent theories on GxE effects have implicated epigenetic mechanisms in disease risk (Meaney, 2010; Szyf, 2007). Epigenetic mechanisms such as DNA methylation, chromatin

remodelling, and histone tail modification, are processes that can modify gene expression without altering the DNA sequence itself (Portela & Esteller, 2010). Epigenetic mechanisms are also believed to be environmentally responsive. As such, epigenetic mechanisms may provide a physiological mechanism for gene-environment interaction effects (Toyokawa, Uddin, Koenen, & Galea, 2012).

DNA methylation. The most commonly studied epigenetic mechanism is DNA methylation—a process by which methyl groups are attached to cytosine bases in CpG dinucleotides (Inbar-Feigenberg, Choufani, Butcher, Roifman, & Weksberg, 2013). In general, DNA methylation at the promotor region of a gene inhibits transcription, resulting in attenuated gene expression (Szyf, 2007). Several studies show that environmental impacts affect DNA methylation processes, and consequently, influence phenotypes via modified gene expression. For example, one study reported that rat pups who experienced low levels of maternal nurturance in the first week of life showed increased levels of methylation at the glucocorticoid receptor compared to rat pups who experienced high levels of maternal nurturance (Meaney & Szyf, 2005). The neglected rat pups showed reduced expression of the glucocorticoid receptor, and consequently, a heightened stress response. The preceding findings were translated to a human study that investigated methylation levels at the glucocorticoid receptor in post-mortem hippocampal tissue of individuals who committed suicide and experienced childhood abuse (McGowan et al., 2009). Individuals who committed suicide and were abused showed increased methylation levels and decreased expression of the glucocorticoid receptor, compared to controls and those who committed suicide but were not abused. Several other studies have investigated DNA methylation levels in relation to psychiatric illness (e.g., Liu, Jiao, Wang, & Yuan, 2018). Many such studies focus on disorders such as schizophrenia (e.g., Nishioka, Bundo, Kasai, &

Iwamoto, 2012) or bipolar disorder (e.g., Fries et al., 2016) while relatively few have focused on eating disorders, or AN in particular (Hübel et al., 2018). Studies that have investigated DNA methylation in individuals with AN are reviewed below.

Global methylation. Studies examining global methylation levels (over the entire genome, or over selected genomic regions) in people with AN have produced inconsistent (and sometimes contradictory) findings. Two studies showed that, compared to healthy controls, individuals with AN exhibited decreased levels of methylation (Liu, Jiao, Wang, & Yuan, 2018), while another study observed increased levels (Booij et al., 2015). Yet another study failed to detect any significant differences in global methylation levels between individuals with AN and healthy controls (Saffrey, Novakovic, & Wade, 2014). The divergent findings may be attributed to the varied methods of measurement or differences in clinical samples used in these studies. Regardless, insights from global methylation measures may be limited as they do not provide information on specific regions that contribute to disease (Hübel et al., 2018).

Studies of methylation in candidate genes. Other studies have investigated DNA methylation levels at sites corresponding to genes with a theoretical link to AN. Findings from such studies have suggested that, compared to healthy controls, individuals with AN exhibit hypermethylation at sites associated with alpha synuclein (linked to folate sensitivity; Frieling et al., 2007), as well as with dopamine neurotransmission (Frieling et al., 2010). Another study observed hypermethylation in individuals with AN at a site corresponding to the oxytocin receptor (associated with attachment and emotional functioning; Kim, Kim, Kim, & Treasure, 2014). The preceding findings provide initial support for the role of altered methylation profiles in the etiology of AN. However, results of candidate gene studies need to be interpreted with

caution as they are invariably based on underpowered sample, rendering findings notoriously hard to replicate.

Epigenome-wide association studies. The "gold standard" of epigenetic research is epigenome-wide association studies (EWASs) that interrogate the entire epigenome for disorder-relevant variations (Yilmaz et al., 2015). Only three published EWASs have investigated site-specific methylation levels in individuals with AN.

One epigenome-wide study by our own group (Booij et al., 2015) compared individuals with active AN to non-eating disordered individuals. Analyses yielded 14 differentially methylated sites. Many of the implicated sites corresponded to genes that may be relevant to physiological functioning in AN. For example, two of the differentially methylated sites corresponded to the *NR1H3* gene, which is associated with cholesterol and lipid-related functions. In addition, within individuals with AN, the age of illness onset and the chronicity of illness were both associated with methylation levels. One of the 12 sites that were associated with the age of illness onset corresponded to the *SP6* gene, which is involved in the maintenance of hair, skin, and teeth– features that may deteriorate with long-standing exposure to AN. Among 142 differentially methylated sites that were associated with the chronicity of illness, some corresponded to genes involved in psychiatric functioning including neurotransmitter systems previously implicated in the etiology of AN, such as oxytocin (Kim et al., 2014) and serotonin (Devlin et al., 2002; Grice et al., 2002; Karwautz et al., 2011). Further associations were observed between chronicity of illness and genes involved in AN-relevant domains such as anxiety, social behaviour, immunity, and organ functioning.

A subsequent investigation by our group (Steiger et al., 2019) compared site-specific methylation levels across individuals with active AN, individuals remitted from AN, and

individuals with no eating disorder. Those with active AN exhibited many differentially methylated sites compared to either comparison group. Some of the implicated sites corresponded to genes associated with psychiatric functioning (e.g., serotonin mechanisms), physiological functioning (e.g., lipid and glucose processes), immune functioning, and bone health. Notably, 28 probes were common to active versus non-eating disordered and active versus remitted comparisons. The direction of methylation effects at such probes were in opposite directions: individuals with active AN exhibited increased methylation compared to individuals that were non-eating disordered and individuals remitted from AN exhibited decreased methylation compared to individuals with active AN. The preceding suggests, encouragingly, that altered methylation levels in individuals with AN may be "reset" once remission is achieved (Steiger et al., 2019). Additionally, we observed that remitted and non-eating disordered individuals did not exhibit any significant differences in methylation levels, indicating comparable epigenetic profiles in these control groups. In the same study, we observed that, within individuals with AN, chronicity of illness was associated with methylation levels at sites corresponding to genes linked to serotonergic and glutamatergic neurotransmission (e.g., *5-HT*$_{2A}$ and *GRM2*) as well as wound healing and connective tissue disorders (*TNXB*; Kaufman & Butler, 2016; Zweers et al., 2003).

One other epigenome-wide study examined site-specific methylation among individuals with AN and each of two control groups—healthy population individuals and "lean" individuals with a low BMI but no AN (Kesselmeier et al., 2018). The authors observed 81 differentially methylated sites in comparisons between AN and population groups, and 51 differentially methylated sites between AN and lean groups. One differentially methylated site that was observed between individuals with AN and population groups, corresponds to the *CSGALNACT1*

gene (associated with cartilage and bone-tissue development). The authors suggested that within individuals with AN, altered methylation at the *CSGALNACT1* gene, may contribute to decreased bone strength (a common symptom observed in AN-patients). This latter finding complements our own study by showing that some differentially methylated sites between AN and control groups correspond to genes associated with bone health (Steiger et al., 2019). Lastly, the authors observed effects for the *NR1H3* and *TNXB* genes, which were also isolated in our own studies (Booij et al., 2015; Steiger et al., 2019).

All three EWASs that have investigated site-specific methylation levels in individuals with AN showed alterations compared to individuals who are not ill. In particular, findings complement the results of a GWASs described previously (Duncan et al., 2017) by implicating sites that correspond to genes associated with psychiatric, physiological, and immunological domains. Findings are consistent with the idea that DNA methylation processes are relevant to AN. Further work is needed to investigate the factors that may impact DNA methylation levels in individuals with AN.

One-Carbon Metabolism Pathway

Nutritional status is known to have a direct and powerful influence upon DNA methylation levels (ElGendy, Malcomson, Lara, Bradburn, & Mathers, 2018). The pathway for such influences involves the one-carbon metabolism pathway, in which specific nutrients, such as folate and methionine, donate methyl to allow for DNA methylation (see Figure 1). Alongside specialized enzymes, the nutrients in the one-carbon metabolism pathway facilitate several biochemical reactions and generate S-adenosylmethionine (SAM), the universal methyl donor (Anderson, Sant, & Dolinoy, 2012; Niculescu, Craciunescu, & Zeisel, 2006; Selhub, 1999). In the final step of one-carbon metabolism, DNA methyltransferase enzymes (DNMTs) bind methyl

groups from SAM to CpG sites, catalyzing 5-methyl cytosine: the product of methylated DNA (Anderson et al., 2012; Niculescu et al., 2006).

Studies investigating the association between nutrition and disease have assessed how levels of one-carbon metabolism nutrients impact DNA methylation levels (Anderson et al., 2012; Canani et al., 2011). In general, DNA methylation relies on the availability of nutrients that are methyl-donors (i.e., folate, B12, betaine, choline, and methionine). However, the relationship between levels of nutrients involved in the one-carbon metabolism pathway and methylation levels is not always straightforward; deficiencies in nutrients are not always associated with decreased methylation levels, and an excess of nutrients is not always associated with increased methylation levels (Anderson et al., 2012).

Folate. Folate is a water-soluble B-vitamin that is obtained through diet, or via synthetic folic acid supplementation (Iyer & Tomar, 2009). In the one-carbon metabolism pathway (See Figure 1), folate is first converted to dihydrofolate (DHF), then to tetrahydrofolate (THF): a highly versatile one-carbon donor (Newman & Maddocks, 2017). In its active form (5-methyltetrahydrofolate), THF donates a methyl group, thereby enabling the conversion of homocysteine to methionine (Anderson et al., 2012). One study reported that folate intake was significantly associated with methylation levels at 1413 probes in breast cancer tumour cells (Christensen et al., 2010). Many implicated probes corresponded to genes previously linked to cancer. Other reports suggest that folate deficiency is associated with global hypomethylation in human lymphocyte DNA (Jacob et al., 1998; Rampersaud, Kauwell, Hutson, Cerda, & Bailey, 2000). Another study involving individuals diagnosed with colorectal cancer reported that folate supplementation was associated with increased global methylation levels in leukocyte DNA (Pufulete, 2005). In contrast, individuals free of colorectal cancer exhibited *decreased* global

methylation levels after receiving folate supplements (Pufulete et al., 2005). The latter discrepancy suggests that the relationship between levels of methyl donors and global methylation levels may vary by population. The preceding underscores the importance of accounting for potential clinical group effects when assessing the relationship between nutrients in the one-carbon metabolism pathway and DNA methylation levels.

Methionine. Methionine, an essential amino acid obtained through the diet, is involved in the building of proteins, and in immunological, metabolic, and digestive processes (Martínez et al., 2017). In the one-carbon metabolism pathway (See Figure 1), methionine can be metabolized from homocysteine, and is a precursor to SAM (Anderson et al., 2012). One study showed that methionine levels were associated with methylation at sites corresponding to genes implicated in various types of cancer (Vineis et al., 2011). The authors observed that associations between methionine levels and methylated sites in former smokers differed from associations between methionine levels and methylated sites in non-smokers (Vineis et al., 2011). One again, the preceding highlights that the relationship between nutrients in the one-carbon metabolism pathway and DNA methylation levels may be moderated by group.

B12. B12, another water-soluble nutrient obtained through the diet, is a coenzyme in the one-carbon metabolism pathway (See Figure 1) and a precursor to methionine synthase, an enzyme that helps convert homocysteine into methionine (Miller, 2003). B12 is involved in the synthesis of myelin and the maintenance of red blood cells (Briani et al., 2013; Olsen et al., 2009). One study reported that maternal serum B12 levels were associated with methylation levels both in maternal-blood and cord-blood at sites corresponding to the insulin-like growth factor (IGF2) gene (Ba et al., 2011). A study with rodents reported that B12 deficiency was associated with hypomethylation in the mucous membrane of the colon (Choi et al., 2004).

Given that B12 is an essential cofactor in one-carbon metabolism pathway reactions, it is unsurprising that studies have found a link between B12 and DNA methylation levels.

Choline. Choline is obtained through the diet, is a building block for acetylcholine, and essential to lipid metabolism and cell-membrane integrity (Zeisel, 2004). In the one-carbon metabolism pathway (See Figure 1), choline is oxidized into betaine which serves as a methyl donor during the conversion of homocysteine into methionine (Holm, Ueland, Kvalheim, & Lien, 2003). One study examining liver cancer cells (Jiang, Greenwald, & Jack-Roberts, 2016), reported that choline supplementation tended to be associated with global hypermethylation (Jiang et al., 2016). In two rodent studies, maternal choline deficiency was associated with hypomethylation in the fetal brain (Kovacheva et al., 2007; Niculescu et al., 2006), but with hypermethylation in the liver—supporting the notion that associations between levels of one-carbon metabolism nutrients and methylation are tissue-specific.

Betaine. Betaine is obtained through the diet and synthesized via choline. In the one-carbon metabolism pathway (See Figure 1), betaine donates a methyl group during the conversion of homocysteine into methionine (Holm et al., 2003). Betaine can also be metabolized into dimethylglycine (DMG), another nutrient in the one-carbon metabolism pathway. One study observed that rats receiving betaine supplementation exhibited an improvement in fatty liver. The authors attributed the improvement to restored methylation and gene expression levels (Wang et al., 2014). Another study observed that chickens fed a betaine-supplemented diet exhibited altered methylation levels at multiple CpG sites in adipose tissue (Xing, Kang, & Jiang, 2011). Finally, a study also investigating adipocytes in chickens reported that betaine supplementation was associated with site-specific hyper and hypomethylation (Konycheva et al., 2011). Although studies suggest an association between betaine and DNA methylation levels,

human studies are needed to better understand the relationship. Previous studies on the link between one-carbon metabolism nutrients and DNA methylation have largely focused on folate and methionine, while nutrients such as betaine and DMG have received less attention.

Association of one-carbon metabolism nutrients with DNA methylation levels in AN. To our knowledge, only two studies have examined the association between one-carbon metabolism nutrients and DNA methylation levels in individuals with AN. Both studies utilized indices of global methylation levels. One such study by Tremmolizo and colleagues (2014) found no correlation between plasma levels of homocysteine, B12, and folate and global methylation levels. Another study by Frieling et al. (2007) showed that individuals with AN who had high levels of homocysteine displayed low global methylation levels. Studies that investigate site-specific methylation levels may provide further insight into the relationship between nutrients in the one-carbon metabolism pathway and DNA methylation processes.

Nutrient levels in individuals with AN. In individuals with AN, levels of the nutrients involved in the one-carbon metabolism pathway are not well established. The majority of nutrition-related studies in AN focus on micronutrients that are not involved in one-carbon metabolism, or on macronutrients such as fats, and carbohydrates (Chiurazzi et al., 2017; Setnick, 2010). Among those studies that do examine one-carbon metabolism nutrients, some suggest that individuals with AN have a low intake of (and consequent deficiency in) folate, although findings to this effect are mixed (Hadigan et al., 2000; Setnick, 2010). One study observed that plasma levels of B12 were elevated in patients with AN compared to healthy controls (Corbetta et al., 2015). Another study comparing choline levels in cerebrospinal fluid between groups with and without AN reported that choline levels were not significantly different; however, in individuals with AN, choline levels were inversely correlated with illness

severity (Gerner et al., 1984). Finally, some studies have shown that individuals with AN exhibit elevated levels of methionine and other amino acids (hyperaminoacidemia; Moyano, Vilaseca, Artuch, & Lambruschini, 1998).

Relationship between one-carbon metabolism nutrients and psychological symptoms. Various findings have supported the idea that levels of one-carbon metabolism nutrients are linked to psychological symptoms. For instance, studies associate deficiencies in folate and B12 with symptoms of depression (e.g., Coppen & Bolander-Gouaille, 2005). One study suggested that individuals with chronic depression exhibit elevated frontal-lobe choline levels (Portella et al., 2011). Another study suggested that combined supplementation of betaine and SAM may be an effective therapy-adjunct for individuals who have a poor response to anti-depressants (Di Pierro, Orsi, & Settembre, 2015). Animal studies have shown an association between diets deficient in folate, methionine, and choline and anxiety symptoms (Konycheva et al., 2011; Tomizawa et al., 2015). Finally, one study showed that mice subjected to a folate-deficient diet exhibited increased impulsive behaviours compared to mice fed a folate-sufficient diet (Ash et al., 2013). To date, there has been no demonstration that variations in levels of one-carbon metabolism nutrients are associated with psychopathological indices in individuals with AN.

The Present Thesis

This thesis explored the pathway between nutrients involved in one-carbon metabolism and DNA methylation levels in individuals with AN. Therefore, we compared plasma nutrient levels and DNA methylation levels between three groups: individuals with active AN (AN-Active), individuals who were remitted from AN (AN-Remitted), and non-eating disordered (NED) individuals. We then assessed whether or not the relationship between levels of one-carbon metabolism nutrients and DNA methylation differed across these groups. In other words,

this thesis investigates the correspondence between nutritional state and DNA methylation profiles as a function of AN diagnosis.

Procedures in all studies were approved by the Douglas Mental Health University Institute Research Ethics Board. All participants provided written consent prior to study participation.

Study 1

Goals and Hypotheses

Investigations of the factors that contribute to DNA methylation patterns, such as the one-carbon metabolism pathway, may provide insights into disease risk. This study aimed to establish whether or not levels of the nutrients involved in the one-carbon metabolism pathway differed in individuals with active AN, compared to levels in individuals in two control groups. To do so, we compared plasma levels of folate, B12, choline, betaine, methionine and DMG across AN-Active, AN-Remitted and NED individuals. In AN-Active individuals we also assessed whether or not nutrient levels differed between individuals with AN-restrictive and binge/purge types. Finally, we assessed whether or not nutrient levels were associated with self-reported symptoms of eating-disorders, depression, anxiety, and impulsivity.

Given that AN is associated with food restriction and general malnutrition, we anticipated that AN-Active individuals would exhibit significantly lower plasma levels of folate, B12, choline, and betaine, methionine and DMG compared to AN-Remitted and NED individuals. Considering the link between nutrient levels and psychiatric symptoms that has been purported in the literature, we also anticipated that nutrient levels would be significantly associated with symptoms of eating disorders, depression, anxiety, and impulsivity.

Methods

Participants. Individuals with active AN recruited at the Eating Disorders Continuum at the Douglas Mental Health University Institute included 53 women who were diagnosed as having AN according to the DSM-5 and had a Body Mass Index (BMI) of ≤17.5 (AN-Active group). Of these, 30 were diagnosed as having AN-restrictive type and 23 were diagnosed as having AN-binge/purge type. Diagnoses were based on the Eating Disorders Examination (EDE) interview (Fairburn, Cooper, & O'Connor, 2008). We also recruited 40 women who once met full DSM-5 criteria for AN, but who no longer met criteria for an eating disorder (according to the EDE interview) and had maintained a relatively normal BMI for at least 1 year (AN-Remitted group). Specifically, all individuals in the AN-Remitted group had a BMI of at least 18.5 (commonly considered normal weight; World Health Organization) with the exception of one individual who had a BMI of 18.43, but exhibited no eating symptoms on the basis of other tests. Lastly, we recruited 36 non-eating disordered women through public and university-based announcements (NED group). According to the Structural Clinical Interview for DSM diagnoses (SCID), NED women had no eating disorder or other psychiatric illnesses. NED women were not taking any psychotropic medications. To preserve the representability of the sample, we included 29 of 53 individuals (54.72%) in the AN-Active group and 14 of 40 individuals (35%) in the AN-Remitted group who were taking psychoactive medication. Data on participants' age, BMI, chronicity of illnesses, medication use, and cigarette smoking are presented in Table 1. Participants did not significantly differ with respect to age but did differ with respect to BMI. As expected, AN-Active individuals (AN-restrictive and AN-binge/purge types) had lower BMIs than AN-Remitted and NED individuals. Consistent with reported differences in prevalence rates of cigarette smoking (Anzengruber et al., 2006), individuals with AN-binge/purge type were

more likely to smoke than the other groups, and individuals with AN-restrictive type were less likely to smoke than the other groups.

Measures. Eating disorder (ED) symptoms were assessed using the EDE interview, a widely used semi-structured interview with Cronbach alphas ranging from 0.67 to 0.90 (Fairburn et al., 2008). To estimate chronicity of illness (i.e., number of months since ED symptoms began), trained interviewers constructed a retrospective timeline of participants' ED symptom history and then utilized items from the EDE interview to approximate the point in time at which ED symptoms seemed to have met DSM-5 thresholds for an ED diagnosis. Since the study began before the release of DSM-5, we assessed psychiatric comorbidity using the Structured Clinical Interview for DSM-IV (SCID) and Structured Clinical Interview for DSM-IV Axis II for Borderline and Obsessive-Compulsive Personality Disorder (First, Gibbon, & Spitzer, 1997; First, Spitzer, & Gibbon, 2002). However, we updated all psychiatric diagnoses in accordance with DSM-5 criteria. Body Mass Index (BMI) was calculated using anthropometric measures (Kg/m^2). Participants completed the following self-report measures:

The Eating Disorder Examination Questionnaire (EDE-Q) is derived from the EDE interview and has a total of 41 items, 23 of which contribute to the determination of scores on four subscales: Dietary Restraint, Eating Concerns, Weight Concerns, and Shape Concerns. Cronbach alphas on these scales range from .78 to .93 (Fairburn et al., 2008; Mond, Hay, Rodgers, Owen, & Beumont, 2004).

To assess general psychopathological characteristics in our participants, we applied several well-known scales tapping psychiatric symptoms or traits. These included:

The Dimensional Assessment of Personality Pathology -Basic Questionnaire (DAPP-BQ), which provides a comprehensive assessment of DSM personality concepts and has 282

items that yield 18 subscales (Livesley, Jackson, & Schroeder, 1991; Schroeder, Wormworth, & Livesley, 1992). Cronbach Alphas range from .87 to .94 (Schroeder et al., 1992). For the purpose of this thesis, we retained the Anxiousness subscale.

The Center for Epidemiologic Studies Depression Scale (CES-D) contains 20 items assessing the major facets of depression (Eaton, Muntaner, Smith, Tien, & Ybarra, 2004; Randloff, 1977). Reported Cronbach alphas range between .85 to .90 (Randloff, 1977).

The Barratt Impulsiveness Scale (BIS-11) assesses impulsivity has 30 items that yield 3 factors: attentional, motor, and non-planning. Cronbach alphas range between .79 to .83 (Patton, Stanford, & Barratt, 1995). Data on participants' self-reported symptoms are provided in Table 2.

Biochemical assays. To characterize levels of nutrients involved in the one-carbon metabolism pathway in our participants, we measured plasma levels of folate, B12, betaine, choline, methionine and DMG. As in other studies with individuals with AN (e.g., Barron et al., 2017), non-fasting blood levels were used in this study. Requesting that participants fast before giving blood would have compromised the safety of AN-Active individuals. Whole blood samples were collected in EDTA tubes and centrifuged to separate plasma from blood cells. The plasma was then frozen at -80 °C until analysis. Plasma folate and vitamin B12 concentrations were measured by AccuBind® ELISA kit (cat# 7825-300B, Monobind. Lake Forest, CA) following the manufacturer's protocol. Choline, betaine, DMG and methionine were analyzed simultaneously in one aliquot by tandem mass spectrometry (LC-MS/MS). An internal standard solution containing deuterated isotopomers of each nutrient was added to each aliquot. Samples were deproteinized with acetonitrile. Supernatant was obtained using Atlantis HILIC Column (Waters. Milford, MA) and was separated on an Agilent 1290 UPLC (Agilent. Palo Alta, CA)

coupled with an Agilent 6460 tandem quadrupole mass spectrometer. To measure verified ion pairs for each nutrient, each analyte was assayed in multiple reaction-monitoring modes. Each compound was quantitated against the ion pair of its own isotopomer internal standard. Results were quantitated using Agilent MassHunter software. For each participant, the concentrations of each metabolite were measured in duplicates, and a mean was calculated. In 4 cases, folate concentrations obtained from the AccuBind® ELISA kit had one out of two sample dilutions that were either above or below the assay's range of sensitivity. In such cases, we used the mean of the viable concentration and the kit's upper or lower limit as appropriate. In 2 cases, the concentration of vitamin B12 in the samples exceeded the upper range of the assay. In such cases, the assay kit's upper limit value was assigned. For 5 individuals from the AN-Active group, only post-treatment nutrient data were available. For these participants, we imputed pre-treatment values for each nutrient using the mean from the AN-Active group.

Data analyses. Groups were compared on descriptive variables using one-way ANOVAs and chi-square tests conducted with SPSS 23. Data on nutrient levels of folate, B12, betaine, choline, methionine and DMG were assessed for normality using graphical representations and Shapiro–Wilk tests. One folate value was 2 standard deviations above the mean, and was deleted from analyses. Otherwise, all assumptions of normality were met.

Nutrient levels were compared across AN-Active, AN-Remitted, and NED groups using a one-way MANOVA (in which all 6 nutrient levels were entered) and subsequent univariate ANOVAs. Based on findings from previous studies (e.g., Chiurazzi et al., 2017; Hadigan et al., 2000) power estimates suggest that a sample size of 42 would be sufficient to detect an effect at $\alpha = .05$. Given that the present sample size is 129, we believe that our study should have had power sufficient to detect an existent effect. We first tested for overall differences among groups

(AN-Active vs. AN-Remitted vs. NED). Significant effects were then subjected to pairwise comparisons using independent t tests with a Bonferroni correction for multiple comparisons. Within the group of AN-Active individuals, we compared nutrient levels between individuals with AN-restrictive and AN-binge/purge types using an independent samples t-test. Finally, we assessed the association between nutrient levels and self-reported symptoms of eating disorders, depression, anxiety and impulsivity. We selected these symptoms because the literature suggests they may be associated with levels of nutrient involved in one-carbon metabolism (Ash et al., 2013; Coppen & Bolander-Gouaille, 2005; H. Frieling et al., 2005; Konycheva et al., 2011; Tomizawa et al., 2015). Prior to analyses, data from the EDE-Q, CES-D, DAPP-BQ (anxiousness scale), and BIS-11 questionnaires were assessed for assumptions of normality using graphical representations (e.g., normality plots) and Shapiro–Wilk tests; all assumptions of normality were met. Diagnosis (AN-Active, AN-Remitted, NED) was dummy-coded. We conducted separate hierarchical linear regression analyses with self-reported scores from the EDE-Q, CES-D, DAPP-BQ (anxiousness scale), and BIS-11 as a criterion variable. In step 1, diagnosis, age, and BMI were entered as predictor variables. The AN-Active was used as a reference in analyses. In step 2, plasma nutrient levels of folate, B12, betaine, choline, methionine and DMG were entered as predictor variables.

Results

Group comparisons on measures of plasma nutrient levels. Table 3 shows the plasma nutrient levels in AN-Active, AN-Remitted and NED groups. To compare nutrient levels across AN-Active, AN-Remitted and NED groups, we ran a one-way MANOVA followed by univariate ANOVAs. Results from the one-way MANOVA revealed a significant multivariate effect, F (12, 228) = 3.54, $p < .001$; Wilk's $\Lambda = 0.710$, partial $\eta^2 = .157$. Univariate tests isolated significant

univariate differences in B12 (F (2, 119) = 4.71; p =.011; partial η^2 = .073) and betaine (F (2, 119) = 3.82; p = .025; partial η^2 = .060. Planned comparisons using Bonferroni at p <.05, suggested that AN-Active individuals had higher B12 levels than NED individuals. Uncorrected pairwise comparisons (using Fisher's Least Significant Difference at p <.05) suggested that AN-Active individuals had higher betaine levels than AN- Remitted individuals and NED individuals. However, these results did not survive a Bonferroni correction. Results suggest that despite being malnourished and depending on specific nutrient, AN-Active individuals exhibit stable or elevated levels of nutrients involved in one-carbon metabolism.

Table 4 depicts values of plasma nutrient levels in individuals with AN-restrictive and AN-binge-purge type. To compare nutrient plasma levels between these groups we ran independent samples t-tests. No significant group differences on plasma nutrient levels were observed.

Association between plasma nutrient levels and clinical indices. Table 5 depicts the association between predictor variables (diagnosis, age, BMI, nutrient levels) and self-reported symptoms on clinical questionnaires. To assess whether plasma nutrient levels are associated with self-reported symptoms of eating disorders, depression, anxiety, and impulsivity, we ran hierarchical regression analyses. Each questionnaire (EDE-Q, CES-D, DAPP-BQ [anxiousness], BIS-11) was analyzed separately. Diagnosis, age, and BMI were entered as predictors in step 1, and plasma nutrient levels of folate, B12, betaine, choline, methionine and DMG were entered as predictors in step 2. With respect to the EDE-Q, DAPP-BQ (anxiousness), and CES-D scales, models with predictors from step 1 (i.e., age, BMI, diagnosis) as well as from step 2 (nutrient levels) significantly predicted symptoms (p < .05). However, nutrient levels of folate, B12, betaine, choline, methionine, and DMG did not significantly predict self-reported symptoms on

any such scale over and above diagnosis, age, and BMI (change in R^2 was non-significant, $p > .05$). With respect to the BIS-11 attentional score, only the model with predictors from step 1 (age, BMI, diagnosis) was significant. With respect to the BIS-11 motor, non-planning, and total scores, none of the predictor variables were significant ($p > .05$).

Discussion (Study 1)

We compared plasma levels of folate, B12, betaine, choline, methionine and dimethylglycine (DMG) across groups of women with active AN, women remitted from AN, and women with no eating-disorder history. We also investigated whether or not nutrient levels were associated with symptoms of eating disorders, depression, anxiety, and impulsivity.

AN-Active versus AN-Remitted versus NED. Results revealed various group-based differences as to plasma nutrient levels—but patterns of findings were surprising in the respect that actively ill individuals were found to have unexpected elevations of B12, and possibly, of betaine. On the assumption that plasma nutrient levels should correspond to an individual's nutritional state, we had expected the converse—namely that actively ill people would display decreased nutrient levels. In other words, we expected that individuals with active AN, given that they are malnourished, would exhibit lower plasma nutrient levels compared to individuals who were not actively malnourished (i.e., remitted and non-eating disordered individuals). Instead, our results indicated that individuals with active AN exhibited higher plasma levels of B12 than did non-eating disordered individuals, and possibly, higher betaine levels than did non-eating disordered and remitted individuals. In contrast, individuals with active AN exhibited plasma levels of folate, choline, methionine and DMG that were comparable to levels exhibited by remitted and non-eating disordered individuals. Such findings suggest that in individuals with AN, plasma nutrient levels may not reflect malnourished states. One possible explanation is that

metabolic adaptations develop during malnourished states in order to preserve nutrient and energy levels (Galgani & Ravussin, 2008; Moyano et al., 1998; Palova, Charvat, Masopust, Klapkova, & Kvapil, 2007).

Our finding that individuals with active AN displayed elevated plasma levels of B12 and betaine ran contrary to our initial expectation, and appears to defy the logic that plasma nutrient levels should be reduced (and not elevated) in malnourished individuals. We are unable to ascertain which explanation is most viable, but we can think of two ways to account for results. The first centers on choline regulation. Based on the same logic that plasma nutrient levels should be reduced in malnourished individuals, we had expected to find reduced levels of choline in actively ill individuals and, instead, find choline levels in these individuals to be comparable to those in AN-Remitted or NED individuals. To account for this unexpected pattern of findings, we offer the speculation that some regulatory process, acting to compensate for effects of malnutrition, may be at work. In formulating our proposal, we note that previous findings have been consistent with the idea that—because choline is so essential to brain development and cell-membrane integrity (Zeisel, 2004)—the body will protect choline levels during states of malnutrition by increasing choline flux—i.e., by finding other sources of choline than those available from direct dietary sources. As a case in point, one study observed that mice fed a choline-deficient diet still exhibited normal choline levels in both plasma and brain samples (Li, Agellon, & Vance, 2007). The study authors suggested that when choline levels are depleted due to decreased dietary intake, organs such as the intestines and kidneys mobilize choline supplies to replenish the brain. Based on the preceding, we offer the speculation that choline flux may have been increased in our AN-Active participants due to their actively malnourished state. If so, then increased choline flux could be driving, as a precursor, increases in levels of

nutrients that occur later in the one-carbon metabolism pathway—namely betaine and B12. In other words, we may be observing the effects of a metabolic adaptation, occurring specifically due to actively ill participants' malnourished state, to preserve nutrient and energy levels. Lending credence to our proposal, previous animal and human studies have noted that choline and B12 levels are closely associated (Compher, Kinosian, Stoner, Lentine, & Buzby, 2002; Edelstein & Guggenheim, 1971). Likewise, since betaine is directly metabolized from choline, levels of choline and betaine would be expected to be tightly linked. A second explanation for elevated B12 levels makes reference to liver dysfunction—a hypothesis that has been proposed by previous authors (Corbetta et al., 2015). However, we consider the first statement more likely (centered on choline flux) on the grounds that we verified liver function in active individuals using indices of alanine aminotransferase (ALT) enzymes, and found anomalies in only 3 cases (see Table 3).

AN-restrictive versus AN binge/purge type. We sought to determine whether or not levels of nutrients involved in one-carbon metabolism would differ between individuals that regularly engage in binging and/or purging behaviours (AN- binge/purge type) compared to individuals that only restrict (AN-restrictive type). Consistent with previous studies (e.g., Levine et al., 2007), we observed no significant differences in plasma nutrient levels between AN-subtype groups.

Association between plasma nutrient levels and self-reported symptoms. Previous studies suggest that nutritional deficits and, more specifically, deficiencies in nutrients involved in one-carbon metabolism, are associated with symptoms such as depression, anxiety, and impulsivity (Ash et al., 2013; Coppen & Bolander-Gouaille, 2005; Konycheva et al., 2011; Tomizawa et al., 2015). In the present study, we did not observe a significant association

between levels of plasma nutrients and scores on measures of eating- disorder symptoms, depression, anxiety, or impulsivity. The preceding discrepancy in findings may be because prior studies generally reported an association between *deficiencies* in one-carbon nutrient levels and psychopathological symptoms. Even malnourished participants in this study exhibited plasma nutrient levels that were similar, or elevated to those observed in healthy individuals. This being the case, it is possible that nutritional adaptations that may occur during the acute stage of AN outweigh the effects of malnutrition observed in NED populations.

Study 2

Goals and Hypotheses

Our second study compared the relationship between levels of nutrients involved in one-carbon metabolism and DNA methylation levels across AN-Active, AN-Remitted and NED groups. We first conducted a comparison of site-specific methylation levels across diagnostic groups. Given findings from our previous studies (Booij et al., 2015; Steiger et al., 2019), we anticipated that AN-Active individuals would exhibit differentially methylation levels at various DNA sites compared both to AN-Remitted and NED individuals. Next, we explored the association between levels of nutrients and DNA methylation, and assessed whether the association varied across diagnostic groups. Considering that AN-Active individuals are malnourished, and have been shown to exhibit altered methylation profiles compared to non-actively ill individuals (Booij et al., 2015; Steiger et al., 2019), we anticipated that AN-Active individuals would exhibit a nutrient-methylation relationship that differed from AN-Remitted and NED individuals.

Methods

Participants. This study included a subset of 107 women (34 AN-Active individuals, 39 AN-Remitted individuals, and 34 NED individuals) from Study 1 for whom we had available data both on plasma nutrients and DNA methylation.

Measures. ED symptoms, psychiatric comorbidity and BMI were assessed using measures identical to those described in Study 1.

Biochemical assays. Procedures for biochemical assays used to measure plasma nutrient levels of folate, B12, betaine, choline, methionine and DMG in this study are described in Study 1.

DNA methylation processing and filtering. As described elsewhere (Steiger et al., 2019), whole blood was collected in EDTA tubes and DNA was extracted from leukocytes using the Qiagen extraction kit. Analyses were conducted at the Genome Quebec Innovation Centre using the Infinium Human Methylation 450 BeadChip Kit (n = 52 samples) or the Infinium MethylationEPIC BeadChip Kit (n = 55 samples). Two different arrays were utilized because Illumina discontinued its 450 BeadChip kit; although both manufacturer information and an independent study support the validity of combining the arrays (Moran, Arribas, & Esteller, 2016). Only probes present in both array types were considered in the analysis. DNA was first checked for quality using picogreen and then bisulfate converted using EZ96 DNA Methylation-Gold Kit (Zymo Research). Samples were then transferred to BCD and then MSA4 plates, and neutralized before overnight amplification. The MSA4 plates were fragmented, precipitated and resuspended 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 (Illumina Inc.).

Data analyses. Analyses were conducting using a similar approach to that described by Booij et al (2015) and Steiger et al (2019). Briefly, Illumina arrays were analyzed using the ChAMP – bioconductor package in R including normalization. The initial data set included 452,567 probes obtained from 107 participants. Probes located on the Y chromosome, or that exhibited methylation with a standard deviation of less than 0.05 (that might reflect random technical variation), were eliminated. We have applied such data reduction strategies in previous studies (Booij et al., 2015; Steiger et al., 2019). The final data set included 41,233 probes (9.1% of the original probes). We applied a cell composition correction implemented in Minfi-R (FlowSorted.Blood.EPIC), based on the reference methylation profiles of separated peripheral blood mononuclear cells (Houseman et al., 2012). Probe intensities were then converted to β values, and the CpGs were annotated using the Ensembl genome database (human GCRh37 assembly). Next, individual β values were compared across groups using general linear models with each methylation β value as the dependent variable, group as the independent variable and age, smoking, psychotropic medication use, and estimated cell proportions as covariates. Overall differences among groups (AN-Active versus AN-Remitted versus NED) were first tested using an ANOVA, followed by pairwise comparisons to study differences between specific groups (active versus NED, active versus remitted, remitted versus NED). FDR corrections were applied for each set of analyses (i.e., within ANOVAs, analyses of covariance, pairwise contrasts, linear regression) with Q set at < 0.01.

The individual beta values obtained were subjected to further analyses in order to investigate the association between methylation levels and levels of nutrients involved in one-carbon metabolism, and importantly, whether or not the association between methylation and nutrient levels differed as a function of group (Active versus Remitted versus NED). To

investigate such associations, we ran separate general linear models for each nutrient. For each analysis, DNA methylation was the outcome and the nutrient of investigation was the independent variable. Age, smoking, psychotropic medication use, and estimated cell proportions were used as covariates.

To compare the relationship between nutrient and methylation levels across diagnostic groups (Active versus Remitted versus NED), we reran each of the 6 analyses adding a Group by Nutrient interaction term to the model (i.e., group * nutrient). Significant interaction effects were followed by subsequent paired comparisons (active vs. NED; remitted, remitted versus NED) to further investigate and interpret the interaction effects. In the present thesis, we focus primarily on such interaction effects (i.e., does the association between methylation and nutrients involved in one-carbon metabolism differ as a function of diagnosis). FDR corrections were applied to these analyses with Q set at <0.01.

Results

Group comparisons on DNA methylation levels. Table 6 shows results from the ANOVA and pairwise comparisons testing differences in methylation levels across groups. To compare site-specific methylation levels across AN-Active, AN-Remitted and NED groups, we conducted a one-way ANOVA for the 3 groups. Results revealed differential methylation ($Q < 0.01$) on 11 probes, corresponding to 11 genes. For 9 out of 11 such probes, there was at least one significant pairwise comparison between groups (AN-Active versus NED, AN-Active versus AN-Remitted; $Q<0.01$).

AN-Active versus NED individuals. Pairwise comparisons between AN-Active and NED individuals isolated 8 differentially methylated probes ($Q<0.01$) corresponding to 8 different genes (*RPTOR, GTDC1, SULT1C2, FTSJD2, DOT1L, LRRC8D, CCDC48, GDPD5*). Several of

these genes appear to be disorder-relevant. To start, *RPTOR* is responsive to nutrient levels and regulates cell growth. *GDPD5* is associated with glycerol metabolism. *SULT1C2* is involved in the sulfate conjugation of multiple hormones and neurotransmitters. *DOT1L* encodes a histone methyltransferase protein that helps preserve healthy cartilage (Monteagudo et al., 2017). The remaining implicated probes corresponded to genes (*GTDC1, FTSJD2, LRRC8D, CCDC48)* that do not have known protein functions. Methylation levels at all 8 differentially methylated probes were higher in AN-Active individuals compared to NED individuals.

AN-Active versus AN-Remitted individuals. Pairwise comparisons between AN-Active and AN-Remitted individuals revealed differential methylation at 10 probes ($Q < 0.01$), corresponding to 9 genes (*DOT1L, STAT3 (2 probes), C5orf63, LETM2, C11orf41, LUZP1, CCDC48, FTSJD2, JAZF1*). Some implicated probes corresponded to genes that may be disorder-relevant. For instance, the *STAT3* gene is associated with cellular processes including cell-growth and cell-death. *JAZF1* gene (involved in transcriptional repression), has also been implicated in glucose and lipid metabolism (Liao, Wang, Qi, & Xiao, 2019). *DOT1L* (as described above) is implicated in cartilage health (Monteagudo et al., 2017). The remaining implicated probes corresponded to genes (*C5orf63, LETM2, C11orf41, LUZP1, CCDC48, FTSJD2*) that do not have known protein functions. DNA methylation levels at all 10 differentially methylated probes were significantly lower in AN-Remitted individuals compared to AN-Active individuals.

Three probes, corresponding to the *DOT1L, CCDC48* and *FTSJD2* genes, were common to AN-Active versus NED and AN-Active versus AN-Remitted comparisons. Interestingly, the direction of methylation effects at these 3 probes ran in opposite directions, so as to suggest hypermethylation in AN-Active individuals, and a lowering of methylation levels in AN-

Remitted individuals (i.e., AN-Active individuals exhibited increased methylation compared to NED individuals, and AN-Remitted individuals exhibited decreased methylation compared to AN-Active individuals). Such findings suggest that methylation levels in active individuals may become elevated, but that any such elevations may be restored upon remission.

AN-Remitted versus NED individuals. Comparisons between AN-Remitted and NED individuals revealed no differentially methylated probes. Such findings suggest that methylation profiles in individuals remitted from AN resemble those of non-eating disordered individuals. At present, we cannot ascertain the causality of observed effects (i.e., whether methylation levels in active individuals are restored to "normal" once remission is achieved, or whether changes in methylation levels precede remission).

Interaction between nutrient level and diagnostic group on DNA methylation levels. To explore the relationship between levels of nutrients involved in the one-carbon metabolism pathway and site-specific DNA methylation levels across AN-Active, AN-Remitted and NED groups, we ran general linear models separately for each nutrient, with an interaction term for diagnostic group. For B12, methionine, betaine, and DMG levels, significant interaction effects between nutrient levels and diagnostic group on DNA methylation levels were observed and are depicted in Appendix Tables 1-4. No significant interaction effects between folate levels and diagnostic category or choline levels and diagnostic category on DNA methylation levels were found ($Q > 0.01$).

B12. Significant interaction effects between B12 levels and diagnostic group on DNA methylation levels are depicted in Appendix Table 1. To compare the relationship between B12 and methylation levels across diagnostic groups, we ran a general linear model with a group by

B12 interaction term (group * B12). Results indicated significant interaction effects, suggesting that diagnostic status influenced the strength of association between B12 and methylation levels.

Pairwise comparisons between AN-Active and NED individuals indicated 108 probes (corresponding to 101 genes) at which the relationship between B12 and methylation levels differed significantly. At 84 of these probes (approximately 78% of implicated probes), the relationship between B12 and methylation levels was concordant between AN-Active and NED individuals: That is, AN-Active and NED individuals both exhibited a positive relationship between B12 and methylation levels at 67 probes, and both exhibited a negative relationship between B12 and methylation levels at 17 probes. In either case, the strength of the relationship between B12 and methylation levels was stronger in AN-Active individuals (i.e., the observed effect was larger in AN-Active individuals compared to NED individuals). Among the probes in question, several corresponded to genes that may be relevant to AN pathology such as *RPTOR* (implicated in cell growth) and *STARD3NL* (implicated in cholesterol transport). Other such probes corresponded to genes associated with psychiatric status such as glutamatergic neurotransmission (*GRM5),* and autism spectrum disorder (*SLC25A12*; Liu et al., 2015).

Pairwise comparisons between AN-Active and AN-Remitted individuals indicated 21 probes (corresponding to 21 genes) at which the relationship between B12 and methylation levels was significantly different. The pattern of effects resembled that seen in comparisons between AN-Active and NED individuals. At 13 probes (approximately 62% of implicated probes), the relationship between B12 and methylation levels was concordant between AN-Active and AN-Remitted individuals: AN-Active and AN-Remitted individuals both exhibited a positive relationship between B12 and methylation levels at 5 probes, and both exhibited a negative relationship between B12 and methylation levels at 8 probes. In either case, the strength

of the relationship between B12 and methylation levels was generally stronger in AN-Active individuals. Among the probes in question, some correspond to genes that may be relevant to AN pathology such as *TRIO* (implicated in such as cell-growth), *GREB1* (involved in estrogen-responsive processes), and *OR2T10* (involved in olfaction).

Methionine. Significant interaction effects between methionine levels and diagnostic group on DNA methylation levels are depicted in Appendix Table 2. To compare the relationship between methionine and methylation levels across diagnostic groups, we ran a general linear model with a group by methionine interaction term (group * methionine). Results indicated significant interaction effects, suggesting that diagnostic status influenced the strength of association between methionine and methylation levels at many probes.

Pairwise comparisons between AN-Active and NED individuals indicated 149 probes (corresponding to 145 genes) at which the relationship between methionine and methylation levels was significantly different. The pattern of effects resembled that seen in comparisons concerning B12. At 106 probes (approximately 71% of implicated probes), the relationship between methionine and methylation levels was concordant between AN-Active and NED individuals: AN-Active and NED individuals both exhibited a positive relationship between methionine and methylation levels at 69 probes, and both exhibited a negative relationship between methionine and methylation levels at 37 probes. In either case, the strength of the relationship between methionine and methylation levels was stronger in AN-Active individuals (i.e., the observed effect was larger in AN-Active individuals compared to NED individuals) with the exception of 1 probe. Among the probes in question, several corresponded to genes that may be relevant to AN including those associated with physiological processes such as cholesterol and lipid-related functions (*NR1H3*), glucose metabolism (*HK1*) and appetite

(*GHRL*). Other such probes corresponded to genes associated with dopamine neurotransmission (*DRD2*), as well as inflammation and pain sensitivity (*SCN11A*).

Betaine and DMG. With respect to betaine and DMG, significant interaction effects between nutrient levels and diagnostic group on DNA methylation levels are depicted in Appendix Table 3, and Appendix Table 4, respectively. To compare the relationship between nutrient and methylation levels across diagnostic groups, we ran a general linear model separately for betaine and DMG, with a group by nutrient interaction term (group * nutrient). A small number of findings indicated significant interaction effects, suggesting that diagnostic status influenced the strength of association between nutrient and methylation levels. However, no systematic pattern was observed.

Main effects (in the absence of interaction effects). To explore the relationship between nutrient levels and DNA methylation levels, we ran general linear models separately for each nutrient. Results indicated that methionine levels were associated with DNA methylation levels at 7,205 probes ($Q < 0.01$), at which no significant interaction effects were detected. In other words, at these probes, the relationship between methionine and DNA methylation levels was not affected by diagnostic group. Results show expected associations between methionine and DNA methylation; as a precursor to SAM (the universal methyl donor for mammalian methylation processes; Selhub, 1999), methionine is essential to methyl group transfers within the one-carbon metabolism pathway (Waterland, 2006). Findings validate the measures used to assess the correspondence between nutrients involved in one-carbon metabolism pathway and DNA methylation. No significant main effects between folate and DNA methylation levels or choline and DNA methylation levels were observed ($Q > 0.01$).

Discussion (Study 2)

In this study, we investigated the correspondence between nutrients involved in the one-carbon metabolism pathway and DNA methylation patterns across groups of women with active AN, women remitted from AN, and women with no eating-disorder history. We first compared epigenome-wide methylation profiles across diagnostic groups. Building on Study 1, we then compared the association between levels of nutrients involved in the one-carbon metabolism pathway and site-specific DNA methylation levels across active, remitted and non-eating disordered groups.

Group differences on DNA methylation levels. Comparisons between AN-Active and NED groups indicated differential methylation on 8 different probes, corresponding to 8 different genes: *RPTOR, GTDC1, SULT1C2, FTSJD2, DOT1L, LRRC8D, CCDC48, GDPD5*. Among implicated genes, some may be relevant to physiological processes in AN. For instance, *RPTOR* encodes a regulatory protein (raptor) of mTOR complex 1, which is responsive to nutrient levels and can inhibit cell-growth (Kim et al., 2002). We observed that AN-Active individuals exhibited hypermethylation at the *RPTOR* probe compared to NED individuals, which may suggest that *RPTOR* gene expression is reduced in active individuals. Although the preceding is speculative, reduced expression of *RPTOR* could conceivably affect the functioning of the mTOR 1 pathway, and decrease cellular-growth—an effect that would be consistent with reduced body mass in individuals with AN. Another implicated gene, *GDPD5*, is associated with glycerol metabolism. Glycerol can be converted to glucose through the break-down of fat cells (adipose lipolysis; Berg, Tymoczko, & Stryer, 2002a). The preceding may be an important process during prolonged periods of starvation, when glucose levels are depleted (Berg, Tymoczko, & Stryer, 2002b). Indeed, early studies report that the conversion rate of glucose from glycerol is increased during fasted states (Baba, Zhang, & Wolfe, 1995; Bortz, Paul, Haff,

& Holmes, 1972). Altered methylation levels at the *GDPD5* probe in AN-Active individuals may coincide with altered glycerol metabolism, and glucose levels. *DOT1L* encodes a histone methyltransferase protein that is involved in cartilage health; in a mouse model, the loss of *DOT1L* compromised cartilage cells (chondrocytes) and led to osteoarthritis (Monteagudo et al., 2017). The authors attributed the effect to impaired inhibition of Wnt signalling pathways, whose altered activation is linked to osteoarthritis (Lories, Corr, & Lane, 2013; Zhu et al., 2009). The association between cartilage health and eating disorders has been underexplored (Donaldson & Gordon, 2015). However, studies have reported that female athletes who have symptoms that parallel those in AN (i.e., menstrual irregularities, energy deficiency with or without disordered eating, as well as low bone mineral density; Nazem & Ackerman, 2012) may sustain more severe cartilage-related injuries (and musculoskeletal injuries in general), than athletes without such symptoms (Thein-Nissenbaum, Rauh, Carr, Loud, & McGuine, 2012). Consistent with high rates of bone disease (Misra, Golden, & Katzman, 2016) as well as autoimmune disorders (i.e., rheumatoid arthritis; Raevuori et al., 2014) in individuals with AN, hypermethylation at the *DOT1L* probe in AN-Active individuals may impair inhibition of the Wnt signalling pathways, and contribute to cartilage damage. *SULT1C2* is involved in sulfate conjugation, while *GTDC1, FTSJD2, LRRC8D,* and *CCDC48* genes do not have known protein functions.

Our analyses also revealed 10 probes, corresponding to 9 different genes, at which methylation levels differed between AN-Active and AN-Remitted individuals: *DOT1L, STAT3, C5orf63, LETM2, C11orf41, LUZP1, CCDC48, FTSJD2, JAZF*. Two implicated probes corresponded to a member of the signal transducers and activators of transcription proteins (*STAT3*). Several studies have shown a role of STAT3 in body-weight regulation (Raevuori et al., 2014). For instance, *STAT3* can activate ciliary neurotrophic factor (*CNTF*) which has been

shown to mimic the effects of leptin: a hormone associated with appetite suppression and weight loss (Mattson, 2001). Correspondingly, polymorphisms of *STAT3* have been associated with such indices as BMI, weight circumference and obesity (Ma, Wang, Chen, Ou, & Zou, 2014). Other studies have suggested that *STAT3* may be heavily implicated in the muscle wasting characteristic of cachexia syndrome (Bonetto et al., 2012 ; Ma et al., 2017). The *JAZF1* gene is involved in diverse transcriptional regulation. Of interest, *JAZF1* is involved in glucose and lipid metabolism and may be responsive to circulating nutrient levels (Liao, Wang, Qi, & Xiao, 2019). *JAZF1* has been implicated in the development of obesity (Meng et al., 2018), and type 2 diabetes mellitus (T2DM; Siddiqui, Musambil, & Usmani, 2014; Taneera et al., 2012). One study investigating mice fed a high fat diet, observed that increased expression of *JAZF1* was associated with decreased body weight as well as decreased lipid accumulation in the liver (Jang et al., 2014). That *JAZF1* is involved in lipid metabolism and gluconeogenesis supports the possibility that altered methylation levels of *JAZF1* are relevant to physiological recovery from AN. Consistent with the idea that some features of AN may be epigenetically regulated, present findings suggest that appetite as well as weight-related processes in individuals with AN may be associated with altered methylation profiles. As previously described, the *DOT1L* gene encodes a histone methyltransferase protein, associated with cartilage health (Monteagudo et al., 2017). The direction of methylation effects at the *DOT1L* probe in group comparisons indicated that active individuals exhibited increased methylation compared to non-eating disordered individuals and that remitted individuals exhibited decreased methylation compared to active individuals. The preceding may suggest that altered functioning of the *DOT1L* gene during the active stage of illness, is restored upon remission. More studies are needed that directly evaluate

cartilage health in individuals with active AN and in individuals remitted from AN. The *C5orf63, LETM2, C11orf41, LUZP1, CCDC48,* and *FTSJD2* genes do not have known protein functions.

We note that out of the 8 differentially methylated probes identified in active versus non-eating disordered comparisons, 6 were identified in our previous EWAS (Steiger et al., 2019). Out of the 10 total differentially probes identified in active versus remitted comparisons, 9 were implicated in our previous EWAS (Steiger et al., 2019). Although participants in this study represented a substantial subset of those included in our earlier report, consistency across these studies does suggest a degree of stability of the findings. With respect to the directions of methylation effects observed in the present study, comparisons between active and non-eating disordered individuals revealed higher DNA methylation levels in the active group at all 8 differentially methylated probes. In contrast, comparisons between active and remitted individuals indicated that DNA methylation levels were lower in the remitted group at all 10 differentially methylated probes – a general pattern also observed in our previous EWAS (Steiger et al., 2019). Viewed together, these findings suggest that it is possible to differentiate individuals with active AN from remitted and non-eating disordered individuals on the basis of DNA methylation profiles—suggesting that illness-induced changes in methylation profiles seen in individuals with AN may be restored upon remission. Such findings may be clinically relevant as they might indicate potential markers for illness staging and targets for novel treatments.

The association between nutrient and DNA methylation levels across groups. Results showed a relationship between nutrient levels and methylation that is complex, and sometimes hard to characterize. However, certain patterns of correspondence between nutrient and methylation levels did lend themselves to cautious preliminary interpretation, which we offer here reservedly.

Our findings on the association between B12 and methylation suggested that, at affected probes, individuals with active AN exhibited a stronger association between B12 levels and DNA methylation than did either non-eating disordered individuals or individuals who were in remission from AN. (For instance, on 84 probes, or 74%, individuals with AN exhibited a stronger nutrient-to-methylation link than did non-eating disordered individuals). Similarly, our findings pertaining to the link between methionine levels and methylation suggested a stronger association between nutrient and methylation levels in AN-Active individuals than in NED individuals. A general theme would thus seem to be that some as-yet unknown factor may amplify the connection between nutrient and methylation levels in individuals in an actively ill state.

Genes implicated in possible alterations of the link between nutrients and methylation had diverse functions, but generally seemed to include genes acting in brain function, emotion regulation, metabolism and immunity. In this respect, our findings isolate genes with comparable functions to those observed in a previous study by our group (Steiger et al., 2019) and in a large-scale GWAS study documented by Duncan et al. (2017). Notably, 11 probes identified in the present investigation mapped onto genes that were previously identified by Steiger et al. (2019).

At this point, we can only speculate about why individuals with AN might exhibit a stronger association than do non-eating disordered individuals between plasma B12 and methionine levels, on the one hand, and DNA methylation levels, on the other. As we proposed earlier (see Discussion of Study 1), we speculate that individuals with AN may develop adaptations aimed at buffering against detrimental consequences of malnutrition. For instance, nutrient stores may be mobilized from peripheral organs in order to compensate for restricted dietary intake. Such adaptations, might affect the magnitude of correspondence between

nutrients involved in one-carbon metabolism and DNA methylation. Findings pertaining to B12 suggest that the relationship between nutrients and methylation in individuals with AN, may differ from non-eating disordered as well as remitted individuals. If our account is correct, then any alteration in the strength of association between nutrients and methylation would represent an illness related adaptation, and not a factor acting in illness susceptibility. However, even if secondary to active illness, the processes could act to heighten illness sequelae, or illness entrenchment.

Although the exact mechanism that may alter the link between one-carbon metabolism nutrients and DNA methylation is currently unidentified, we briefly note an additional observation that may provide insight into its operation: several probes that were identified in analyses involving B12 were also identified in analyses involving methionine. Similarly, several probes that were identified in analyses involving betaine, were also identified in the analyses involving dimethylglycine (DMG). The observed overlap between B12 and methionine as well as betaine and DMG may not be random, and instead, may be attributable to the fact that nutrients in the one-carbon metabolism pathway help generate other nutrients in the pathway (Anderson et al., 2012). For instance, B12 helps facilitate the conversion of homocysteine into methionine, while betaine is converted into DMG when it donates methyl to homocysteine (Obeid, 2013). Present findings support the possibility that nutrients that participate in the same set of biochemical reactions in the one-carbon metabolism pathway, share similar associations to DNA methylation. As a result, such nutrients may be similarly susceptible to alterations induced by AN.

General Discussion

Strengths and Limitations

Among its strengths, the present study may help clarify processes that connect malnutrition to epigenetic factors, and ultimately, to disorders like AN. In addition, our study included controls for several variables that may impact methylation readings, such as smoking and psychotropic medication use. Finally, we employed statistical corrections to help offset high rates of false-positive results.

Our study also has several limitations, which need to be held in mind while interpreting findings. First, our sample size is small which may have limited the power that was needed to detect all significant effects. Second, we utilized a peripheral tissue (leukocytes) to measure DNA methylation levels. Given that DNA methylation is tissue-specific, the relevance of our findings to brain functions becomes suspect. Although not feasible in reality, investigations of DNA methylation profiles in brain tissue would better elucidate any epigenetic effects that were relevant to a psychiatric condition like AN. In the same vein, we note, however, that epigenetic profiles derived from peripheral tissue may still be meaningful with respect to AN – given that it is a condition that affects tissues throughout the whole body. Furthermore, evidence supports good levels of correspondence between the brain and periphery in terms of epigenetic mechanisms (Booij, Wang, Lévesque, Tremblay, & Szyf, 2013; Szyf, 2012; Szyf, 2014). Second, our studies did not include indices of gene expression, preventing any assessments of the functional significance of altered methylation levels. Lastly, present findings do not provide mechanistic explanations that specify the cause and effect relationship between nutrient and methylation levels. Future studies should investigate the functional significance of altered methylation levels in AN, as well as the potential mechanisms that alter the relationship between nutrient and methylation levels.

Conclusions

The investigations reported in this thesis provide some novel indications concerning the processes by which epigenetic mechanisms may contribute to AN. Findings suggest that despite being malnourished, individuals with AN exhibit stable or elevated levels of nutrients involved in the one-carbon metabolism pathway—a pathway integral to DNA methylation. We have interpreted the initially surprising finding as implying a compensatory adaptation to malnutrition, in which there may be increased flux of choline access from bodily tissues to the brain via the bloodstream.

Individuals with AN also exhibited differentially methylated probes compared both to individuals remitted from AN and individuals without a history of eating disorders. Many such implicated probes corresponded to genes associated with nutritional and metabolic processes that may relevant to the physiological functioning in AN.

Finally, individuals with AN exhibited an altered relationship between nutrients involved in the one-carbon metabolism pathway and methylation levels, compared both to individuals remitted from AN and individuals without a history of eating disorders – providing initial evidence of illness-induced effects on the nutrient-methylation relationship. To our knowledge, our study is the first to examine the relationship between levels of one-carbon metabolism nutrients and site-specific DNA methylation patterns in individuals with AN. Previous studies involving individuals with AN concerned the relationship between levels of one-carbon metabolism nutrients and global methylation levels (Helge Frieling et al., 2007; Tremolizzo et al., 2014); present findings suggest that the relationship between nutrients in the one-carbon metabolism pathway and DNA methylation is intricate and site-specific. Future work is needed to elucidate the mechanisms that may alter the relationship in question.

Together, findings suggest that indices of nutrients involved in one-carbon metabolism pathway and methylation levels may be useful markers of illness stage as well as recovery. Findings also suggest that nutritional rehabilitation may help restore epigenetic functioning in individuals with AN, and contribute to recovery.

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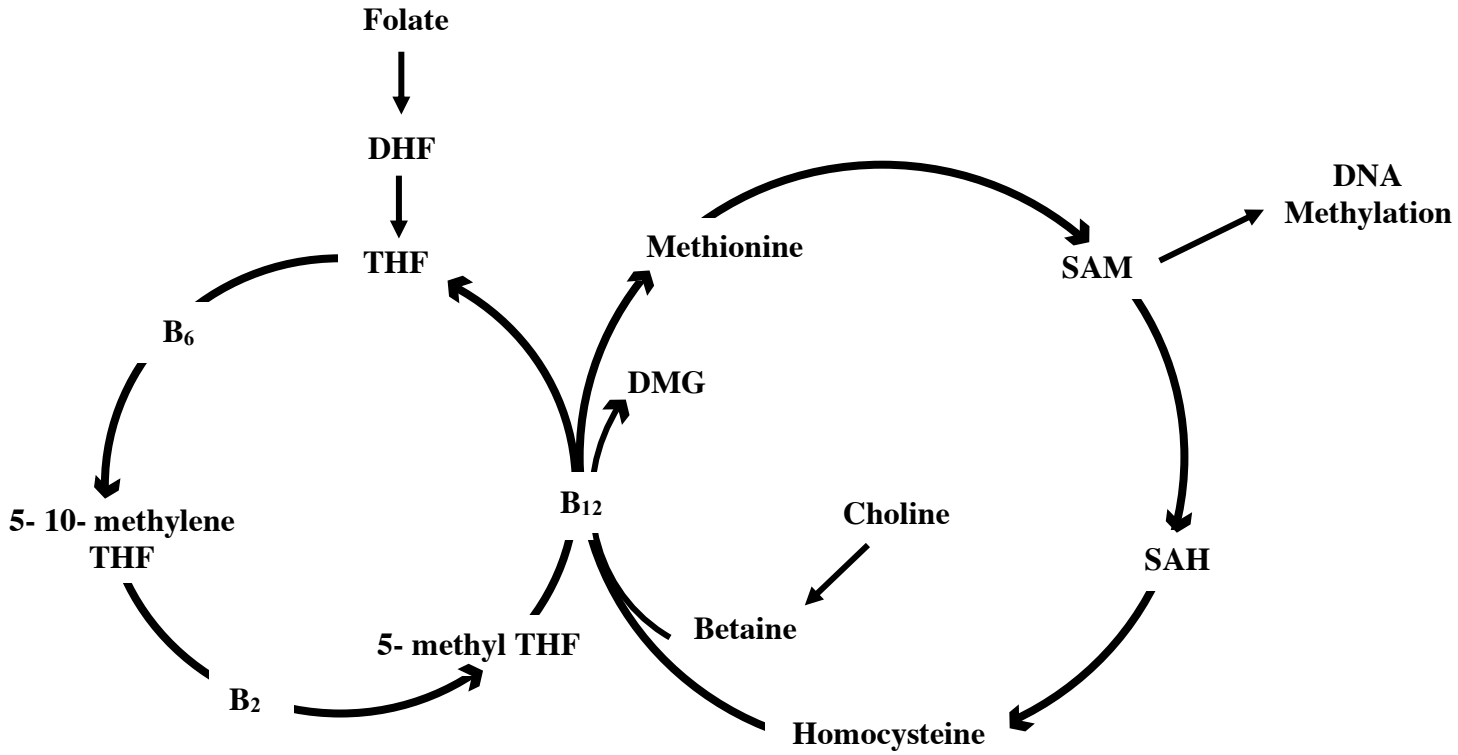

Figure 1. Figure from Anderson et al. (2012). Vitamin B_6 serves as a cofactor in the conversion of THF into 5,10-methylene THF. Vitamin B_2 is converted into FAD, which serves as a cofactor in the conversion of 5,10-methylene THF to 5-methyl THF. Vitamin B_{12} serves as a precursor to methionine synthase, which helps facilitates the conversion of homocysteine into methionine. DHF= dihydrofolate; DMG= dimethylglycine; FAD= flavin adenine dinucleotide; SAM= S-adenosylmethionine; SAH= S-adenosylhomocysteine; THF= tetrahydrofolate.

Table 1

Participant characteristics, medication use, and cigarette smoking

	AN-R (*n*=30)	AN-BP (*n*=23)	AN-Remitted (*n*=40)	NED (*n*=36)
	Mean ± SD (Range)			
Characteristics				
Age[1]	26.57 ± 9.50 (18-53)	23.83 ± 5.88 (18-37)	26.55 ± 5.62(19-38)	25.36 ± 5.91 (18-38)
BMI[2]	14.53 ± 1.37 [a] (11.60-17.12)	16.08 ± 1.11 [b] (13.10-17.50)	21.73 ± 2.75 [d] (18.43-30.99)	23.31 ± 2.96 [c] (18.79-30.60)
Chronicity[3]	101.47± 104.37 (12-456)	100.43± 65.58 (12-264)	89.70± 53.60 (12-288)	NA
	Frequency (% of group)			
Medication Use	14 (46.70)	14 (60.90)	14 (35)	0
Antidepressants	14 (46.67)	11 (47.83)	12 (30)	0
Antipsychotics	4 (13.33)	7 (30.43)	3 (7.5)	0
Hypnotics	1(3.33)	0	0	0
Mood stabilizers	0	1 (4.35)	0	0
Smokers[4]	1 (3.33 [a])	9 (39.13 [b])	5 (12.5 [ab])	5 (13.89 [ab])

AN-R = Anorexia Nervosa Restrictive Type; AN-BP = Anorexia Nervosa Binge/Purge Type; BMI= body mass index; NA= not applicable; NED = Non-eating disordered; n.s.= not significant; SD= standard deviation

Means with different superscript letters differed at $p < 0.05$.

[1] F (3, 125) = .42 , n.s.

[2] F (3, 125) = 105.56, p <.001

[3] F (2,90) = .25, n.s.

[4] χ^2 (3) = 13.54, p <.05

Table 2

Participant scores on self-report questionnaires

	AN-Active (*n*=53)		AN-Remitted (*n*=40)		NED (*n*=36)		
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*F*
Eating Disorders Examination Questionnaire							
Dietary restraint	3.57 [a]	1.85	0.58 [b]	0.80	0.48 [b]	1.04	64.97**
Eating concerns	2.93 [a]	1.70	0.72 [b]	1.27	0.26 [b]	0.83	43.24**
Weight concerns	4.15 [a]	1.57	1.51 [b]	1.35	0.68 [d]	0.92	72.22**
Shape concerns	4.55 [a]	1.40	1.86 [b]	1.47	0.86 [d]	0.79	88.46**
Total score	3.84 [a]	1.37	1.16 [b]	1.07	0.58 [d]	0.83	89.43**
DAPP-BQ							
Anxiousness	3.94[a]	0.77	3.24[b]	0.96	2.14[d]	0.80	43.04**
CES-D	35.09[a]	12.73	15.61[b]	12.31	7.80[d]	8.26	59.31**
Barratt Impulsiveness Scale							
Attentional	2.39[a]	0.51	2.29[ab]	0.49	2.03[b]	0.40	5.58*
Motor	2.16	0.46	2.12	0.55	1.97	0.36	1.65
Non-planning	1.90	0.39	2.07	0.53	2.09	0.4	2.21
Total score[1]	63.45	10.07	64.38	13.11	60.97	9.66	0.88

[1]Calculated as a pro-rated cumulative score; AN-Active= active Anorexia Nervosa; AN-Remitted= remitted from Anorexia Nervosa; NED= non-eating disordered; NA= not applicable; Different superscript letters indicate mean difference at $p<.05$; **$p<.001$; *$p<.05$

Table 3

Analysis of plasma nutrient levels in different diagnostic groups and of ALT values in AN-Active individuals

	AN-Active (*n*=52)		AN-Remitted (*n*=37)		NED (*n*=33)		
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Partial Eta Squared*
Folate [1]	9.39	5.91	12.25	7.26	11.59	5.74	0.041
B12 [2]	698.89[a]	516.08	641.50[ab]	381.18	411.73[b]	302.59	0.073
Choline [3]	6.78	2.27	7.19	2.72	7.40	2.59	0.014
Betaine [4]	42.84	21.26	33.05	20.30	32.46	17.40	0.060
Methionine[5]	28.27	28.78	31.03	38.06	27.55	34.12	0.006
DMG [6]	1.32	0.73	1.55	0.66	1.61	0.70	0.037
	Frequency (% of group) [c]						
ALT (5-60 μl)	N :50 (94.34)		NA		NA		
	A :3 (5.66)		NA		NA		
	B :0		NA		NA		

List-wise deletion resulted in the loss of 7 people from analyses. Different superscript letters indicate mean difference at $p<.05$. *Frequency (% of group)* [c] =number and proportion of individuals within normal (N) range, above (A) range, or below (B) range of ALT reference range (5-60 μl). ALT= Alanine aminotransferase; AN-Active= active Anorexia Nervosa; AN-Remitted= remitted from Anorexia Nervosa; N= normal; NA= not applicable; NED= non-eating disordered; n.s.= not significant

[1] F (2, 119) = 2.54 , n.s.
[2] F (2, 119) = 4.71, $p< 0.05$
[3] F (2, 119) = 0.84, n.s.
[4] F (2, 119) = 3.82 , n.s.
[5] F (2, 119) = 0.38, $p< 0.05$
[6] F (2, 119 = 2.31, n.s.

Table 4

Independent t-tests comparing plasma nutrient levels between individuals with AN-restrictive and AN-binge/purge type

	AN-R (*n*=30)		AN-BP (*n*=23)				
	Mean	*SD*	*Mean*	*SD*	*t*	*df*	*p*
Folate	9.96	5.74	8.62	6.18	0.80	50	0.43
B12	786.66	590.72	579.21	372.75	1.45	50	0.15
Choline	6.61	2.21	7.0	2.37	-0.62	51	0.54
Betaine	45.43	25.75	39.47	13.15	1.01	51	0.32
Methionine	28.60	31.39	27.84	25.67	0.10	51	0.93
DMG	1.41	0.87	1.19	0.51	1.06	51	0.30

AN-R = Anorexia Nervosa Restrictive Type; AN-BP = Anorexia Nervosa; Binge/Purge Type; DMG= dimethylglycine; SD= standard deviation

Table 5

Hierarchical regression analysis of predictors on self-reported symptoms

			Unstandardized Coefficients		Standardized Coefficients				*p* value		
Variable	*Step*	*Predictor*	*B*	*SE*	*ß*	*p*		*Δ R²*	*Δ R²*	*F*	*p*
EDEQ-R	1	Age	0.01	0.02	0.03	0.62	0.57	0.57	0.00	33.33	<.0001
		BMI	0.07	0.06	0.14	0.25					
		Rem	-3.47	0.49	-0.77	<.0001					
		NED	-3.73	0.56	-0.83	<.0001					
	2						0.58	0.01	0.94	12.95	<.0001
		Age	0.01	0.02	0.03	0.69					
		BMI	0.06	0.06	0.12	0.35					
		Rem	-3.48	0.53	-0.77	<.0001					
		Con	-3.70	0.60	-0.82	<.0001					
		Folate	0.03	0.03	-0.08	0.30					
		B12	0.00	0.00	-0.01	0.95					
		Betaine	0.00	0.01	-0.00	0.96					
		Choline	0.04	0.07	0.05	0.63					
		Methionine	0.00	0.01	0.02	0.82					
		DMG	-0.09	0.22	-0.03	0.68					
EDEQ-E	1	Age	-0.02	0.02	-0.07	0.33	0.47	0.47	<.0001	21.81	<.0001
		BMI	0.07	0.06	0.17	0.21					
		Rem	-2.73	0.50	-0.67	<.0001					
		Con	-3.26	0.56	-0.82	<.0001					
	2						0.49	0.02	0.71	8.89	<.0001
		Age	-0.02	0.02	-0.07	0.39					
		BMI	0.09	0.06	0.21	0.15					
		Rem	-2.88	0.53	-0.71	<.0001					
		NED	-3.34	0.59	-0.85	<.0001					
		Folate	0.02	0.03	0.08	0.37					
		B12	0.00	0.00	0.02	0.88					
		Betaine	0.00	0.01	-0.03	0.97					
		Choline	-0.08	0.07	-0.11	0.23					

		Methionine	0.01	0.01	0.14	0.21				
		DMG	0.25	0.22	0.10	0.26				
EDEQ-W	1	Age	-0.00	0.02	-0.01	0.91	0.64	0.64	<.0001	42.30	<.0001
		BMI	0.15	0.05	0.31	0.01				
		Rem	-3.66	0.46	-0.79	<.0001				
		NED	-4.69	0.52	-1.06	<.0001				
	2						0.66	0.02	0.62	17.10	<.0001
		Age	-0.01	0.02	-0.02	0.80				
		BMI	0.14	0.06	0.29	0.02				
		Rem	-3.79	0.49	-0.82	<.0001				
		NED	-4.85	0.55	-1.10	<.0001				
		Folate	0.04	0.02	0.12	0.09				
		B12	0.00	<.0001	-0.10	0.49				
		Betaine	-0.01	0.01	-0.10	0.39				
		Choline	0.04	0.07	0.06	0.51				
		Methionine	0.00	0.01	0.02	0.85				
		DMG	0.09	0.20	0.03	0.68				
EDEQ-S	1	Age	0.00	0.02	0.00	0.003	0.67	0.67	<.0001	50.46	<.0001
		BMI	0.12	0.05	0.25	0.97				
		Rem	-3.56	0.44	-0.78	<.0001				
		NED	-4.74	0.50	-1.06	<.0001				
	2						0.68	0.01	0.95	19.46	<.0001
		Age	-0.00	0.02	-0.00	0.97				
		BMI	0.11	0.05	0.24	0.04				
		Rem	-3.62	0.47	-0.8	<.0001				
		NED	-4.78	0.53	-1.06	<.0001				
		Folate	0.03	0.02	0.08	0.26				
		B12	0.00	0.00	-0.02	0.84				
		Betaine	-0.00	0.01	-0.03	0.69				
		Choline	0.02	0.07	0.02	0.78				
		Methionine	0.00	0.01	-0.00	0.99				
		DMG	0.01	0.20	0.00	0.96				
EDEQ-T	1	Age	0.00	0.02	0.00	0.97	0.67	0.67	<.0001	50.46	<.0001

		BMI	0.12	0.05	0.25	0.02				
		Rem	-3.56	0.44	-0.78	<.0001				
		NED	-4.74	0.50	-1.06	<.0001				
	2						0.68	0.01	0.93	19.46	<.0001
		Age	-0.00	0.02	-0.00	0.97				
		BMI	0.11	0.05	0.24	0.04				
		Rem	-3.62	0.47	-0.79	<.0001				
		NED	-4.78	0.53	-1.06	<.0001				
		Folate	0.03	0.02	0.08	0.26				
		B12	0.00	0.00	-0.02	0.84				
		Betaine	-0.00	0.01	-0.03	0.69				
		Choline	0.02	0.07	0.02	0.78				
		Methionine	0.00	0.01	-0.00	0.99				
		DMG	0.01	0.20	0.00	0.96				
DAPP-BQ Anx.	1	Age	-0.00	0.01	-0.01	0.89	0.46	0.46	<.0001	20.77	<.0001
		BMI	-0.00	0.04	-0.01	0.93				
		Rem	-0.71	0.31	-0.29	0.02				
		NED	-1.84	0.35	-0.74	<.0001				
	2						0.02	0.02	0.82	8.34	<.0001
		Age	-0.01	0.01	-0.04	0.61				
		BMI	0.00	0.04	0.00	0.98				
		Rem	-0.71	0.33	-0.29	0.03				
		NED	-1.82	0.37	-0.74	<.0001				
		Folate	0.01	0.02	0.06	0.51				
		B12	0.00	0.00	0.03	0.77				
		Betaine	0.00	0.01	0.10	0.30				
		Choline	0.01	0.05	0.02	0.84				
		Methionine	0.00	0.00	-0.00	0.97				
		DMG	0.03	0.14	0.02	0.85				
CES-D	1	Age	0.08	0.16	0.04	0.59	0.54	0.54	<.0001	29.00	<.0001
		BMI	0.26	0.49	0.06	0.59				
		Rem	-20.64	4.13	-0.57	<.0001				
		NED	-30.22	4.73	-0.83	<.0001				

	2						0.55	0.02	0.80	11.56	<.0001
		Age	0.05	0.17	0.02	0.79					
		BMI	0.23	0.52	0.06	0.65					
		Rem	-21.03	4.43	-0.58	<.0001					
		NED	-30.28	4.99	-0.83	<.0001					
		Folate	0.22	0.21	0.08	0.29					
		B12	0.00	0.00	0.03	0.78					
		Betaine	0.01	0.07	0.01	0.93					
		Choline	0.60	0.62	0.09	0.33					
		Methionine	-0.01	0.06	-0.02	0.87					
		DMG	-0.51	1.83	-0.02	0.78					
BIS11-A	1	Age	-0.01	0.01	-0.07	0.47	0.12	0.12	0.01	3.30	0.01
		BMI	0.01	0.02	0.11	0.52					
		Rem	-0.14	0.17	-0.13	0.40					
		NED	-0.49	0.20	-0.45	0.02					
	2						0.17	0.05	0.54	1.82	0.07
		Age	-0.01	0.01	-0.11	0.28					
		BMI	0.01	0.02	0.07	0.68					
		Rem	-0.12	0.18	-0.12	0.47					
		NED	-0.50	0.21	-0.47	0.02					
		Folate	0.01	0.01	0.08	0.49					
		B12	0.00	0.00	-0.13	0.32					
		Betaine	0.00	0.00	0.04	0.76					
		Choline	0.02	0.03	0.10	0.46					
		Methionine	-0.00	0.00	-0.09	0.56					
		DMG	0.01	0.10	0.01	0.92					
BIS11-M	1	Age	-0.00	0.01	-0.05	0.59	0.04	0.04	0.37	1.08	0.37
		BMI	-0.00	0.02	-0.03	0.88					
		Rem	0.02	0.17	0.02	0.91					
		NED	-0.18	0.19	-0.18	0.35					
	2						0.09	0.04	0.64	0.86	0.58
		Age	-0.00	0.01	-0.06	0.60					
		BMI	-0.01	0.02	-0.09	0.63					

		Rem	0.08	0.18	0.08	0.66				
		NED	-0.14	0.20	-0.14	0.50				
		Folate	0.00	0.01	-0.01	0.96				
		B12	0.00	0.00	-0.07	0.63				
		Betaine	0.00	0.00	0.02	0.85				
		Choline	0.03	0.03	0.19	0.18				
		Methionine	0.00	0.00	0.03	0.82				
		DMG	-0.10	0.07	-0.16	0.17				
BIS11-NP	1	Age	0.00	0.01	0.04	0.67	0.06	0.06	0.21	1.50	0.21
		BMI	-0.02	0.02	-0.19	0.28					
		Rem	0.34	0.16	0.35	0.04					
		NED	0.33	0.18	0.35	0.07					
	2						0.12	0.06	0.43	1.20	0.30
		Age	0.00	0.01	0.05	0.60					
		BMI	-0.03	0.02	-0.28	0.14					
		Rem	0.41	0.17	0.42	0.17					
		NED	0.36	0.19	0.37	0.06					
		Folate	0.00	0.01	0.01	0.90					
		B12	0.00	0.00	-0.30	0.02					
		Betaine	0.00	0.00	0.08	0.52					
		Choline	-0.02	0.02	-0.10	0.45					
		Methionine	0.00	0.00	0.21	0.16					
		DMG	-0.01	0.07	-0.01	0.90					
BIS11-T	1	Age	-0.05	0.15	-0.03	0.77	0.19	0.04	0.48	0.88	
		BMI	-0.17	0.46	-0.07	0.72					
		Rem	3.08	3.92	0.13	0.43					
		NED	-1.72	4.49	-0.07	0.70					
	2						0.28	0.04	0.63	0.79	
		Age	-0.06	0.16	-0.04	0.73					
		BMI	-0.38	0.49	-0.15	0.43					
		Rem	4.59	4.17	0.19	0.27					
		NED	-1.18	4.72	-0.05	0.80					
		Folate	0.06	0.20	0.03	0.78					

B12	-0.01	0.00	-0.22	0.10
Betaine	0.03	0.06	0.06	0.62
Choline	0.27	0.58	0.07	0.64
Methionine	0.03	0.05	0.09	0.57
DMG	-1.04	1.72	-0.07	0.55

Active Anorexia Nervosa was used as a reference in analyses; SE= Standard error; Δ R^2= R^2 change; *p* value Δ R^2= significant R^2 change; EDEQ= Eating Disorder Examination Questionnaire (R=dietary restraint; E= eating concerns; W=weight concerns; S=shape concerns; T= total); DAPP-BQ (Anx)= The dimensional Assessment of Personality Pathology- Basic Questionnaire (anxiousness scale); CES-D= The Center for Epidemiological Studies Depression Scale; BIS11= The Barratt Impulsiveness Scale (A= attentional; M= motor; NP= non-planning; T= total).

Table 6

Probes (and corresponding genes) on which methylation levels differ significantly (Q < .01) in ANOVA and pairwise comparisons of AN-Active vs. AN-Remitted vs. NED.

				ANOVA	AN-Active vs. NED		AN-Active vs. AN-Remitted	
CpG site	Gene	Name	Function	Q-value	Effect	Q-value	Effect	Q-value
cg04173586	DOT1L	DOT1 like histone lysine methyltransferase	The protein encoded by this gene is a histone methyltransferase that methylates lysine-79 of histone H3.	1.65E-05	0.112951	0.002893	-0.097642	0.000549
cg06983052	LRRC8D	leucine rich repeat containing 8 VRAC subunit D		6.64E-04	0.049142	0.004674		
cg25197194	CCDC48	EF-hand and coiled-coil domain containing 1		6.64E-04	0.064103	0.004674		
cg14422240	FTSJD2	cap methyltransferase 1		6.64E-04	0.067308	0.001105	-0.047185	0.009952
cg23597162	JAZF1	JAZF zinc finger 1	This gene encodes a nuclear protein with three C2H2-type zinc fingers, and functions as a transcriptional repressor.	6.64E-04			-0.070082	0.009952

Table 6 *Continued*

				ANOVA	AN-Active vs. NED		AN-Active vs. AN-Remitted	
CpG site	Gene	Name	Function	Q-value	Effect	Q-value	Effect	Q-value
cg17833746	STAT3	signal transducer and activator of transcription 3	The encoded protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis.	7.92E-04			-0.067219	0.000560
cg17870520	GTDC1	Glycosyl-transferase like domain containing 1		2.33E-03	0.090459	0.000122		
cg25570328	SULT1C2	sulfotransferase family 1C member 2	Sulfotransferase enzymes catalyze the sulfate conjugation of many hormones, neurotransmitters, drugs, and xenobiotic compounds.This gene encodes a protein that belongs to the SULT1 subfamily, responsible for transferring a sulfo moiety from PAPS to phenol-containing compounds.	3.85E-03	0.048277	0.000698		

Table 6 *Continued*

				ANOVA	AN-Active vs. NED		AN-Active vs. AN-Remitted	
CpG site	Gene	Name	Function	Q-value	Effect	Q-value	Effect	Q-value
cg22091236	RPTOR	regulatory associated protein of MTOR complex 1	This gene encodes a component of a signaling pathway that regulates cell growth in response to nutrient and insulin levels.	3.85E-03	0.045784	0.000021		
cg00968616	CUEDC1			5.69E-03				
cg21899461	STK39			7.39E-03				
cg21583440	GDPD5	glycerophosphodiester phosphodiesterase domain containing 5	Glycerophosphodiester phosphodiesterases such as GDPD5 are involved in glycerol metabolism		0.071360	0.004674		
cg00207226	C5orf63	chromosome 5 open reading frame 63					-0.049254	0.005873
cg05487134	STAT3	signal transducer and activator of transcription 3	The encoded protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis.				-0.066609	0.006483

Table 6 *Continued*

				ANOVA	AN-Active vs. NED		AN-Active vs. AN-Remitted	
CpG site	Gene	Name	Function	Q-value	Effect	Q-value	Effect	Q-value
cg26232451	LETM2	leucine zipper and EF-hand containing transmembrane protein 2					-0.037246	0.008247
cg03479289	C11orf41						-0.051520	0.009952

AN-Active= active Anorexia Nervosa; AN-Remitted= remitted from Anorexia Nervosa; ANOVA = analysis of variance; Genes and gene functions are taken from the NCBI gene database https://www.ncbi.nlm.nih.gov/gene. (Empty cells imply that clearly established functions are lacking as per the NCBI gene database).

Appendix

Table A1

Probes (and corresponding genes) at which methylation levels are significantly associated (Q < .01) with an interaction between B12 levels and diagnostic category

AN-Active versus NED-Controls						
CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg11674404	WDR17	WD repeat domain 17	This gene encodes a WD repeat-containing protein and is thought to be a candidate gene for retinal disease.	0.00026	0.00000	0.00000
cg23262213	SLC25A12	solute carrier family 25 member 12	The encoded protein is involved in the exchange of aspartate for glutamate across the inner mitochondrial membrane. Polymorphisms in this gene may be associated with autism.	-0.00030	-0.00001	0.00001
cg14796318	CCL5	C-C motif chemokine ligand 5	The encoded chemokine functions as a chemoattractant for blood monocytes, memory T helper cells and eosinophils. It causes the release of histamine from basophils and activates eosinophils	0.00022	0.00000	0.00001
cg06090161	SLC38A10	solute carrier family 38 member 10		-0.00028	-0.00001	0.00001

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg19689828	GLYATL2	glycine-N-acyltransferase like 2		0.00021	0.00000	0.00001
cg02652202	GNB5	G protein subunit beta 5	This gene encodes a beta subunit, which are important regulators of alpha subunits, as well as of certain signal transduction receptors and effectors.	-0.00032	0.00001	0.00001
cg24740515	SKI	SKI proto-oncogene	This gene encodes a protein that functions as a repressor of TGF-beta signaling, and may play a role in neural tube development and muscle differentiation.	-0.00026	-0.00001	0.00010
cg15641339	HDGFL1	HDGF like 1		-0.00022	0.00001	0.00020
cg21357996	MYH7B	myosin heavy chain 7B	This gene encodes a heavy chain of myosin II, which catalyzes ATP hydrolysis and interacts with actin, and a tail domain in which heptad repeat sequences promote dimerization by interacting to form a rod-like alpha-helical coiled coil.	-0.00019	0.00000	0.00020
cg09714852	PTPRN2	protein tyrosine phosphatase receptor type N2		-0.00045	-0.00002	0.00020

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg03809898	MCHR2	melanin concentrating hormone receptor 2		-0.00028	0.00001	0.00027
cg12175087	LY6E	lymphocyte antigen 6 family member E		-0.00013	-0.00001	0.00027
cg22168386	TNFRSF19	TNF receptor superfamily member 19	The encoded receptor is capable of inducing apoptosis by a caspase-independent mechanism, and it is thought to play an essential role in embryonic development.	-0.00018	0.00000	0.00027
cg11864201	TBC1D16	TBC1 domain family member 16		-0.00020	-0.00001	0.00027
cg26932839	RPTOR	regulatory associated protein of MTOR complex 1	This gene encodes a component of a signaling pathway that regulates cell growth in response to nutrient and insulin levels.	-0.00021	-0.00001	0.00027
cg23999224	SAMD4B	sterile alpha motif domain containing 4B		-0.00023	0.00000	0.00027

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg06080194	PMEPA1	prostate transmembrane protein, androgen induced 1	The encoded protein suppresses the androgen receptor and transforming growth factor beta signaling pathways though interactions with Smad proteins.	-0.00019	-0.00001	0.00027
cg06746829	PADI2	peptidyl arginine deiminase 2	This gene encodes an enzyme which catalyzes the post-translational deimination of proteins by converting arginine residues into citrullines in the presence of calcium ions.	0.00018	0.00000	0.00031
cg26570714	OR2T10	olfactory receptor family 2 subfamily T member 10	Olfactory receptors interact with odorant molecules in the nose, to initiate a neuronal response that triggers the perception of a smell.	-0.00020	-0.00001	0.00031
cg22673542	OR2T10	olfactory receptor family 2 subfamily T member 10	Olfactory receptors interact with odorant molecules in the nose, to initiate a neuronal response that triggers the perception of a smell.	-0.00020	0.00001	0.00031
cg24208604	CBFA2T3	CBFA2/RUNX 1 translocation partner 3	This gene encodes a member of the myeloid translocation gene family which recruits a range of corepressors to facilitate transcriptional repression.	-0.00021	-0.00001	0.00031
cg18121684	SERPINB13	serpin family B member 13	The encoded protein inhibits the activity of cathepsin K and is itself transcriptionally repressed by RUNX1	-0.00026	0.00000	0.00031

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg01608477	PTPRA	protein tyrosine phosphatase receptor type A	The protein encoded by this gene has been shown to dephosphorylate and activate Src family tyrosine kinases, and is implicated in the regulation of integrin signaling, cell adhesion and proliferation.	-0.00025	0.00000	0.00031
cg14420230	LAMC2	laminin subunit gamma 2	Laminins are the major noncollagenous constituent of basement membranes. They have been implicated in a wide variety of biological processes including cell adhesion, differentiation, migration, signaling, neurite outgrowth and metastasis.	-0.00026	-0.00001	0.00032
cg12547959	TRIO	trio Rho guanine nucleotide exchange factor	The encoded protein promotes the reorganization of the actin cytoskeleton, thereby playing a role in cell migration and growth.	0.00014	-0.00001	0.00033
cg26411409	TCP10L2	t-complex 10 like 2		-0.00025	-0.00001	0.00033
cg25532627	TBC1D16	TBC1 domain family member 16		-0.00019	-0.00001	0.00041
cg00821186	OR2T11	olfactory receptor family 2 subfamily T member 11 (gene/pseudogene)	Olfactory receptors interact with odorant molecules in the nose, to initiate a neuronal response that triggers the perception of a smell.	-0.00022	0.00000	0.00041

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg24357890	HRH1	histamine receptor H1	Histamine is a ubiquitous messenger molecule released from mast cells, enterochromaffin-like cells, and neurons. The protein encoded by this gene mediates the contraction of smooth muscles, the increase in capillary permeability due to contraction of terminal venules, the release of catecholamine from adrenal medulla, and neurotransmission in the central nervous system.	-0.00022	0.00000	0.00041
cg06038472	RAB43	RAB43, member RAS oncogene family		-0.00022	-0.00001	0.00041
cg25222324	C4orf36	chromosome 4 open reading frame 36		-0.00020	0.00000	0.00041
cg11464571	GNAI1	G protein subunit alpha i1	The encoded protein is part of a complex that responds to beta-adrenergic signals by inhibiting adenylate cyclase.	-0.00022	-0.00001	0.00041

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg25960479	GRM5	glutamate metabotropic receptor 5	The encoded protein is a metabotropic glutamate receptor, whose signaling activates a phosphatidylinositol-calcium second messenger system. This protein may be involved in the regulation of neural network activity and synaptic plasticity. Glutamatergic neurotransmission is involved in most aspects of normal brain function and can be perturbed in many neuropathologic conditions.	-0.00018	-0.00001	0.00041
cg26162657	C14orf143	EF-hand calcium binding domain 11		-0.00023	0.00000	0.00041
cg22453634	IGF1R	insulin like growth factor 1 receptor	The insulin-like growth factor I receptor plays a critical role in transformation events. It is highly overexpressed in most malignant tissues where it functions as an anti-apoptotic agent by enhancing cell survival.	-0.00020	0.00000	0.00041
cg12473257	PMEPA1	prostate transmembrane protein, androgen induced 1	The encoded protein suppresses the androgen receptor and transforming growth factor beta signaling pathways though interactions with Smad proteins.	-0.00019	0.00000	0.00041

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg14655532	KIAA0495	TP73 antisense RNA 1		-0.00024	-0.00002	0.00042
cg26313699	OR1G1	olfactory receptor family 1 subfamily G member	Olfactory receptors interact with odorant molecules in the nose, to initiate a neuronal response that triggers the perception of a smell.	-0.00017	-0.00001	0.00042
cg23429340	SETMAR	SET domain and mariner transposase fusion gene	The encoded protein binds DNA and functions in DNA repair activities including non-homologous end joining and double strand break repair.	-0.00024	-0.00001	0.00042
cg06103394	PAQR4	progestin and adipoQ receptor family member 4		0.00015	0.00000	0.00043
cg19879537	C12orf34	family with sequence similarity 222 member A		-0.00021	-0.00002	0.00043
cg20659584	BRD9	bromodomain containing 9		-0.00020	-0.00001	0.00044

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg18455772	PKDREJ	polycystin family receptor for egg jelly	This protein may play a role in human reproduction.	-0.00023	-0.00001	0.00049
cg04024799	LOC646627	LY6/PLAUR domain containing 8		0.00012	0.00002	0.00053
cg19339902	HTRA3	HtrA serine peptidase 3		0.00008	0.00001	0.00055
cg19835595	SYT4	synaptotagmin 4		-0.00022	-0.00001	0.00057
cg17331757	CTNNA2	catenin alpha 2		-0.00027	-0.00003	0.00058
cg25670567	GALNT9	polypeptide N-acetylgalactosaminyltransferase 9	This gene initiates mucin-type O-linked glycosylation in the Golgi apparatus by catalyzing the transfer of GalNAc to serine and threonine residues on target proteins.	-0.00017	-0.00001	0.00059
cg21635265	B4GALT7	beta-1,4-galactosyltransferase 7	The enzyme encoded by this gene attaches the first galactose in the common carbohydrate-protein linkage found in proteoglycans.	-0.00023	-0.00003	0.00060

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg03466717	ZNF423	zinc finger protein 423	The protein encoded by this gene functions as a DNA-binding transcription factor by using distinct zinc fingers in different signaling pathways.	0.00017	0.00001	0.00061
cg09227795	DDAH1	dimethylarginine dimethylaminohydrolase 1	The encoded enzyme plays a role in nitric oxide generation by regulating cellular concentrations of methylarginines, which in turn inhibit nitric oxide synthase activity.	-0.00011	0.00000	0.00061
cg04820440	STARD3NL	STARD3 N-terminal like	The encoded protein binds cholesterol molecules and may play a role in endosomal cholesterol transport.	-0.00019	-0.00001	0.00061
cg09045574	DNMBP	dynamin binding protein	This gene regulates the configuration of cell junctions.	-0.00018	0.00000	0.00061
cg05639937	DCLK1	doublecortin like kinase 1	The encoded protein is involved in several different cellular processes, including neuronal migration, retrograde transport, neuronal apoptosis and neurogenesis.	-0.00024	-0.00001	0.00061
cg10648125	HERC2	HECT and RLD domain containing E3 ubiquitin protein ligase 2	This gene belongs to the HERC gene family that encodes a group of unusually large proteins, which contain multiple structural domains.	-0.00024	0.00000	0.00061

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg04854451	MRPL46	mitochondrial ribosomal protein L46	Mammalian mitochondrial ribosomal proteins are encoded by nuclear genes and help in protein synthesis within the mitochondrion.	0.00020	0.00001	0.00061
cg07177972	CHSY1	chondroitin sulfate synthase 1	This gene encodes an enzyme that plays critical roles in the biosynthesis of chondroitin sulfate, a glycosaminoglycan involved in many biological processes including cell proliferation and morphogenesis.	-0.00018	0.00000	0.00061
cg15542639	PANX1	pannexin 1	The protein encoded by this gene belongs to the innexin family, which are the structural components of gap junctions.	-0.00018	0.00000	0.00066
cg23575099	LGALS3	galectin 3	The encoded protein plays a role in numerous cellular functions including apoptosis, innate immunity, cell adhesion and T-cell regulation. The protein exhibits antimicrobial activity against bacteria and fungi.	-0.00013	0.00000	0.00066
cg19263847	NCRNA00200	long intergenic non-protein coding RNA 200		-0.00011	-0.00001	0.00068

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg03328615	GRIN3B	glutamate ionotropic receptor NMDA type subunit 3B	The protein encoded by this gene is a subunit of an N-methyl-D-aspartate (NMDA) receptor. The encoded protein forms a heterotetramer with GRIN1 to create an excitatory glycine receptor.	-0.00023	-0.00001	0.00071
cg04910505	ADARB2	adenosine deaminase RNA specific B2	This gene encodes a member of the double-stranded RNA adenosine deaminase family of RNA-editing enzymes and may play a regulatory role in RNA editing.	-0.00019	0.00000	0.00073
cg10613426	ELSPBP1	epididymal sperm binding protein 1	The protein encoded by this gene belongs to the sperm-coating protein family of epididymal origin.	-0.00043	-0.00006	0.00073
cg02351082	C5orf60	chromosome 5 open reading frame 60		0.00019	0.00001	0.00074
cg12774454	ABLIM2	actin binding LIM protein family member 2		-0.00023	0.00000	0.00076
cg08434399	EBF3	EBF transcription factor 3	The encoded protein inhibits cell survival through the regulation of genes involved in cell cycle arrest and apoptosis.	-0.00024	-0.00001	0.00080
cg08233148	METRNL	meteorin like, glial cell differentiation regulator		-0.00039	0.00005	0.00082

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg09365094	PTPRN2	protein tyrosine phosphatase receptor type N2		-0.00020	-0.00001	0.00084
cg02220481	MYH11	myosin heavy chain 11	The protein encoded by this gene functions as a major contractile protein, converting chemical energy into mechanical energy through the hydrolysis of ATP.	-0.00024	-0.00001	0.00084
cg06736160	PALM	paralemmin	The product of this gene is implicated in plasma membrane dynamics in neurons and other cell types.	-0.00018	-0.00001	0.00084
cg19497388	ACSS1	acyl-CoA synthetase short chain family member 1	This gene encodes a mitochondrial acetyl-CoA synthetase enzyme.	-0.00027	-0.00002	0.00085
cg26592560	PANX1	pannexin 1	The protein encoded by this gene belongs to the innexin family. Innexin family members are the structural components of gap junctions.	-0.00021	-0.00002	0.00091
cg10469906	MVP	major vault protein	The encoded protein may play a role in multiple cellular processes. The encoded protein also plays a role in multidrug resistance.	-0.00023	-0.00001	0.00092
cg04615639	TBC1D16	TBC1 domain family member 16		-0.00018	0.00000	0.00093

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg08343075	DBNDD1	dysbindin domain containing 1		-0.00018	0.00000	0.00097
cg12567418	PRR22	proline rich 22		-0.00019	0.00000	0.00097
cg00453864	TMPRSS9	transmembrane serine protease 9	This gene enhances the invasive capability of pancreatic cancer cells and may be involved in cancer progression.	-0.00017	0.00000	0.00098
cg05372765	C6orf114	glucose-fructose oxidoreductase domain containing 1		-0.00014	-0.00001	0.00106
cg16956665	STT3A	STT3 oligosaccharyltransferase complex catalytic subunit A	The protein encoded by this gene functions in the endoplasmic reticulum to transfer glycan chains to asparagine residues of target proteins.	-0.00062	0.00003	0.00121
cg21654314	CHMP6	charged multivesicular body protein 6	This gene encodes a member of the chromatin-modifying protein/charged multivesicular body protein family, which degrade surface receptors, and in biosynthesis of endosomes.	0.00021	0.00002	0.00143

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg21048763	TBC1D16	TBC1 domain family member 16		-0.00018	0.00000	0.00318
cg23230830	LOC646627	LY6/PLAUR domain containing 8		0.00008	0.00000	0.00344
cg25979829	GGT1	gamma-glutamyltransferase 1	The enzyme encoded by this gene is a type I gamma-glutamyltransferase that catalyzes the transfer of the glutamyl moiety of glutathione to a variety of amino acids and dipeptide acceptors.	-0.00020	0.00000	0.00356
cg08621778	ARL8A	ADP ribosylation factor like GTPase 8A		0.00010	0.00003	0.00401
cg22524346	TPST1	tyrosylprotein sulfotransferase 1		-0.00022	0.00001	0.00404
cg24805759	TBC1D16	TBC1 domain family member 16		-0.00017	-0.00005	0.00404

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg21826272	EPHX1	epoxide hydrolase 1	Epoxide hydrolase is a critical biotransformation enzyme that converts epoxides from the degradation of aromatic compounds to trans-dihydrodiols which can be conjugated and excreted from the body.	-0.00012	-0.00001	0.00411
cg16603012	APRT	adenine phosphoribosyltransferase	The encoded enzyme catalyzes the formation of AMP and inorganic pyrophosphate from adenine and 5-phosphoribosyl-1-pyrophosphate (PRPP). It also produces adenine as a by-product of the polyamine biosynthesis pathway.	0.00017	0.00001	0.00411
cg03810198	MXRA7	matrix remodeling associated 7		0.00053	0.00000	0.00431
cg09675820	TBC1D16	TBC1 domain family member 16		-0.00018	-0.00001	0.00470
cg24992817	B3GNT1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2	The encoded enzyme is involved in the biosynthesis of poly-N-acetyllactosamine chains.	-0.00020	-0.00002	0.00506

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg15177604	POLR1B	RNA polymerase I subunit B	Eukaryotic RNA polymerase I (pol I) is responsible for the transcription of ribosomal RNA (rRNA) genes and production of rRNA, the primary component of ribosomes.	0.00027	-0.00001	0.00538
cg25234117	PLCH1	phospholipase C eta 1	PLCH1 is an enzyme that cleaves phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2) to generate second messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG)	0.00007	0.00001	0.00578
cg02326253	NUDT1	nudix hydrolase 1	The encoded protein helps prevent mutations that may result in carcinogenesis or neurodegeneration.	-0.00014	-0.00001	0.00578
cg05681757	FGD4	FYVE, RhoGEF and PH domain containing 4	This gene encodes a protein that is involved in the regulation of the actin cytoskeleton and cell shape. It is also involved in the activation of CDC42 via the exchange of bound GDP for free GTP.	0.00009	0.00000	0.00578
cg14780837	SYNGR1	synaptogyrin 1	This gene encodes an integral membrane protein associated with presynaptic vesicles in neuronal cells. The exact function of this protein is unclear.	0.00009	0.00002	0.00578

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg17514168	OR2T11	olfactory receptor family 2 subfamily T member 11 (gene/pseudogene)	Olfactory receptors interact with odorant molecules in the nose, to initiate a neuronal response that triggers the perception of a smell.	-0.00015	-0.00001	0.00579
cg19736040	SHC2	SHC adaptor protein 2		-0.00017	-0.00001	0.00579
cg06384865	ARSJ	arylsulfatase family member J	Sulfatases, such as ARSJ, are involved in hormone biosynthesis, modulation of cell signaling, and degradation of macromolecules	-0.00015	0.00001	0.00590
cg26944949	LOC554203	JPX transcript, XIST activator	JPX is a nonprotein-coding RNA that appears to participate in X chromosome inactivation.	-0.00019	-0.00001	0.00609
cg25048531	LOC440839	(No gene available)		-0.00012	-0.00002	0.00651
cg11338389	COX8A	cytochrome c oxidase subunit 8A	The protein encoded by this gene couples the transfer of electrons from cytochrome c to molecular oxygen, with the concomitant production of a proton electrochemical gradient across the inner mitochondrial membrane.	0.00020	0.00000	0.00696

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg13332114	MUC4	mucin 4, cell surface associated	Glycoproteins play important roles in the protection of the epithelial cells and have been implicated in epithelial renewal and differentiation. This gene encodes an integral membrane glycoprotein.	-0.00011	0.00000	0.00748
cg24405543	C10orf47	proline and serine rich 2		-0.00022	0.00000	0.00762
cg20264068	CR1L	complement C3b/C4b receptor 1 like		0.00018	-0.00002	0.00765
cg14491535	JAZF1	JAZF zinc finger 1	This gene encodes a nuclear protein with three C2H2-type zinc fingers, and functions as a transcriptional repressor.	0.00011	0.00002	0.00923
cg04968013	CACNB2	calcium voltage-gated channel auxiliary subunit beta 2	This gene encodes a subunit of a voltage-dependent calcium channel protein that is a member of the voltage-gated calcium channel superfamily.	-0.00029	0.00000	0.00923
cg08940505	TBC1D16	TBC1 domain family member 16		-0.00015	0.00002	0.00923

Table A1 *continued*

AN-Active versus AN-Remitted						
CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg21601498	FBXO42	F-box protein 42	SCF complexes, formed by SKP1, cullin, and F-box proteins, act as protein-ubiquitin ligases.	-2.13E-06	-3.24E-06	2.57E-06
cg25793243	GREB1	growth regulating estrogen receptor binding 1	This gene is an estrogen-responsive gene that is an early response gene in the estrogen receptor-regulated pathway. It is thought to play an important role in hormone-responsive tissues and cancer.	1.31E-06	3.89E-06	2.57E-06
cg20934215	PDZRN3	PDZ domain containing ring finger 3 x2	The encoded protein may function in vascular morphogenesis and the differentiation of adipocytes, osteoblasts and myoblasts.	5.37E-06	-9.64E-06	2.57E-06
cg24131452	DIP2C	disco interacting protein 2 homolog C x2		9.19E-06	2.98E-06	2.57E-06
cg22647566	FAM160B1	family with sequence similarity 160 member B1		-6.04E-06	4.55E-06	2.93E-05

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg11674404	WDR17	WD repeat domain 17	This gene encodes a WD repeat-containing protein. It is abundantly expressed in retina and testis, and is thought to be a candidate gene for retinal disease.	2.55E-04	7.00E-06	6.54E-05
cg14796318	CCL5	C-C motif chemokine ligand 5	The encoded chemokine, a member of the CC subfamily, functions as a chemoattractant for blood monocytes, memory T helper cells and eosinophils. It causes the release of histamine from basophils and activates eosinophils.	2.19E-04	-1.23E-05	9.15E-04
cg02652202	GNB5	G protein subunit beta 5	This gene encodes a beta subunit, which are important regulators of alpha subunits, as well as of certain signal transduction receptors and effectors.	-3.17E-04	-3.06E-06	1.08E-03

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg23262213	SLC25A12	solute carrier family 25 member 12	The encoded protein localizes to the mitochondria and is involved in the exchange of aspartate for glutamate across the inner mitochondrial membrane. Polymorphisms in this gene may be associated with autism.	-2.98E-04	1.19E-05	2.03E-03
cg19689828	GLYATL2	glycine-N-acyltransferase like 2		2.12E-04	-2.34E-05	2.07E-03
cg06090161	SLC38A10	solute carrier family 38 member 10		-2.79E-04	-9.44E-06	2.07E-03
cg10986455	GDPD2	glycerophosphodiester phosphodiesterase domain containing 2	The encoded protein hydrolyzes glycerophosphoinositol to produce inositol 1-phosphate and glycerol. This protein may have a role in osteoblast (bone) differentiation and growth.	4.45E-05	3.30E-05	2.07E-03
cg20276743	ACACA	acetyl-CoA carboxylase alpha	ACC is a biotin-containing enzyme which catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, the rate-limiting step in fatty acid synthesis.	1.25E-04	-1.47E-04	4.77E-03

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg22673542	OR2T10	olfactory receptor family 2 subfamily T member 10	Olfactory receptors interact with odorant molecules in the nose, to initiate a neuronal response that triggers the perception of a smell.	-2.01E-04	-1.60E-05	8.54E-03
cg12547959	TRIO	trio Rho guanine nucleotide exchange factor	The encoded protein promotes the reorganization of the actin cytoskeleton, thereby playing a role in cell migration and growth.	1.37E-04	3.12E-05	8.54E-03
cg15641339	HDGFL1	HDGF like 1		-2.16E-04	-7.03E-06	8.54E-03
cg03809898	MCHR2	melanin concentrating hormone receptor 2		-2.84E-04	-2.18E-05	8.54E-03
cg10421029	FBXL19	F-box and leucine rich repeat protein 19	The encoded protein is reported to bind to the transmembrane receptor interleukin 1 receptor-like 1 and regulate its ubiquitination and degradation.	2.96E-05	-2.38E-05	8.54E-03
cg21766592	SLC1A5	solute carrier family 1 member 5	The SLC1A5 gene encodes a sodium-dependent neutral amino acid transporter that can act as a receptor for RD114/type D retrovirus	1.05E-05	-7.19E-06	8.54E-03

Table A1 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg21357996	MYH7B	myosin heavy chain 7B	This gene encodes a heavy chain of myosin II, which includes a globular motor domain, which catalyzes ATP hydrolysis and interacts with actin.	-1.91E-04	-2.67E-05	8.54E-03
cg03485694	EFCAB4B	calcium release activated channel regulator 2A		-2.80E-05	-3.84E-05	9.49E-03
NED-Controls versus AN-Remitted						
CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg21601498	FBXO42	F-box protein 42	SCF complexes, formed by SKP1, cullin, and F-box proteins, act as protein-ubiquitin ligases.	0.00013	0.00000	0.00000
cg25793243	GREB1	growth regulating estrogen receptor binding 1 x2	This gene is an estrogen-responsive gene that is an early response gene in the estrogen receptor-regulated pathway. It is thought to play an important role in hormone-responsive tissues and cancer.	-0.00030	0.00000	0.00000
cg24131452	DIP2C	disco interacting protein 2 homolog C		-0.00030	0.00000	0.00000

Table A1 *continued*

CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg20934215	PDZRN3	PDZ domain containing ring finger 3	The encoded protein may function in vascular morphogenesis and the differentiation of adipocytes, osteoblasts and myoblasts.	-0.00030	-0.00001	0.00000
cg22647566	FAM160B1	family with sequence similarity 160 member B1		-0.00029	0.00000	0.00000
cg21766592	SLC1A5	solute carrier family 1 member 5	The SLC1A5 gene encodes a sodium-dependent neutral amino acid transporter that can act as a receptor for RD114/type D retrovirus	0.00011	-0.00001	0.00001
cg03485694	EFCAB4B	calcium release activated channel regulator 2A		0.00015	-0.00004	0.00014
cg10986455	GDPD2	glycerophosphodiester phosphodiesterase domain containing 2	The encoded protein hydrolyzes glycerophosphoinositol to produce inositol 1-phosphate and glycerol. This protein may have a role in osteoblast differentiation and growth.	-0.00020	0.00003	0.00014

Table A1 *continued*

CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg25607249	SLC1A5	solute carrier family 1 member 5	The SLC1A5 gene encodes a sodium-dependent neutral amino acid transporter that can act as a receptor for RD114/type D retrovirus	0.00007	0.00000	0.00389

AN-Active= active anorexia nervosa group. AN-Remitted= remitted from anorexia nervosa group. NED-Controls= non-eating disordered controls. Effect AN= effect in AN-Active group. Effect Rem= effect in AN-Remitted group. Effect NED= effect in NED-Controls. *Genes and gene functions are taken from the NCBI gene database https://www.ncbi.nlm.nih.gov/gene. (Empty cells imply that clearly established functions are lacking as per the NCBI gene database).*

Table A2

Probes (and corresponding genes) at which methylation levels are significantly associated (Q < .01) with an interaction between methionine levels and diagnostic category

AN-Active versus NED-Controls						
CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg21630843	SGMS2	sphingomyelin synthase 2	The protein encoded by this gene is an enzyme that catalyzes the formation of sphingomyelin, primarily at the cell membrane.	-0.004385	-0.000538	4.04E-09
cg02652202	GNB5	G protein subunit beta 5	This gene encodes a beta subunit of G proteins. Beta subunits are important regulators of alpha subunits, as well as of certain signal transduction receptors and effectors.	-0.003416	-0.000168	8.89E-07
cg14796318	CCL5	C-C motif chemokine ligand 5	The ended chemokine, a member of the CC subfamily, functions as a chemoattractant for blood monocytes, memory T helper cells and eosinophils. It causes the release of histamine from basophils and activates eosinophils.	0.002301	9.60E-05	8.89E-07

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg23262213	SLC25A12	solute carrier family 25 member 12	The encoded protein localizes to the mitochondria and is involved in the exchange of aspartate for glutamate across the inner mitochondrial membrane.	-0.003112	-0.000321	1.45E-06
cg24342013	RB1	RB transcriptional corepressor 1	The protein encoded by this gene is a negative regulator of the cell cycle and was the first tumor suppressor gene found. The encoded protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure.	-0.002675	-0.000645	4.21E-06
cg19689828	GLYATL2	glycine-N-acyltransferase like 2		0.002079	0.000331	3.27E-05
cg11724156	BUD31	BUD31 homolog		-0.003046	-0.000287	0.000146
cg20137461	GPR160	G protein-coupled receptor 160		-0.002272	-0.000255	0.000189

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg10539616	CNOT7	CCR4-NOT transcription complex subunit 7	The encoded protein provides a therapeutic target for enhancing its antimicrobial activity against foreign agents.	-0.002939	0.000163	0.000248
cg07834743	ADAMTS16	ADAM metallopeptidase with thrombospondin type 1 motif 16	The encoded preproprotein is proteolytically processed to generate the mature protein, which may inhibit chondrosarcoma cell proliferation and migration. This gene may regulate blood pressure.	0.001958	-4.84E-06	0.000291
cg07206630	FGF1	fibroblast growth factor 1	The encoded protein functions as a modifier of endothelial cell migration and proliferation, as well as an angiogenic factor. It is thought to be involved in organogenesis.	-0.001521	8.58E-05	0.000291
cg21488538	CNGA3	cyclic nucleotide gated channel alpha 3	This gene encodes a member of the cyclic nucleotide-gated cation channel protein family which is required for normal vision and olfactory signal transduction.	0.001589	-0.000548	0.000527

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg14397416	LOC645752	golgin A6 family member A pseudogene		-0.001713	-0.000478	0.000527
cg02480602	IFNGR1	interferon gamma receptor 1	This gene (IFNGR1) encodes the ligand-binding chain (alpha) of the gamma interferon receptor. A genetic variation in IFNGR1 is associated with susceptibility to Helicobacter pylori infection.	-0.002716	0.000514	0.000633
cg05110629	NEK1	NIMA related kinase 1	The protein encoded by this gene is a serine/threonine kinase involved in cell cycle regulation.	-0.001533	-0.000295	0.000676
cg02835561	CNN3	calponin 3	This encoded protein is associated with the cytoskeleton but is not involved in contraction.	-0.002732	0.000134	0.000690
cg18051316	RDBP	negative elongation factor complex member E	It has not been demonstrated that the encoded protein binds RNA.	-0.001692	3.86E-06	0.000690

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg17568962	TEAD3	TEA domain transcription factor 3	This gene product is a member of the transcriptional enhancer factor (TEF) family of transcription factors, and is involved in the transactivation of the chorionic somatomammotropin -B gene enhancer.	0.000564	0.000177	0.000690
cg20016411	DRD2	dopamine receptor D2	This gene encodes the D2 subtype of the dopamine receptor. This G-protein coupled receptor inhibits adenylyl cyclase activity.	0.001350	0.000564	0.000690
cg04099420	RIPK1	receptor interacting serine/threonine kinase 1	The encoded protein plays a role in inflammation and cell death in response to tissue damage, pathogen recognition, and as part of developmental regulation.	-0.001134	-0.000187	0.000700
cg01255913	TSPAN17	tetraspanin 17		0.001604	0.000632	0.000714
cg25945676	SLC12A7	solute carrier family 12 member 7		-0.001651	0.000394	0.000820

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg17714861	PRKAG2	protein kinase AMP-activated non-catalytic subunit gamma 2	This gene is a member of the AMPK gamma subunit family. AMPK is an important energy-sensing enzyme that functions by inactivating key enzymes involved in regulating de novo biosynthesis of fatty acid and cholesterol.	-0.001744	3.22E-06	0.000820
cg10023652	PACRG	parkin coregulated	The parkin co-regulated gene protein forms a large molecular complex with chaperones, including heat shock proteins 70 and 90, and chaperonin components.	-0.002172	0.000238	0.000868
cg14448919	AHRR	aryl-hydrocarbon receptor repressor	The protein encoded by this gene participates in the aryl hydrocarbon receptor signaling cascade, which mediates dioxin toxicity, and is involved in regulation of cell growth and differentiation.	-0.001658	0.000143	0.000879
cg02739870	SLC12A7	solute carrier family 12 member 7		0.000665	0.000122	0.000879

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg11464763	MAD1L1	mitotic arrest deficient 1 like 1	MAD1L1 is a component of the mitotic spindle-assembly checkpoint that prevents the onset of anaphase until all chromosome are properly aligned at the metaphase plate.	-0.001143	-0.000210	0.000879
cg19525418	CHD7	chromodomain helicase DNA binding protein 7	This gene encodes a protein that contains several helicase family domains.	-0.002253	0.000443	0.000879
cg10382221	MIR152	microRNA 152	microRNAs (miRNAs) are short (20-24 nt) non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs.	0.001786	-0.000187	0.000879
cg15248035	CCIN	calicin	The encoded protein contains kelch repeats and a BTB/POZ domain and is necessary for normal morphology during sperm differentiation.	0.000904	0.000414	0.001012

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg20985758	PDXP	pyridoxal phosphatase	Pyridoxal 5-prime-phosphate (PLP) is the active form of vitamin B6 that acts as a coenzyme in maintaining biochemical homeostasis. The preferred degradation route from PLP to 4-pyridoxic acid involves the dephosphorylation of PLP by PDXP.	-0.001628	-0.000796	0.001032
cg13370754	SSBP2	single stranded DNA binding protein 2	This gene encodes a subunit of a protein complex that is involved in the DNA damage response and maintenance of genome stability. The encoded protein may also play a role in telomere repair.	-0.002649	0.000619	0.001236
cg04911180	TBL1XR1	transducin beta like 1 X-linked receptor 1	The protein encoded by this gene is required for transcriptional activation by a variety of transcription factors.	-0.001339	0.000133	0.001285
cg12808359	WNK4	WNK lysine deficient protein kinase 4	The encoded kinase regulates the activities of several types of ion channels, cotransporters, and exchangers involved in electrolyte flux in epithelial cells	0.001559	-0.000495	0.001316

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg24376810	ELK1	ETS transcription factor ELK1	The protein encoded by this gene is a nuclear target for the ras-raf-MAPK signaling cascade.	0.001412	0.000384	0.001316
cg07658508	SLC26A1	solute carrier family 26 member 1	This gene is a member of a family of sulfate/anion transporter genes.	-0.001144	-0.000210	0.001484
cg08409113	WNK4	WNK lysine deficient protein kinase 4	The encoded kinase regulates the activities of several types of ion channels, cotransporters, and exchangers involved in electrolyte flux in epithelial cells	0.002200	-0.000439	0.001546
cg19442647	ZXDC	ZXD family zinc finger C		0.002162	0.000653	0.001712
cg02425140	C17orf97	chromosome 17 open reading frame 97		0.002050	-0.000618	0.001712
cg19249811	SVIL	supervillin	The encoded protein appears to aid in both myosin II assembly during cell spreading and disassembly of focal adhesions.	-0.001972	0.000328	0.001722

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg18148314	ACOT7	acyl-CoA thioesterase 7	The encoded protein hydrolyzes the CoA thioester of palmitoyl-CoA and other long-chain fatty acids.	0.000885	0.000400	0.001877
cg02421033	CACNA1H	calcium voltage-gated channel subunit alpha1 H	This gene encodes a T-type member of the alpha-1 subunit family, a protein in the voltage-dependent calcium channel complex.	-0.001835	-0.000387	0.001877
cg03780356	EIF4G1	eukaryotic translation initiation factor 4 gamma 1	The protein encoded by this gene is a component of the multi-subunit protein complex EIF4F. This complex facilitates the recruitment of mRNA to the ribosome, which is a rate-limiting step during the initiation phase of protein synthesis.	0.001528	0.000211	0.001937
cg11661235	THEM5	thioesterase superfamily member 5		-0.002572	8.25E-05	0.001990

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg18402034	CLIC1	chloride intracellular channel 1	Chloride channels are a diverse group of proteins that regulate fundamental cellular processes including stabilization of cell membrane potential, transepithelial transport, maintenance of intracellular pH, and regulation of cell volume.	-0.002289	0.000507	0.001990
cg25313204	SLC22A3	solute carrier family 22 member 3		0.001655	0.000250	0.001990
cg04910505	ADARB2	adenosine deaminase RNA specific B2 (inactive)	This gene encodes a member of the double-stranded RNA adenosine deaminase family of RNA-editing enzymes and may play a regulatory role in RNA editing.	-0.001875	-0.000177	0.002015

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg07630255	MPI	mannose phosphate isomerase	Phosphomannose isomerase catalyzes the interconversion of fructose-6-phosphate and mannose-6-phosphate and plays a critical role in maintaining the supply of D-mannose derivatives, which are required for most glycosylation reactions.	0.000935	0.000524	0.002015
cg13537353	ATP8A2	ATPase phospholipid transporting 8A2	The protein encoded by this gene is thought to aid in generating and maintaining asymmetry in membrane lipids.	0.001823	1.61E-05	0.002137
cg08308214	PECI	enoyl-CoA delta isomerase 2	The protein encoded is a key mitochondrial enzyme involved in beta-oxidation of unsaturated fatty acids.	-0.002366	-0.000331	0.002292
cg18182148	GFI1	growth factor independent 1 transcriptional repressor	The encoded protein plays a role in diverse developmental contexts, including hematopoiesis and oncogenesis.	0.002155	0.000586	0.002309

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg09631415	CDH11	cadherin 11	This gene encodes a type II classical cadherin from the cadherin superfamily, integral membrane proteins that mediate calcium-dependent cell-cell adhesion.	0.001359	0.000705	0.002721
cg09618893	EHMT2	euchromatic histone lysine methyltransfera se 2	This gene encodes a methyltransferase that methylates lysine residues of histone H3. Methylation of H3 at lysine 9 by this protein results in recruitment of additional epigenetic regulators and repression of transcription.	0.001539	-0.000554	0.003013
cg18637761	PITPNM2	phosphatidylino sitol transfer protein membrane associated 2		0.000573	0.000309	0.003013

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg18135087	TUFT1	tuftelin 1	Tuftelin is an acidic protein that is thought to play a role in dental enamel mineralization and is implicated in caries susceptibility. It is also thought to be involved with adaptation to hypoxia, mesenchymal stem cell function, and neurotrophin nerve growth factor mediated neuronal differentiation.	-0.002473	0.000249	0.003020
cg08233148	METRNL	meteorin like, glial cell differentiation regulator		-0.003693	0.000506	0.003020
cg12426467	C7orf28A	CCZ1 homolog, vacuolar protein trafficking and biogenesis associated		-0.001760	-0.000362	0.003134
cg11302624	RGS14	regulator of G protein signaling 14	This gene encodes a member of the regulator of G-protein signaling family. Acting as a GTPase activating protein (GAP), the protein increases the rate of conversion of the GTP to GDP.	-0.003633	-0.002025	0.003275

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg01372366	PTPRJ	protein tyrosine phosphatase receptor type J	The encoded protein was shown to negatively regulate T cell receptor signaling possibly through interfering with the phosphorylation of Phospholipase C Gamma 1 and Linker for Activation of T Cells.	0.000772	0.000307	0.003275
cg14311481	SART3	spliceosome associated factor 3, U4/U6 recycling protein	The encoded protein is thought to be involved in the regulation of mRNA splicing.	-0.001995	-0.000491	0.003275
cg16223079	PPCDC	phosphopantothenoylcysteine decarboxylase	Biosynthesis of coenzyme A (CoA) from pantothenic acid (vitamin B5) is an essential universal pathway in prokaryotes and eukaryotes. PPCDC, one of the last enzymes in this pathway, converts phosphopantothenoylcysteine to 4-prime-phosphopantetheine.	0.000540	5.22E-05	0.003314

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg05251269	SLC9A3R2	SLC9A3 regulator 2	The encoded protein plays a role in intestinal sodium absorption by regulating the activity of the sodium/hydrogen exchanger 3, and may also regulate the cystic fibrosis transmembrane regulator (CFTR) ion channel.	0.000580	0.000559	0.003314
cg17863679	ZNF711	zinc finger protein 711		0.001436	0.000184	0.003314
cg22152931	PRKRIP1	PRKR interacting protein 1		0.000733	0.000284	0.003350
cg02787087	IRX6	iroquois homeobox 6		0.001377	-2.00E-05	0.003675
cg21384971	COPZ2	coatomer protein complex subunit zeta 2	The encoded protein is a subunit of the coatomer protein complex, a seven-subunit complex that functions in the formation of COPI-type, non-clathrin-coated vesicles.	0.001175	0.000494	0.003675

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg18546006	TRIM13	tripartite motif containing 13	This gene is located on chromosome 13 within the minimal deletion region for B-cell chronic lymphocytic leukemia.	0.000505	0.000140	0.003730
cg06901711	PDIA3P	protein disulfide isomerase family A member 3 pseudogene 1		-0.002658	-0.001367	0.004016
cg26310000	KLK11	kallikrein related peptidase 11	Growing evidence suggests that many kallikreins are implicated in carcinogenesis and some have potential as novel cancer and other disease biomarkers.	-0.001340	-0.000694	0.004016
cg19003337	KRT17	keratin 17	This gene encodes the type I intermediate filament chain keratin 17, expressed in nail bed, hair follicle, sebaceous glands, and other epidermal appendages.	0.001096	0.000649	0.004042
cg01396391	SMYD3	SET and MYND domain containing 3	This gene encodes a histone methyltransferase which functions in RNA polymerase II complexes by an interaction with a specific RNA helicase.	0.001726	0.000107	0.004123

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg01500402	MLST8	MTOR associated protein, LST8 homolog		0.000648	0.000509	0.004260
cg14699728	NPAS4	neuronal PAS domain protein 4	NXF is a transcriptional regulator, which is involved in a wide range of physiologic and developmental events.	0.001338	-5.69E-05	0.004284
cg00261690	SNHG3-RCC1	regulator of chromosome condensation 1		0.000852	0.000640	0.004366
cg03693925	MICB	MHC class I polypeptide-related sequence B	This gene is involved in activating the cytolytic response of natural killer (NK) cells, CD8 alphabeta T cells, and gammadelta T cells which express the receptor.	-0.001376	-2.35E-06	0.004366
cg07576664	PARD3	par-3 family cell polarity regulator	PARD family members affect asymmetrical cell division and direct polarized cell growth	-0.002811	-0.001724	0.004426
cg14306819	ERCC3	ERCC excision repair 3, TFIIH core complex helicase subunit	This gene encodes an ATP-dependent DNA helicase that functions in nucleotide excision repair.	0.000768	0.000259	0.004609

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
ch.14.190588F	PRKD1	protein kinase D1	The protein encoded by this gene is involved in many cellular processes, including Golgi body membrane integrity and transport, cell migration and differentiation, MAPK8/JNK1 and Ras pathway signaling, MAPK1/3 pathway signaling, cell survival, and regulation of cell shape and adhesion.	0.001331	0.001327	0.004609
cg01919963	CNIH2	cornichon family AMPA receptor auxiliary protein 2	The protein encoded by this gene is an auxiliary subunit of the ionotropic glutamate receptor of the AMPA subtype. AMPA receptors mediate fast synaptic neurotransmission in the central nervous system.	0.001034	-1.27E-05	0.004613
cg05063104	SLC5A8	solute carrier family 5 member 8	SLC5A8 has been shown to transport iodide and short-chain fatty acids. In kidney, SLC5A8 functions as a high-affinity sodium-coupled lactate transporter involved in reabsorption of lactate and maintenance of blood lactate levels.	0.001111	0.000336	0.004613

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg03126694	LPAR6	lysophosphatidic acid receptor 6	The protein encoded by this gene belongs to the family of G-protein coupled receptors.	0.000600	0.000114	0.004646
cg23669081	HOXB7	homeobox B7	The encoded nuclear protein functions as a sequence-specific transcription factor that is involved in cell proliferation and differentiation.	-0.003480	0.000630	0.004805
cg21646366	RYR1	ryanodine receptor 1	The encoded protein functions as a calcium release channel in the sarcoplasmic reticulum but also serves to connect the sarcoplasmic reticulum and transverse tubule.	-0.002752	0.000714	0.004805
cg11313429	ALOX12P2	arachidonate 12-lipoxygenase pseudogene 2		-0.001738	-0.001114	0.004879
cg02743650	IGSF22	immunoglobulin superfamily member 22		0.002307	0.000202	0.004990
cg06352352	C16orf70	chromosome 16 open reading frame 70		0.000687	0.000319	0.004990

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg22225307	FCHO1	FCH domain only 1		0.001362	0.000367	0.005093
cg00244920	RPL32P3	ribosomal protein L32 pseudogene 3		-0.001987	0.000550	0.005204
cg11795975	HSPA12A	heat shock protein family A (Hsp70) member 12A		0.001254	-0.000119	0.005204
cg07458170	ZBTB44	zinc finger and BTB domain containing 44		0.001168	0.000573	0.005204
cg02877575	NTM	neurotrimin	The encoded protein may promote neurite outgrowth and adhesion via a homophilic mechanism.	0.001275	0.000775	0.005204
cg04128307	WDR59	WD repeat domain 59		0.001474	-9.75E-05	0.005523
cg10946263	C5orf33	NAD kinase 2, mitochondrial	This gene encodes a mitochondrial kinase that catalyzes the phosphorylation of NAD to yield NADP.	-0.001364	-8.71E-05	0.005630

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg04498349	GRIN2A	glutamate ionotropic receptor NMDA type subunit 2A	The encoded protein is an N-methyl-D-aspartate (NMDA) receptor subunit. NMDA receptors are involved in long-term potentiation, an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning.	0.000906	-0.000198	0.005630
cg03530210	WDR47	WD repeat domain 47		0.001781	0.001176	0.006146
cg24531401	INTS1	integrator complex subunit 1	INTS1 is a subunit of the Integrator complex, which mediates 3-prime end processing of small nuclear RNAs U1 and U2	0.000965	0.000633	0.006296
cg21558409	WNK4	WNK lysine deficient protein kinase 4	The encoded kinase regulates the activities of several types of ion channels, cotransporters, and exchangers involved in electrolyte flux in epithelial cells	0.001541	0.000805	0.006331

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg23600177	BTBD8	BTB domain containing 8		-0.001326	-0.000758	0.006778
cg26843872	TBC1D10B	TBC1 domain family member 10B	TBC1D10B functions as a GTPase-activating proteins (GAP) for several proteins of the Rab family	0.000535	0.000215	0.006778
cg08198187	AMN1	antagonist of mitotic exit network 1 homolog		0.001109	0.000631	0.006919
cg25257677	SLC39A14	solute carrier family 39 member 14	This gene encodes a member of the the SLC39A family of divalent metal transporters that mediates the cellular uptake of manganese, zinc, iron, and cadmium. It is an important transporter of nontransferrin-bound iron and a critical regulator of manganese homeostasis.	-0.002652	-0.002416	0.007053
cg22356063	FAM194B	glutamate rich 6B		-0.002763	-0.001518	0.007053

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg15998406	EFNA2	ephrin A2	The encoded EPH receptor and EPH-related receptors have been implicated in mediating developmental events, particularly in the nervous system.	0.001421	0.000365	0.007069
cg18135379	OLFML2B	olfactomedin like 2B		-0.002924	-0.002203	0.007165
cg06940195	SRPK2	SRSF protein kinase 2		0.001614	0.000442	0.007165
cg23218559	AMH	anti-Mullerian hormone	This protein plays a role in Leydig cell differentiation and function and follicular development in adult females.	0.001536	-5.74E-05	0.007165

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg02405193	BTBD12	SLX4 structure-specific endonuclease subunit	This gene encodes a protein that functions as an assembly component of multiple structure-specific endonucleases. These endonuclease complexes are required for repair of specific types of DNA lesions and critical for cellular responses to replication fork failure.	0.000792	0.000328	0.007166
cg27570256	LOC100270710	*(discontinued)*		0.000970	0.000962	0.007192
cg10101634	CMTM7	CKLF like MARVEL transmembrane domain containing 7	This gene acts as a tumor suppressor that regulates G1/S transition in the cell cycle, and epidermal growth factor receptor/protein kinase B signaling during tumor pathogenesis	0.001279	-0.000250	0.007274

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg15237829	TNIK	TRAF2 and NCK interacting kinase	The protein encoded by this gene is a serine/threonine kinase that functions as an activator of the Wnt signaling pathway, which plays important roles in carcinogenesis and embryonic development.	-0.002616	-0.000889	0.007350
cg13572782	MBP	myelin basic protein	The protein encoded by the classic MBP gene is a major constituent of the myelin sheath of oligodendrocytes and Schwann cells in the nervous system.	-0.002428	-0.000478	0.007372
cg11474778	RAD54B	RAD54 homolog B	This protein binds to double-stranded DNA, and displays ATPase activity in the presence of DNA.	0.001396	0.000696	0.007472
cg07086380	TNFAIP8	TNF alpha induced protein 8		0.000809	0.000210	0.007480
cg25212701	C1orf226	chromosome 1 open reading frame 226		-0.001386	-0.000672	0.007793

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg25237894	C2orf82	secondary ossification center associated regulator of chondrocyte		-0.002108	0.000445	0.007793
cg07515565	GMDS	GDP-mannose 4,6-dehydratase	GDP-mannose 4,6-dehydratase (GMD; EC 4.2.1.47) catalyzes the first step in the synthesis of GDP-fucose from GDP-mannose, using NADP+ as a cofactor.	-0.003645	-0.001049	0.007793
cg04180483	TRIM39	tripartite motif containing 39	The function of this protein has not been identified.	-0.001319	-0.000856	0.007793
cg25310700	ASB13	ankyrin repeat and SOCS box containing 13	The protein encoded by this gene is a member of the ankyrin repeat and SOCS box-containing (ASB) family of protein, which serve to couple suppressor of cytokine signalling (SOCS) proteins and their binding partners with the elongin B and C complex, possibly targeting them for degradation.	0.000526	0.000680	0.007793

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg08605326	CA10	carbonic anhydrase 10	This gene encodes a protein that belongs to the carbonic anhydrase family of zinc metalloenzymes, which catalyze the reversible hydration of carbon dioxide in various biological processes.	0.001593	-6.36E-05	0.007793
cg05883299	BHLHB9	basic helix-loop-helix family member b9	The encoded protein may be involved in the survival of neurons.	-0.001408	-8.27E-05	0.007793
cg13921921	ARHGEF2	Rho/Rac guanine nucleotide exchange factor 2	The encoded protein may form complex with G proteins and stimulate rho-dependent signals.	0.000548	0.000148	0.007897
cg03825810	SCN11A	sodium voltage-gated channel alpha subunit 11	This gene encodes one member of the sodium channel alpha subunit gene family. It mediates brain-derived neurotrophic factor-evoked membrane depolarization and is a major effector of peripheral inflammatory pain hypersensitivity.	0.001128	0.000594	0.007897

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg14600987	TNNT3	troponin T3, fast skeletal type	The binding of Ca(2+) to the trimeric troponin complex initiates the process of muscle contraction. This gene encodes fast skeletal troponin T protein; also known as troponin T type 3.	0.000593	0.000226	0.007907
cg09228599	BAT2L1	proline rich coiled-coil 2B		-0.002475	-0.001142	0.008370
cg21631428	QRFPR	pyroglutamylated RFamide peptide receptor		0.001207	0.000248	0.008740
cg03165014	ITGBL1	integrin subunit beta like 1		0.002077	-0.000003	0.008740
cg17522207	NKPD1	NTPase KAP family P-loop domain containing 1		0.001993	-0.000390	0.008740
cg08690459	NR1H3	nuclear receptor subfamily 1 group H member 3	The NR1 family members are key regulators of macrophage function, controlling transcriptional programs involved in lipid homeostasis and inflammation.	0.000540	0.000111	0.008814

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg24866700	MIR1227	microRNA 1227	microRNAs are short non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms.	0.000472	0.000145	0.008830
cg05627639	CHST8	carbohydrate sulfotransferase 8	The encoded protein is responsible for sulfation of GalNAc on luteinizing hormone (LH), which is required for production of the sex hormones.	0.001368	0.001117	0.008841
cg06442162	SLC26A7	solute carrier family 26 member 7	This gene is one member of a family of sulfate/anion transporter genes.	-0.001496	-0.000592	0.008870
cg12897164	FBXO32	F-box protein 32	F-box proteins function in phosphorylation-dependent ubiquitination.	0.000815	0.000519	0.008870
cg21752357	RGPD1	RANBP2 like and GRIP domain containing 1		0.001701	0.001044	0.008958
cg07630301	MYO3A	myosin IIIA	The protein encoded by this gene plays an important role in hearing in humans.	0.001663	0.001151	0.009117

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg20737388	DNAJB13	DnaJ heat shock protein family (Hsp40) member B13	This gene encodes a member of the heat shock protein 40 co-chaperone family.	0.002752	0.000258	0.009139
cg11748260	DHX16	DEAH-box helicase 16	This gene encodes a DEAD box protein, which is a functional homolog of fission yeast Prp8 protein involved in cell cycle progression.	0.000511	0.000331	0.009250
cg06748146	HK1	hexokinase 1	Hexokinases phosphorylate glucose to produce glucose-6-phosphate, the first step in most glucose metabolism pathways.	0.001065	0.000417	0.009280

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg05844788	GHRL	ghrelin and obestatin prepropeptide	This gene encodes the ghrelin-obestatin preproprotein that is cleaved to yield two peptides, ghrelin and obestatin. Ghrelin is a powerful appetite stimulant and plays an important role in energy homeostasis. Ghrelin is thought to regulate multiple activities, including hunger, reward perception via the mesolimbic pathway, gastric acid secretion, gastrointestinal motility, and pancreatic glucose-stimulated insulin secretion.	0.000519	0.000335	0.009636
cg22285621	SSH3	slingshot protein phosphatase 3	The SSH family appears to play a role in actin dynamics by reactivating ADF/cofilin proteins in vivo.	0.000725	-0.000204	0.009636
cg21196487	S100A2	S100 calcium binding protein A2	S100 proteins are involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. This protein may have a tumor suppressor function.	0.000501	0.000259	0.009692

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg09076123	NCF2	neutrophil cytosolic factor 2	This gene encodes a subunit of the multi-protein NADPH oxidase complex found in neutrophils. This oxidase produces a burst of superoxide which is delivered to the lumen of the neutrophil phagosome.	0.000587	0.000339	0.009692
cg10270150	DUSP22	dual specificity phosphatase 22		0.000745	0.000536	0.009692
cg08802652	C6orf64	SAYSVFN motif domain containing 1		-0.001514	-0.000939	0.009692
cg00377727	SEC23A	Sec23 homolog A, coat complex II component	The encoded protein is suggested to play a role in the ER-Golgi protein trafficking.	-0.002911	0.000562	0.009692
cg18963509	WNK4	WNK lysine deficient protein kinase 4	see row 36. The encoded kinase is part of the tight junction complex in kidney cells, and regulates the balance between NaCl reabsorption and K(+) secretion.	0.001406	-0.000422	0.009692

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg13283635	COL9A3	collagen type IX alpha 3 chain	This gene encodes one of the three alpha chains of type IX collagen, the major collagen component of hyaline cartilage.	0.000806	0.000440	0.009692
cg03955859	SPATA5	spermatogenesis associated 5		-0.001171	8.12E-05	0.009874
cg08776660	CRAMP1L	cramped chromatin regulator homolog 1		0.000957	0.000577	0.009874
cg10681804	GGT7	gamma-glutamyltransferase 7	This gene is a member of a gene family that encodes enzymes involved in both the metabolism of glutathione and in the transpeptidation of amino acids.	-0.005898	0.000538	0.009889
AN-Active versus AN-Remitted						
CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg19297688	INSL4	insulin like 4	INSL4 encodes a precursor that undergoes post-translational cleavage to produce 3 polypeptide chains.	-0.000166	0.000219	0.007198

Table A2 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg21601498	FBXO42	F-box protein 42	F-box proteins interact with SKP1 through the F box, and they interact with ubiquitination targets.	3.26E-05	1.67E-05	0.008117
cg20934215	PDZRN3	PDZ domain containing ring finger 3	The encoded protein may function in vascular morphogenesis and the differentiation of adipocytes, osteoblasts and myoblasts.	-0.000195	-0.000262	0.008117
cg24131452	DIP2C	disco interacting protein 2 homolog C		-8.92E-06	-3.86E-05	0.008117
cg25793243	GREB1	growth regulating estrogen receptor binding 1	This gene is an estrogen-responsive gene that is an early response gene in the estrogen receptor-regulated pathway. It is thought to play an important role in hormone-responsive tissues and cancer	-7.66E-05	-8.27E-05	0.008699

NED-Controls versus AN-Remitted

CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg19297688	INSL4	insulin like 4	INSL4 encodes a precursor that undergoes post-translational cleavage to produce 3 polypeptide chains.	-0.003937	0.000219	9.38E-05

Table A2 *continued*

CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg21601498	FBXO42	F-box protein 42	F-box proteins interact with SKP1 through the F box, and they interact with ubiquitination targets.	0.001749	1.67E-05	0.001749
cg25793243	GREB1	growth regulating estrogen receptor binding 1	This gene is an estrogen-responsive gene that is an early response gene in the estrogen receptor-regulated pathway. It is thought to play an important role in hormone-responsive tissues and cancer .	-0.004068	-8.27E-05	0.002181
cg20934215	PDZRN3	PDZ domain containing ring finger 3	The encoded protein may function in vascular morphogenesis and the differentiation of adipocytes, osteoblasts and myoblasts.	-0.004211	-0.000262	0.002181
cg24131452	DIP2C	disco interacting protein 2 homolog C		-0.004020	-3.86E-05	0.002181
cg22647566	FAM160B1	family with sequence similarity 160 member B1		-0.003902	-3.57E-05	0.005341

Table A2 *continued*

CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg25607249	SLC1A5	solute carrier family 1 member 5	The SLC1A5 gene encodes a sodium-dependent neutral amino acid transporter that can act as a receptor for RD114/type D retrovirus.	0.001177	3.89E-05	0.006803

AN-Active= active anorexia nervosa group. AN-Remitted= remitted from anorexia nervosa group. NED-Controls= non-eating disordered controls. Effect AN= effect in AN-Active group. Effect Rem= effect in AN-Remitted group. Effect NED= effect in NED-Controls. *Genes and gene functions are taken from the NCBI gene database https://www.ncbi.nlm.nih.gov/gene. (Empty cells imply that clearly established functions are lacking as per the NCBI gene database).*

Table A3

Probes (and corresponding genes) at which methylation levels are significantly associated (Q < .01) with an interaction between betaine levels and diagnostic category

AN-Active versus NED-Controls						
CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg22160263	ATP10B	ATPase phospholipid transporting 10B (putative)		-0.00377	-0.00033	0.02246
cg09595290	CHST15	carbohydrate sulfotransferase 15	This gene encodes a type II transmembrane glycoprotein that acts as a sulfotransferase to transfer sulfate to the C-6 hydroxal group of chondroitin sulfate.	-0.00382	-0.00019	0.02246
cg02279953	SNORD114-11	small nucleolar RNA, C/D box 114-11		-0.00452	0.00010	0.02612
cg26076846	TANC2	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 2		-0.00438	0.00015	0.02612
AN-Active versus AN-Remitted						
CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg03040441	C3orf66	long intergenic non-protein coding RNA 488		0.00064	0.00046	0.00000

Table A3 *continued*

CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg19166759	IGF2R	insulin like growth factor 2 receptor	The encoded receptor has various functions, including in the intracellular trafficking of lysosomal enzymes, the activation of transforming growth factor beta, and the degradation of insulin-like growth factor 2.	0.00017	0.00019	0.00176
NED-Controls versus AN-Remitted						
CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg03040441	C3orf66	long intergenic non-protein coding RNA 488		-0.00389	0.00046	0.00000
cg08170141	COL6A3	collagen type VI alpha 3 chain	This gene encodes the alpha-3 chain, one of the three alpha chains of type VI collagen, a beaded filament collagen found in most connective tissues. These domains have been shown to bind extracellular matrix proteins, an interaction that explains the importance of this collagen in organizing matrix components.	-0.00208	-0.00012	0.00003

Table A3 *continued*

CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg19166759	IGF2R	insulin like growth factor 2 receptor	The encoded receptor has various functions, including in the intracellular trafficking of lysosomal enzymes, the activation of transforming growth factor beta, and the degradation of insulin-like growth factor 2.	-0.00226	0.00019	0.00003
cg26515689	NCRNA00171	zinc ribbon domain containing 1 antisense, pseudogene		-0.00031	-0.00568	0.00158
cg06606539	MIR572	microRNA 572	microRNAs (miRNAs) are short (20-24 nt) non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs.	0.00092	-0.00587	0.00687

AN-Active= active anorexia nervosa group. AN-Remitted= remitted from anorexia nervosa group. NED-Controls= non-eating disordered controls. Effect AN= effect in AN-Active group. Effect Rem= effect in AN-Remitted group. Effect NED= effect in NED-Controls. *Genes and gene functions are taken from the NCBI gene database https://www.ncbi.nlm.nih.gov/gene. (Empty cells imply that clearly established functions are lacking as per the NCBI gene database).*

Table A4

Probes (and corresponding genes) at which methylation levels are significantly associated (Q < .01) with an interaction between DMG levels and diagnostic category

AN-Active versus NED-Controls						
CpG site	Gene	Name	Function	Effect AN	Effect NED	Q-value
cg21524061	TLR6	toll like receptor 6	The protein encoded by this gene is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity.	-0.14827	0.00142	0.00383
cg14240634	FBRSL1	fibrosin like 1		-0.08204	0.00487	0.00383
cg07252575	DHX40P	DEAH-box helicase 40 pseudogene 1		-0.12542	-0.00589	0.00799

Table A4 *continued*

AN-Active versus AN-Remitted						
CpG site	Gene	Name	Function	Effect AN	Effect Rem	Q-value
cg08170141	COL6A3	collagen type VI alpha 3 chain	This gene encodes the alpha-3 chain, one of the three alpha chains of type VI collagen, a beaded filament collagen found in most connective tissues. These domains have been shown to bind extracellular matrix proteins, an interaction that explains the importance of this collagen in organizing matrix components.	0.00776	0.00380	0.00006
cg03040441	C3orf66	long intergenic non-protein coding RNA 488		0.00439	0.01055	0.00006
NED-Controls versus AN-Remitted						
CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg03040441	C3orf66	long intergenic non-protein coding RNA 488		-0.10502	0.01055	0.00007

Table A4 *continued*

CpG site	Gene	Name	Function	Effect NED	Effect Rem	Q-value
cg08170141	COL6A3	collagen type VI alpha 3 chain	This gene encodes the alpha-3 chain, one of the three alpha chains of type VI collagen, a beaded filament collagen found in most connective tissues. These domains have been shown to bind extracellular matrix proteins, an interaction that explains the importance of this collagen in organizing matrix components.	-0.05279	0.00380	0.00109

AN-Active= active anorexia nervosa group. AN-Remitted= remitted from anorexia nervosa group. NED-Controls= non-eating disordered controls. Effect AN= effect in AN-Active group. Effect Rem= effect in AN-Remitted group. Effect NED= effect in NED-Controls. *Genes and gene functions are taken from the NCBI gene database https://www.ncbi.nlm.nih.gov/gene. (Empty cells imply that clearly established functions are lacking as per the NCBI gene database).*