

Solving the Eating Disorders Puzzle Piece by Piece

Cynthia M. Bulik and Gerome Breen

Albeit slowly, we are beginning to piece together the complex biological puzzle of anorexia nervosa (AN), bulimia nervosa (BN), and binge eating disorder (BED). The article by Lutter *et al.* (1) in this issue of *Biological Psychiatry* brings us one step closer to understanding these illnesses.

To set the stage, heritability estimates for all three eating disorders range from 40% to 65% with a smaller but still considerable environmental contribution to risk (2). However, the overwhelming predominance of sociocultural explanations and rationalizations has meant there have been comparatively few investigations into the biological underpinnings of these anomalous conditions. AN is a particularly perplexing illness, primarily, but not exclusively, affecting young women, compelling them into prolonged negative energy balance, often to the point of death.

To uncover the biological causes of eating disorders, multiple approaches are required. In genetics, the field is advancing with genome-wide association studies of AN, with the first genome-wide significant locus having recently been identified (3) in a gene previously associated with type 1 diabetes and other autoimmune conditions. This work also suggests a previously overlooked role for metabolic abnormalities in the genesis of the disorder, particularly with regard to insulin function and body mass. No genome-wide association studies have been conducted for BN or BED.

In their previous work (4) this investigative team advanced the field using familial linkage analysis with whole-genome and exome sequencing to identify two missense mutations: one in a genome-wide significant linkage region within the estrogen-related receptor alpha gene (*ESRRA*) and another within a suggestive linkage region in the histone deacetylase 4 gene (*HDAC4*). Histone acetylation/deacetylation alters chromosome structure and affects transcription factor access to DNA. HDAC4 represses transcription when tethered to a promoter (<https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=9759>). Specifically, a mutation causing an alanine to threonine substitution at amino acid 786 was found via exome sequencing of a large family with multiple cases of AN and BN as well as subthreshold/unspecified eating disorders. The human HDAC4^{A786T} mutation lies at the junction of two protein domains of HDAC4, is relatively common (at >0.2% in allele frequency), and is predicted by bioinformatic analyses to be either benign or mildly deleterious.

Thus, while the linkage evidence is suggestive and the variant lies on the linkage haplotype, it is unclear if the variant is functionally related to eating disorders. Lutter *et al.* (1) address this issue by studying an equivalent mutation introduced into the mouse HDAC4 protein at amino acid 778

(HDAC4^{A778T}) followed by an array of behavioral tests on heterozygous mice that parallel symptoms characteristic of human eating disorders; namely anxiety, despair, compulsivity, and fat responsiveness. Several behaviors were affected, including weight dysregulation and hyper- rather than hypophagia (more relevant to BN and BED than to AN). Critical for the understanding of AN and BN, the authors did conduct sex-specific studies. In addition, they cleverly introduced an environmental stressor into their paradigms by testing mice either singly or group housed. This latter parameter is relevant given the widespread assumption that eating disorders arise from a perfect storm of both genetic and environmental risk factors.

Potentially informing the grossly disproportionate sex difference in AN and BN, male heterozygous mice displayed no metabolic or behavioral differences in either the individual- or group-housed conditions. Why remains a mystery. However, female mice that were individually housed displayed decreased responding for high-fat (i.e., rewarding) pellets and increased compulsive grooming. These results parallel fat avoidance in AN (5) as well as increased obsessive-compulsive behaviors reflected in clinical comorbidity and twin-based correlations between AN and obsessive-compulsive disorder (6), and single nucleotide polymorphism-based genetic correlations between AN and obsessive-compulsive disorder (7). Observations in group-housed female mice diverged in that they displayed increased weight gain on a high-fat diet, decreased behavioral despair (took them longer to give up in a force swim test), and increased anxiety. The authors interpret the results of the forced swim test to be associated with “jitteriness/anxiety syndrome” rather than behavioral despair. However, the mouse behavior could also reflect the behavioral symptom of heightened physical activity in AN, where patients continue to engage in high levels of physical activity even when acutely ill (8). One important point to raise is that the behaviors observed in these mice cut across eating disorder presentations, displaying abnormalities that alternately reflect AN or BN/BED. Given the frequency with which diagnostic crossover occurs in eating disorders (9), this is not necessarily a problem; however, future work should take care to disambiguate symptoms associated with specific eating disorder presentations.

The authors also collected tissue and conducted a targeted brain gene expression analysis of HDAC4 target genes in cortex tissues from the HDAC4^{A778T} mice as well as previously stored brain tissue for a similar experiment in *Esrrsa*-null mice. Notably, they found gene expression changes in six of seven target genes by task, but only in the group-housed mice. Although this is congruent with a potential gene-environment interaction,

it could also represent the effects of a potential environmental confounder caused by the group housing. While the results of the gene expression analyses are promising, genome-wide RNA sequencing analyses or an analysis of a broader panel of genes could have addressed possible confounding.

Their previous work on ESRRA led them to the hypothesis that disruption of the HDAC-ESRRA activity may function by interrupting mitochondrial function—more specifically, mitochondrial biogenesis. Although they did not provide experimental evidence that the HDAC variant exerts its effects via mitochondrial biogenesis, continued investigation of this hypothesis is worthwhile. Lindfors *et al.* (10) contend that the anorexia and hypothalamic neurodegeneration of the *anx/anx* mouse (an animal model of some facets of AN) may be associated with dysfunction of the mitochondrial complex.

The work by Lutter *et al.* (1) is a valuable contribution to the growing body of literature elucidating the biological etiology of eating disorders. Concerted efforts are required across methodologies to complete the puzzle. It is unconscionable that individuals continue to die from AN. Despite decades of study, our evidence for the treatment of AN remains meager. Although family-based interventions are fairly effective for youth, treatment of adults is unacceptably poor. Moreover, we have no medications that are effective in the treatment of the illness. This comes as no surprise given our incomplete understanding of its biology.

Our recent genome-wide association work demonstrates significant negative genetic correlations between AN and body mass index and other unfavorable metabolic parameters (2), and supports the current findings of Lutter *et al.* These and other findings encourage an increased focus on metabolic aspects of AN. In a world where the prevalence of obesity continues to rise, and nonsurgical treatments for obesity are notoriously ineffective, individuals prone to AN have an unexplained ability to maintain dangerously low body mass indices. Rounding out our understanding of the biology of this extreme end of appetite and weight dysregulation may not only inform the development of effective therapeutics for eating disorders, but also contribute to enhanced understanding of appetite and weight dysregulation in obesity.

Acknowledgments and Disclosures

This work was supported by National Institutes of Health Grant No. U01MH109528 (to PF Sullivan), the Klarman Family Foundation (Anorexia Nervosa Genetics Initiative to CMB), Swedish Research Council Grant No. VR Dnr: 538-2013-8864 (to CMB), UK Medical Research Council Grant No. G0901245 (to GB), National Institute for Health Research Biomedical

Research Centre at South London and Maudsley National Health Service Foundation Trust, and King's College London.

The views expressed are those of the author(s) and not necessarily those of the National Health Service, the National Institute for Health Research, or the Department of Health.

CMB is a grant recipient from and consultant for Shire. GB has received consultancy fees and honoraria payments from Eli Lilly and Illumina Inc.

Article Information

From the Department of Psychiatry (CMB) and Department of Nutrition (CMB), University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; Department of Medical Epidemiology and Biostatistics (CMB), Karolinska Institutet, Stockholm, Sweden; and MRC Social Genetic and Developmental Psychiatry Centre (GB), Institute of Psychiatry Psychology and Neuroscience; Biomedical Research Centre for Mental Health at the Maudsley Hospital, Kings College London, London, United Kingdom.

Address correspondence to Cynthia Bulik, Ph.D., CB #7160, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; E-mail: cbulik@med.unc.edu.

Received Mar 14, 2017; accepted Mar 14, 2017.

References

1. Lutter M, Kahn MZ, Satio K, Davis KC, Kidder IJ, McDaniel L, *et al.* (2017): The eating-disorder associated HDAC4^{A778T} mutation alters feeding behaviors in female mice. *Biol Psychiatry* 81:770–777.
2. Yilmaz Z, Hardaway A, Bulik C (2015): Genetics and epigenetics of eating disorders. *Adv Genom Genet* 5:131–150.
3. Duncan L, Yilmaz Z, Walters R, Goldstein J, Anttila V, Bulik-Sullivan B, Eating Disorders Working Group of the Psychiatric Genomics Consortium (in press): Genome-wide association study reveals first locus for anorexia nervosa and metabolic correlations. *Am J Psychiatry*.
4. Cui H, Moore J, Ashimi SS, Mason BL, Drawbridge JN, Han S, *et al.* (2013): Eating disorder predisposition is associated with ESRRA and HDAC4 mutations. *J Clin Invest* 123:4706–4713.
5. Schebendach JE, Klein DA, Mayer LE, Devlin MJ, Attia E, Walsh BT (2014): Assessment of fat taste in individuals with and without anorexia nervosa. *Int J Eat Disord* 47:215–218.
6. Cederlöf M, Thornton L, Lichtenstein P, Larsson H, Boman M, Rück C, *et al.* (2015): Etiological overlap between obsessive-compulsive disorder and anorexia nervosa: A longitudinal cohort, family and twin study. *World Psychiatry* 14:333–338.
7. Anttila V, Bulik-Sullivan B, Finucane H, Anttila V, Bulik-Sullivan B, Finucane HK, *et al.* (2016): Analysis of shared heritability in common disorders of the brain [published online ahead of print Apr 16]. *bioRxiv*; <http://biorxiv.org/content/early/2016/04/16/048991>.
8. Gümmer R, Giel KE, Schag K, Resmark G, Junne FP, Becker S, *et al.* (2015): High levels of physical activity in anorexia nervosa: A systematic review. *Euro Eat Disord Rev* 23:333–344.
9. Tozzi F, Thornton L, Klump K, Bulik C, Fichter M, Halmi K, *et al.* (2005): Symptom fluctuation in eating disorders: Correlates of diagnostic crossover. *Am J Psychiatry* 162:732–740.
10. Lindfors C, Nilsson IA, Garcia-Roves PM, Zuberi AR, Karimi M, Donahue LR, *et al.* (2011): Hypothalamic mitochondrial dysfunction associated with anorexia in the *anx/anx* mouse. *Proc Natl Acad Sci U S A* 108:18108–18113.