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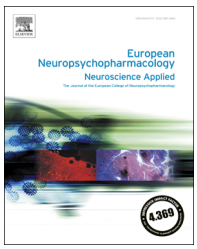

Contemporary views on the genetics of anorexia nervosa

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Abstract
Anorexia nervosa (AN) is a serious mental illness characterized by severe dietary restriction that leads to high rates of morbidity, chronicity, and mortality. Unfortunately, effective treatment is lacking and few options are available. High rates of familial aggregation and significant heritability suggested that the complex etiology of AN is affected by both genetic and environmental factors. In this paper, we review studies that reported common and rare genetic variation that influence susceptibility of AN through candidate gene studies, genome-wide association studies, and sequencing-based studies. We also discuss gene expression, methylation, imaging genetics, and pharmacogenetics to demonstrate that these studies have collectively advanced our knowledge of how genetic variation contributes to AN susceptibility and clinical course. Lastly, we highlight the importance of gene by environment interactions (G × E) and share our enthusiasm for the use of nutritional genomic approaches to elucidate the interaction among nutrients, metabolic intermediates, and genetic variation in AN. A deeper understanding of how nutrition alters genome stability, how genetic variation influences uptake and metabolism of nutrients, and how response to food components affects disordered eating, will lead to personalized dietary interventions and effective nutraceutical and pharmacological treatments for AN.

1. Introduction

Anorexia nervosa (AN) is one of the three formally recognized eating disorders by the American Psychiatric Association in the fifth edition of the Diagnostic and Statistical Manual. AN is a disorder that predominantly affects females (Micali et al., 2013), and is characterized by severe dietary restriction and emaciation that lead to high rates of morbidity, chronicity, and mortality (Arcelus et al., 2011; Chesney et al., 2014; Strober et al., 1997). The pathophysiology and etiology of this disease are unclear, although psychosocial factors have been traditionally speculated as important contributory factors to AN (Lucas, 1981; Woodside, 1993, 1995). Twin and family

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studies found high heritability estimates, demonstrating significant contribution of genetic factors in AN. The familial aggregation (Steinhausen et al., 2015; Strober et al., 2000) coupling to evidence of heritability (Bulik et al., 2006; Thornton et al., 2011; Wade et al., 2000) further strengthens the "biopsychosocial entity" disease model (Lucas, 1981) of this serious illness.

Although AN is less common (prevalence of 0.5–3%) than bulimia nervosa (1–3%) and binge eating disorder (2.4%) (Hoek, 2006; Smink et al., 2012), it is the deadliest eating disorder with an all-cause mortality ranked higher than all other psychiatric disorders with the exception of substance abuse and postpartum depression admission (Chesney et al., 2014). The search for genes that predispose individuals to AN began more than 18 years ago (Campbell et al., 1998; Collier et al., 1997; Hinney et al., 1997a) during an upsurge of interest in discovering what role 5-hydroxytryptamine (5-HT) neurotransmission system plays in the genetics of psychiatric disorders. However, despite years of intensive research and multiple promising leads, not a single gene has been proven as a major risk factor. Furthermore, none of the associated markers found in individual studies has led the way into changing clinical practice.

The primary objective of this review is not to provide an extensive account of the numerous studies done to date. Rather, we aim to illustrate the etiologic uniqueness of AN by highlighting a subset of genetic and related studies and discussing future directions that can translate information on genetic underpinnings into clinical practice. We present these data threaded through a narrative of key genetic and genomic concepts including basic concepts of human genetics and genomics, twin and heritability studies, linkage and association study designs, candidate genes and genome-wide association studies, gene expression and epigenetics such as DNA methylation, gene by environment ($G \times E$) interactions, and the use of multi-domain omics markers to understand molecular functions of genetic risk factors. These genomic- and genetic- factors function together, playing an instrumental role in shaping AN susceptibility, illness course, and outcome. We further propose the use of nutritional genomic approaches (Mutch et al., 2005) to understand how nutrition alters genome stability, how genetic variation influences uptake and metabolism of nutrients, and how response to food components affects disordered eating. Successful implementation of gene-nutrient interaction studies will assist in developing necessary insights into causes and mechanisms of AN, leading to improved etiological understanding of AN, and result in better intervention and treatment strategies.

2. Human genetic variation

The advancement of the Human Genome Project has allowed researchers the use the genome's natural variation to study human biology (Lander et al., 2001). A typical protein-coding gene consists of coding exons that are translated into protein, untranslated regions (UTRs) in the upstream (5-UTR) and downstream (3-UTR) of the transcript region that often contain regulatory control elements that may influence transcriptional efficiency, and several intron regions that may affect alternative splicing of the mRNA. The most common type of genetic variation is termed single nucleotide polymorphisms (SNPs). The first reports linking 5-HT system to AN (Collier et al., 1997; Hinney et al., 1997a) examined common SNPs in the promoter region of the 5-HT2A receptor gene and the 5HT-transporter-linked polymorphic region (5-HTTLPR).

Other sequence variations of interest include microsatellites, which are short or long repetitions of the same sequence motif in tandem (Ellegren, 2004), and insertions or deletions as well as structural variants that affect large chromosomal regions (MacDonald et al., 2014). The microsatellite markers that were found to associate with AN and related phenotypes include allele 13 of the marker D11S911 in the UCP-2/UCP-3 locus in Caucasian women (Campbell et al., 1999), but the association could not be replicated in a sample of Japanese AN patients (Ando et al., 2004). A promoter region microsatellite of AVPR1A, RS3, was associated with Dieting subscale of the Eating Attitudes Test (EAT), and Drive for Thinness subscale of the eating disorders inventory (EDI) (Bachner-Melman et al., 2004). Other studies focused on body weight (Yilmaz et al., 2014) and specific cognitive and behavioral dimensions such as drive for thinness, body dissatisfaction, personal ineffectiveness, perfectionism, and harm avoidance as "subphenotypes" of AN (Frieling et al., 2009; Frieling et al., 2006; Gamero-Villarroel et al., 2015; Mikolajczyk et al., 2010; Root et al., 2011). Identification of AN subphenotype-associated genetic markers may facilitate the discovery of novel susceptibility genes and biological pathways.

The majority of the sequence variation in the genome are located in the introns and are traditionally thought of as "nonfunctional" variants and "neutral" markers. However, intronic variants may affect alternative splicing of the mRNA or act as enhancer of the gene to affect expression of other genes (Pagani and Baralle, 2004). Examples of intronic variants that were implicated in AN risk were identified in the largest meta-analysis of genome-wide association study (GWAS) for AN to date, which comprised of 5551 cases and 21,080 controls (Boraska et al., 2014). Although not meeting the strictest threshold of genome-wide significance ($10(-8)$), SOX2OT intronic variant rs9839776 ($P=3.01 \times 10(-7)$) and PPP3CA intronic variant rs17030795 ($P=5.84 \times 10(-6)$) were the most significantly associated variants in this study (Boraska et al., 2014). It is important to note the phenomenon of linkage disequilibrium (LD), which refers to high correlation among segments of physically close polymorphic sites in the genome. The implication of LD is that the "causal variant" associated with a disease may be "captured" by the detection of a "proxy marker" that is in high LD with the causal variant. Therefore, the two intronic variants reported may be proxy markers for other unscreened yet biological relevant functional variants.

Sequence variation within regulatory regions of the gene, such as promoter and 3'-UTR regions, may form motifs that can affect the gene product by modulation of transcriptional efficiency or post-transcriptional stability. Allelic variants that disrupt these motifs may therefore alter gene expression or protein levels to affect phenotype. In our candidate gene exon-sequencing study (Scott-Van Zeeland et al., 2014), the over-representation of rare variants revealed the EPHX2 as an AN susceptibility gene. Moreover,