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The Integrative Research Approach of Anorexia Nervosa

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Abstract: The prevalence of eating disorders has led to extensive studies on different aspects of this mental illness, ranging from the underlying genetics to animal models. However, given the distinct research methods used in investigations, there are little integration and cross-check across the disciplines, thus withholding the potential unification on the understanding of eating disorders. The aims of this review is to verify the findings from various levels, and also to reach the purpose of discovering existing patterns across the levels and encouraging more cross-level integrative approach. Therefore, this paper reviews the progresses made in fields of genetics, neurobiology, and animal models, and concludes that certain gene variants, brain abnormalities and animal models are associated with eating disorders. It brings forth gene variants identified from an extensive genetic research and points out the possibility of using neuroimaging and then animal models to corroborate the association for future pharmaceutical purposes. It also encourages better communication and cross validation across the fields on technological advances and new findings to better understand eating disorders.

Keywords: eating disorders, integrative approach, neurobiology, genetics, animal model

1. Introduction

Eating disorders (ED) refer to an array of disordered eating behaviors, categorized into subtypes such as bulimia nervosa, anorexia nervosa, and binge eating disorder [1].Repetitive binge eating, which is indicated by consuming a substantial amount food high in sugar, carbohydrates and/or fat in a brief period of time, and the subsequent compensatory purging behaviors, such as inordinate use of laxatives, voluntary vomiting, restrictive eating, or vigorous exercise are hallmarks of bulimia nervosa (BN). Anorexia nervosa (AN) is marked by a considerable reduction in body weight brought on by calorie restriction, a strong fear of weight gain, and false beliefs about size, form, and thinness. Binge eating disorder (BED) is marked by periodic occurrence of out-of-control overeating without resorting to the maladaptive compensating actions that are seen in BN [2].

The understanding of biological etiology of ED, particularly AN, is evolving with the advancement of genomic, neurobiological, and behavioral studies. AN-related biological activities occur at multiple levels, from the subcellular (genetic/epigenetic variations), via cellular (signaling and neural circuits), to organismal implications such as disturbed eating behaviors. These layers of phenotypes are interrelated yet are researched employing relatively distinct scientific methodologies. However, until recently, these techniques have primarily progressed in their respective fields in parallel without proper integration. A hierarchically integrated research method that incorporates biological

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interconnectedness will give a more comprehensive understanding of ED biology and set the groundwork for creating innovative treatments. This paper aims to identify molecular mechanisms of ED in the crucial domains including genomics, neuroimaging, and animal models are addressed in review. The review article can also promote deeper integration of discoveries in order to establish a more cohesive science of ED [3].

2. Genome Wide Association Studies (GWAS) of AN

GWAS endeavor to detect genotype-phenotype correlations by determining the statistical significance of multiple genetic variations over several genomes and a given characteristic or illness [4]. Single-nucleotide polymorphisms (SNPs), the most extensively investigated genetic variations in GWAS, are the marker used by GWAS. Each SNP reflects a single nucleotide variation. SNPs occur regularly once every 1,000 nucleotides on average across the human genome, implying that there are approximately 5 x10 6 SNPs in the human genome. Genomic risk loci, or blocks of connected SNPs that all correlate with the variable of interest in a statistically significantly manner, are generally reported in GWAS. This technique has produced strong connections with a variety of characteristics and disorders, and as the sample size of GWAS grows, the quantity of linked variants is predicted to increase gradually.

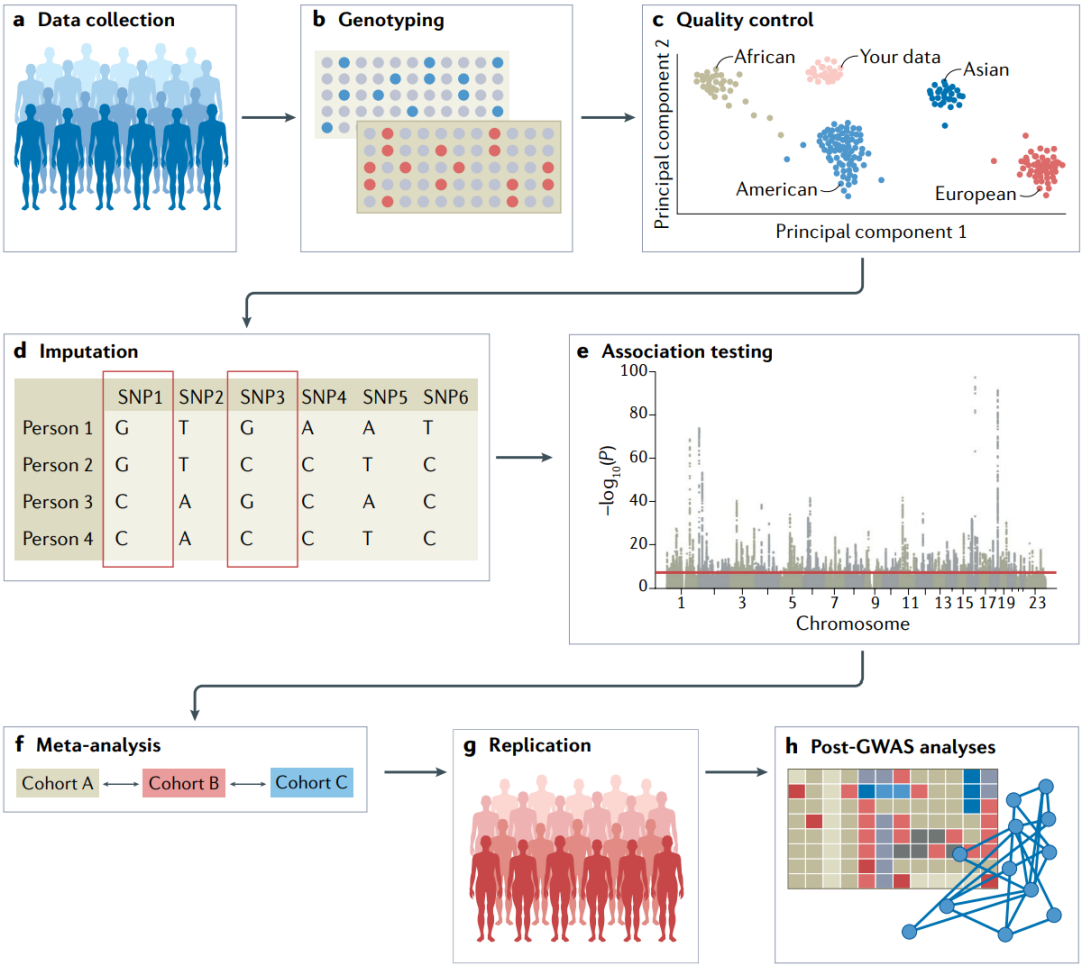

Figure 1: Genome-wide association studies follow the above steps demonstrated in Nature Reviews Methods Primers [4].

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The procedures involved in performing GWAS are outlined below (see Figure 1). A) Data from research cohorts can be obtained via repositories or biobanks. B) Next-generation sequencing techniques (NGS) can be frequently used to gather genotypic statistics for whole-exome sequencing (WES) or whole-genome sequencing (WGS). C) Involving wet-laboratory and dry-laboratory, quality control can process on specific genotypes to retrieve high-quality SNP data. D) With the information from the population of paired reference from sources like the 1000 Genomes Project or TopMed, genotypic data may be narrowed and untyped genotypes can be deduced. E) Using a suitable model, genetic association tests are performed for variation in genes respectively. The product is examined to look for anomalous patterns, thus producing summary statistics. F) Using standardized statistical processes, results from numerous smaller cohorts are pooled. G) Results can be reproduced in an alternate cohort utilizing internal or external replication. H) *in silico* study of GWAS is performed using data from other sources. Afterwards, functional hypotheses can be explored experimentally.

Recently, with the increasing body of genomic related big data, unprecedent discoveries have been made by genome wide association analysis. Major-scale human genome sequencing, for example, has found unusual protein-coding mutations with a substantial effect on body adiposity. By sequencing 645,626 people from Mexico, the United States, and the United Kingdom, researchers noted CALCR, MC4R, GIPR, GPR151, and GPR7516 genes, the five neurologically expressed G protein coupled receptors, that had an exome-wide strong correlation with body mass index (BMI), among the total 16 genes [5]. A protein-truncating variation in GPR75 with a frequency of 4/10,000 was shown to be related with 1.8 kg/m2 lower BMI and a 54% decreased risk of obesity in the heterozygous condition. GPR75 knockout mice were found to be resistant to increase in weight, and GPR75 inhibition may serve as a method of treatment for obesity.

Similar efforts have also been tried on ED research, however, without conclusive findings mainly due to the relatively small cohort sizes. As a result, the most contemporary GWAS of AN, conducted with a large group of people by the Eating Disorders Working Group of the Psychiatric Genomics Consortium (PGC-ED), is examined here. Because GWAS analysis results depend on the scope of the sample, researchers merged samples from the Genetic Consortium for Anorexia Nervosa (GCAN)/Wellcome Trust Case Control Consortium-3 (WTCCC-3), Anorexia Nervosa Genetics Initiative, and the UK Biobank [6]. The final collection contained 33 datasets from 17 nations, totaling 16,992 cases and 55,525 controls of European ancestry. It discovered eight genome-wide significant loci, which includes four single-gene loci: MGMT, CADM1, PTBP2, and FOXP1. The discovery was also further verified using chromatin interaction analyses and/or brain expression quantitative trait loci (eQTL). For instance, CADM1 mediates synaptic assembly and loss of CADM1 protects mice from obesity, promoting a negative energy balance and weight loss. The central nervous system (CNS) was shown to be significantly related with tissue abundance for AN-associated genes. Furthermore, mouse single-cell gene expression statistics indicated substantial correlations between these ED-related genes, striatal medium spiny neurons (GABAergic inhibitory projection neurons that make up a large proportion of the neurons in the striatum), and hippocampal pyramidal neurons (multipolar excitatory projection neurons). Song et al. also reported that AN risk-genes were elevated in modules of coexpression involving extracellular matrix functionalities. The genes were shown to be significantly upregulated in the limbic system, postnatal brain, and non-neuronal types of cells [7].

Watson et al. [6] discovered substantial SNP-based genetic correlations of AN with 447 phenotypes classified as six categories including personality, psychiatric, educational achievement, metabolic, anthropometric, and physical activity. AN exhibited a notably strong genetic correlation with other psychiatric conditions including schizophrenia and obsessive-compulsive disorder (OCD), but a negative one with metabolic and anthropometric variables like leptin, BMI, and fasting insulin. GWAS-identified risk genes for AN and OCD exhibited comparable prefrontal cortex expression changes, indicating that these illnesses may share functional pathways.

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Accordingly, when combined with other bioinformatic techniques, the most major recent scale GWAS analysis reveals that AN is correlated with genes prevalent in the neuron system. And these genes can potentially impact the neurological activity, which lead to the development of ED.

3. Neurobiology of AN

With the introduction of human in vivo neuroimaging methods, people's perception of the brain has altered drastically over the last several decades. Neurobiological research in ED has the potential to produce medical models for improved treatment and diagnosis. With the identification of genes enriched in neuron system significantly associated with ED, it is pivotal to validate their roles in brain structure and function. Magnetic resonance imaging (MRI) is a technology commonly used in brain research to evaluate white matter (WM) and gray matter (GM) volumes, surface area, and cortical thickness [8]. What's more, diffusion weighted imaging and diffusion tensor imaging, both based on MRI, assess water diffusion to assess the intensity of WM connectivity across brain areas and WM tract integrity. Functional MRI (fMRI) is the most extensively used functional brain imaging technology, and it analyzes brain activity by measuring changes in local blood flow. Imaging techniques like single-photon emission computed tomography (SPECT) and positron emission tomography (PET) scan, which employ radioactive substances that are taken up by the body to examine metabolism of glucose and distribution of neurotransmitter receptor, are also common practices.

Numerous neuroimaging research have shown that AN patients have aberrant brain structure and disordered functional activity. However, the finding is inconsistent because the abnormal neural activity has been reported take place in the subcallosal gyrus, the frontal lobe, cingulate cortex, hippocampus/parahippocampus, parietal lobe, and so forth. Small sample sizes, as well as differences in age range, medical comorbidity, length of sickness, clinical symptoms, and data gathering methods, may result in conflicting findings. Therefore, to detect convergent aberrant regional function of AN in neuroimaging, the research team conducted a meta-analysis study on 660 AN patients and 740 controls with Voxel-based morphometry (VBM) and 425 patients with AN and 461 controls with resting-state fMRI [9]. Furthermore, the authors investigated AN patients and controls for their localized alterations in brain function and gray matter volume (GMV) with Seed-based Mapping with Permutation of Subject Images (SDM-PSI), a new approach with a reduced subjective calculation targeting the population impact magnitude. Generally speaking, patients with AN reported GMV in the bilateral median cingulate cortex and left middle occipital gyrus, and even regions beyond them, like the bilateral anterior and posterior cingulate cortex, can be affected as the extension of the effect. Patients with AN also exhibited lower resting-state expression levels in the bilateral anterior and median cingulate cortex, and a higher one in the right parahippocampal gyrus in resting-state fMRI investigations. This comprehensive meta-analysis found deficits in GMV and functional activity in regions like the anterior and median cingulate in AN patients, adding to current insight into the disease's etiology.

fMRI is also utilized to investigate neuropsychological abnormalities among individuals with ED, with particular emphasis on the following aspects: emotion regulation, and reward processing, and cognitive control [10]. Emotional regulation relates to how stimuli elicit emotions and influence behavior. Spanning diagnostic categories, emotions are functionally related with a range of ED behaviors. Reward processing includes concepts such as responsivity (or sensitivity), acquiring, and incentive-dependent decision-making. Cognitive control is typically measured using tasks that assess the capacity to shift between distinct sets of instructions as well as tasks that demand reaction inhibition.

The anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), prefrontal cortex (PFC), and parietal cortex, different regions of the outer layer of the brain, are typical components of cognitive

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control neurocircuitry. As measuring cognitive processing during fMRI scanning, patients with AN often demonstrate hypoactivation of frontoparietal networks when compared to controls. Individuals with AN more frequently made mistakes and demonstrated hypoactivation in frontostriatal circuits during a set-shifting exercise meant to measure the capacity to modify the behaviors when the rules change.

The amygdala, PFC, ventral and dorsal striatum, OFC, parietal cortex, insula, and ACC are among the functional regions engaged in reward processing [11]. Adults with AN, however, were not significantly different from their healthy counterparts in reward system neural activation during one guess task involving money [12]. Individuals with AN showed a proclivity for delayed gratification in a delay-discounting activity, an experiment condition in which participants decide between accepting an instant smaller amount of cash or receiving a greater amount later. The preference for delayed but bigger reward was found to have association with reduced brain activity in areas like the dorsal and striatum ACC [13]. In addition, anomalies in reward learning, or the ability to anticipate rewarding events based on past favorable experiences, have been reported in AN study. Prediction error, in which the brain correlates omission of an expected reward, is one neurological marker correlated with reward learning. Two research [14][15] revealed greater changes in reward circuits in response to both monetary and gustatory incentives in people with AN, implying greater prediction error.

Elevation in adverse effect, including stress, sorrow, anxiety, hostility, shame, and guilt and other unpleasant feelings, has been found to occur before disordered eating behaviors among ED diagnoses [16]. Furthermore, studies have shown that acute mood fluctuations can alter both cognitive processing and the responsiveness of reward [17].

Despite a significant number of evidence of brain abnormality has been accumulated by neurobiology studies on ED, a unified and reliable neuroimaging model for ED diagnosis is still lacking. Genetically defined cohort will be an excellent paradigm for forming a standard neuroimaging of ED. Therefore, the next wave of neurobiological study of AN is to conduct research on individuals with GWAS identified genetic traits. This kind of cohort might give out more consistent and direct conclusion of neuro activities involving in ED.

4. ED and Animal Models

As GWASs on ED cover more data and get more rigorous, genetically engineered or naturally occurring animal models will be necessary to verify the results. Animal models can provide stable and exemplary genetic background, well-controlled exposure to environmental factors, and exact data acquisition and tissue harvest during ED development and recovery. Genes, variations, and mechanisms of pathways identified in animal research can possibly be found and confirmed initially within the same species with the similar genetic makeup, and subsequently be employed in humans Similarly, discovered genes based on extensive human GWAS and predicted circuits from researches on neuroimaging advances may be examined in animal models for fatality and function, possibly across diverse genetic backgrounds. With the advantage of gene editing tools such as CRISPR and base editing, researchers are able to create mouse models by introducing the genetics feature into the mouse genome directly. These animal models will offer powerful tools to rigorously interrogate the role of the engineered gene in the development of AN.

Up to this point, the animal model for ED is limited. The most well-established is the activity-based anorexia (ABA) model, a naturally occurring paradigm for examining AN. The ABA model has crucial AN behavioral components and features such as increased physical activity, depression, anxiety, social phobia, and OCD-like behavior, making it a suitable model for investigating the physiological and behavioral portion of AN. The mice are offered limited food intakes each day, followed by intense physical activity, which causes compulsive-like running, deliberate limitation of

food consumption, lessened anxiety-related behavior, dramatic loss of weight, and mortality without interference. ABA also affects physiology in a way that is like that of AN, such as loss of oestrus, anhedonia, hypothermia, CNS, gastrointestinal, and cardiovascular dysfunction. Further research on ABA, or even new and reliable models, should be invested and looked into, for they guarantee more important progresses and opportunities of application of the findings in other areas.

5. Conclusion

The critical idea of this review is to verify the findings from various levels, and also to reach the purpose of discovering existing patterns across the levels and encouraging more cross-level integrative approach. This will eliminate most of the inconsistency generated by studies merely from one level. For example, gene variants identify from a large scale GWAS analysis must be validated first by the neuroimaging to demonstrate its neurobiological function, and then in an animal model by experimentations on the gain or loss of function. With the cumulation of more genetic data and other medical records, more and more genetic variants will be identified involving in the development of ED. Therefore, groups of AN patients carrying the same gene variants impacting the structure of brain structure will be found. It is important to have these patients studied by neuroimaging and demonstrate the changes in brain structure or functional activities. The multi levels studies will establish the direct connection between gene and brain function. Clear models of brain structure/functional activities of AN may be served as a start point to identify more monogenic and/or multi genetic features related to this disease. Furthermore, engineering mouse models with the findings from large scale GWAS studies by CRISPR or other genome editing tools will provide great opportunity to understand the function of these genes in ED development. With supportive evidence from all these three levels, the gene will become a reliable molecular biomarker for AN diagnosis and treatment. So far, the lack of animal model of ED partially causes the short of effective medical treatment for ED. Multi-level validated engineered animal models will greatly accelerate the development process. In general, the synergic effect generated by the integrative approach brings insightful findings the isolated research approach can never do.

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