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Authors

Levian, Candace
Ruiz, Esmeralda
Yang, Xia

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FOCUS: OBESITY

The Pathogenesis of Obesity from a Genomic and Systems Biology Perspective

Candace Levian, Esmeralda Ruiz, and Xia Yang*

Department of Integrative Biology and Physiology, University of California, Los Angeles

The recent obesity epidemic has imposed significant health, economical, and societal concerns. However, effective preventive and therapeutic strategies are currently lacking, primarily due to a lack of comprehensive understanding of the underlying molecular mechanisms. Recent genome-wide scans of genetic variants, transcriptome, and epigenome have uncovered >50 genetic loci that predispose individuals to obesity and revealed hundreds of genes with altered transcriptional activity and/or epigenetic variations in obesity-related tissues upon various environmental challenges such as high caloric diets, lack of physical activity, and environmental chemicals. These discoveries highlight the importance of genes involved in the control of energy homeostasis and food intake by the central nervous system, as well as genes contributing to lipid metabolism, adipogenesis, fat cell differentiation, and immune response in peripheral tissues, in obesity development. Future studies that are directed to obtain a more comprehensive, systems-level understanding of disease mechanisms and that test novel therapeutic strategies aiming at systems-level normalization of the obesity-related molecular alterations are warranted.

*To whom all correspondence should be addressed: Xia Yang, PhD, Department of Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles, CA 90095; Tele: 310-206-1812; Fax: 310-206-9184; Email: xyang123@ucla.edu.

†Abbreviations: BMI, body mass index; BPA, bisphenol A; CAD, coronary artery disease; chr, chromosome; DDT, dichlorodiphenyltrichloroethane; GWAS, genome-wide association studies; HFD, high fat diet; PPAR, peroxisome proliferator activating receptor; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; TBT, tributyltine.

Keywords: obesity, genomics, genetic risks, environmental risks, high caloric diets, exercise, obesogen, transcriptome, epigenome, integrative genomics, systems biology

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INTRODUCTION

Obesity is symbolized by excessive fat accumulation and recently recognized by the World Health Organization as the No. 5 cause of death worldwide [1]. The term obese is assigned to individuals whose body mass index (BMI†), a measure of fat content in the body, is greater than 30 kg/m². In the past half century, obesity prevalence has been increasing dramatically on a global scale. Today, more than one-third of the U.S. population is obese, with rates that continue increasing not only among adults but also, more alarmingly, in children and adolescent populations, making the United States the most obese country in the world [2]. It is predicted that by 2020 the U.S. obesity rate could reach 86 percent [2]. The rest of the world is following the same trend, as reflected by the fact that the global obesity rate has almost doubled since 1980 [1]. It is, therefore, free of exaggeration to claim that we have entered a worldwide obesity epidemic.

More daunting than the statistics is the long list of pathological conditions and diseases associated with obesity, including hyperlipidemia, heart diseases such as coronary artery disease (CAD) and myocardial infarction, stroke, type 2 diabetes (T2D), hypertension, cancers, low grade and chronic inflammation, fatty liver disease, osteoarthritis, respiratory problems, and neurodegenerative diseases, among which several are top leading causes of death in the United States and in the world [3]. Looking at obesity through an economic perspective indicates that the medical costs of an obese individual is \$1,429 higher than a normal weight individual per year, with 16 to 18 percent of total U.S. health care spending allotted to obese and overweight patients [4]. All of these facts highlight the serious health and economic burdens imposed by obesity and the worsening future outlooks.

So what are the causes of this obesity epidemic? Obesity is predominantly viewed to result from a lifestyle of increased calorie intake and lack of physical activity. This view has a solid ground as the soaring of

obesity prevalence has indeed followed the recent industrialization in human history that enables production of abundant foods along with diminished need for physical activity [5]. Fat mass content is largely determined by the delicate balance between caloric intake and energy consumption required to maintain cell metabolism and function. High caloric foods increase energy intake, and low physical activity reduces energy consumption. As a result, both situations favor energy storage in the adipose tissue, leading to increased fat mass and adiposity.

However, it is apparent that there have to be internal underlying causes that influence obesity in addition to the environmental factors. If not, how do we explain why certain individuals consume more than others and remain lean, while others consume less and gain weight? Twin, adoption, and family studies confirm this mostly observational statement with scientific proof [6,7]. Twin studies, for example, show a 70 to 90 percent similarity of fat mass in identical twins, while fraternal twins have a similarity of 35 to 45 percent, pointing at a genetic contribution to obesity. There is accumulating evidence showing that both genetic and environmental perturbations, as well as the interactions between the two, contribute to obesity development and the epidemic [8]. The fact that the prevalence of obesity among adults and children continues to rise presses for a better understanding of the mechanisms behind obesity, for it holds great promise to developing tailored preventive and therapeutic strategies to ameliorate the negative impact of obesity on human health.

In this review, we will summarize recent progresses in the investigation of the molecular mechanisms underlying obesity development from a genomics perspective that involve genetics (DNA sequences), transcriptome (mRNA levels), and epigenome (modifications of DNA, histone, and chromatin). Specifically, we will first review recent discoveries on the genetic risk factors of obesity and the underlying mechanisms. We will then provide an overview of the genomic impact of various environ-

Table 1. Summary of obesity-related GWAS and genetic loci identified.

Obesity Phenotype	Number of Studies	Number of Suggestive Loci (p-value < 1e-5)	Number of Significant Loci (p-value < 5e-8)
Adiposity	3	8	5
Body Mass Index	17	117	73
Obesity	8	29	10
Obesity (early onset extreme)	1	1	0
Obesity (extreme)	3	19	4
Visceral Fat	1	40	1
Waist Circumference	3	17	2
Waist-Hip Ratio	1	17	15
All Obesity-Related Phenotypes	37	141	57

mental risks of obesity, including diets, physical activity, and environmental chemicals (termed obesogens), to better understand how these external factors interact with the epigenome and transcriptome to confer disease risk. Lastly, we will briefly introduce integrative genomics and systems biology approaches that leverage multiple levels of molecular information from multiple tissues and integrate both genetic and environmental perturbations to offer better understanding of the disease mechanisms. In doing so, we hope to provide a comprehensive view of our current understanding of the genomic basis of obesity and point to potential preventive and therapeutic directions.

GENETIC RISKS OF OBESITY

Genetic Heritability of Obesity

It has long been recognized that genetics plays a large role in obesity, with the genetic heritability of obesity estimated to be 40 to 70 percent [7,9]. Obesity exists in two major types, monogenic and polygenic. Monogenic obesity is primarily caused by gene mutations in single genes and accounts for a small number of extreme early onset obesity cases. Studies of this rare form of obesity have identified genetic variants in less than a dozen genes and provided much of the initial insights into the pathogenesis of obesity [7,10]. The majority of the genes responsible for monogenic obesity are involved in the leptinergic-melanocortinergic pathway. Notable genes include those that

encode leptin (*LEP*), the leptin receptor (*LEPR*), melanocortin 4 receptor (*MC4R*), pro-opiomelanocortin (*POMC*), and brain-derived neurotrophic factor (*BDNF*). Genetic variants in *MC4R* contribute to the most common monogenic form of obesity, accounting for 2 to 3 percent of obesity cases in both childhood and adulthood, whereas rare mutations in *LEP* and *LEPR* demonstrate the strongest effect sizes.

In order to further uncover the genetic risks of obesity, especially the common polygenic form, large-scale, genome-wide association studies (GWAS) have been conducted in the past few years to systematically and objectively investigate the associations between obesity-related phenotypes and genetic variants, primarily in the form of single-nucleotide polymorphisms (SNPs), in thousands to hundreds of thousands of individuals. The development of GWAS have transformed and modernized the way we study genetic effects on various traits and diseases. According to the GWAS catalog [11] that curates SNP-trait associations discovered by published GWAS (accessed on September 3, 2013), 37 studies, many of which are meta-analysis of multiple GWAS, have been conducted to identify the genetic risks of obesity and related phenotypes. Here, we focus on studies conducted on eight obesity-related phenotypic terms in the GWAS catalog, including adiposity, BMI, obesity, obesity (early onset extreme), obesity (extreme), visceral fat, waist circumference, and waist-hip ratio. Of these, the largest GWAS of obesity examined 133,154

Table 2. Significantly genetic loci that are consistently identified in multiple GWAS and across multiple obesity phenotypes.

Locus	Mapped Gene(s)	Official Gene Name	No. of GWAS ^a	Obesity Phenotypes of Focus
16q12.2	<i>FTO</i>	Fat mass and obesity associated	15	Adiposity, BMI, Obesity, Obesity (early onset extreme), Obesity (extreme), and Waist Circumference
18q21.3	<i>MC4R</i>	Melanocortin 4 receptor	8	BMI, Obesity, Obesity (extreme), Waist Circumference
11p14	<i>BDNF</i>	Brain-derived neurotropic factor	4	BMI and Obesity
14q31	<i>NRXN3</i>	Neurexin 3	3	BMI, Waist Circumference, and Obesity
6p12	<i>TFAP2B</i>	Transcription factor AP2 beta	3	BMI, Obesity (extreme), Adiposity
2q33	<i>FLJ20309</i>		3	BMI, Obesity (extreme)
2p25.3	<i>TMEM18</i>	Transmembrane protein 18	3	BMI
19q13.1	<i>KCTD15</i>	Potassium channel tetramerisation domain containing 15	3	BMI
1p31.1	<i>NEGR1</i>	Neural growth regulator 1	3	BMI
3p12	<i>CADM2</i>	Cell adhesion molecule 2	2	BMI, Visceral Fat
6p22.3	<i>CDKAL1</i>	CDK5 regulatory subunit associated protein 1-like 1	2	BMI, Waist-Hip Ratio
1q41	<i>LYPLAL1</i>	Lysophospholipase-like 1	2	Adiposity, Waist-Hip Ratio
2q36	<i>KCNE4</i>	Potassium voltage-gated channel, Isk-related family, member 4	2	BMI, Waist Circumference
21q21	<i>NCAM2</i>	Neural cell adhesion molecule 2	2	Obesity, Visceral Fat
11q12	<i>SLC22A8</i>	Solute carrier family 22m member 8	2	Visceral Fat, BMI
19q13.3	<i>GIPR</i>	Gastric inhibitory polypeptide receptor	2	BMI
4p12	<i>GNPDA2</i>	Glucosamine-6-phosphate deaminase 2	2	BMI
15q23	<i>MAP2K5</i>	Mitogen-activated protein kinase kinase 5	2	BMI
11p11.2	<i>MTCH2</i>	Mitochondrial carrier homolog 2	2	BMI
16p11.2	<i>SH2B1; ATP2A1</i>	SH2B adaptor protein 1; ATPase, Ca ⁺⁺ transporting, cardiac muscle, fast twitch 1	2	BMI

^aNumber of GWAS is represented using the number of unique publications.

individuals and identified 38 genetic loci [12]. As shown in Table 1, to date, a total of 141 suggestive loci (association p-value $< 1 \times 10^{-5}$) have been discovered, of which 57 loci reach genome-wide significance (p-value $< 5 \times 10^{-8}$). Detailed information about all these suggestive and significant loci can be retrieved from the GWAS catalog website at <https://www.genome.gov/26525384>. The large number of genetic loci uncovered from the unbiased genome-wide screening highlights the vast genetic complexity of obesity. Among the phenotypic traits, BMI has been investigated by the largest number of studies and revealed the largest number of significant genetic loci.

Among the 57 significant loci uncovered, 20 are reproducible in ≥ 2 studies and 12 are consistent across ≥ 2 phenotypes (summarized in Table 2). The presence of common loci across various obesity phenotypes supports that these phenotypes share genetic similarity and are capable of capturing obesity features, as expected. Among these, the most significant and consistent locus is on chromosome (chr) 16q12.2 and maps to the gene *FTO*, encoding the “fat mass and obesity associated” protein. This locus is significant in 15 GWAS across six out of the eight obesity phenotypes examined. Other top consistent loci include chr18q21.3 (mapping to *MC4R*, melanocortin 4 receptor), chr11p14 (*BDNF*; brain-derived neurotrophic factor), chr6p12 (*TFAP2B*, transcription factor AP2 beta), and chr14q31 (*NRXN3*, neurexin 3).

Although GWAS is a powerful tool for identifying genetic risks of obesity, all the significant loci uncovered to date together only explain approximately 10 percent of the reported heritability, and a large portion of the genetic heritability of obesity remains elusive [9]. This discrepancy raises an important question: What accounts for the missing heritability of obesity? The exact answer to this question is still unknown but has opened the door to additional studies to test alternative hypotheses. For instance, as GWAS mainly captures common SNPs with a minor allele frequency of > 5 percent, rare variants or structural variants that have not

been examined by GWAS may account for part of the missing heritability [13]. Recent exome and targeted sequencing efforts are directed toward uncovering these genetic events. Another plausible explanation is that a large number of genetic variants with very subtle effects (too subtle to be detected by GWAS with limited sample sizes) collectively contribute to obesity development [13]. These subtle genetic effects can be captured either by increasing population size or by innovative analytical approaches that help expose the needles from the haystack [12,14,15]. Perhaps the most complex of hypotheses is that the remaining heritability of obesity can be explained by interactions between genes and environmental factors, which recently has been tested using systems genetics approaches in animal models under both genetic and environmental perturbations [16,17].

Molecular Mechanisms Underlying Genetic Risks

For monogenic obesity, the genetic risks identified to date primarily affect the functions of genes involved in the leptinergic-melanocortinergic pathway. Disruption of this pathway results in decreased satiety, increased food intake, and energy storage, which ultimately lead to obesity. For instance, leptin, encoded by gene *LEP*, is a vital homeostatic hormone secreted into the bloodstream by adipose tissue in order to signal energy sufficiency to the hypothalamus. Leptin receptor, encoded by *LEPR*, is the key mediator of the actions of leptin. Disruption to this signaling process via genetic perturbations in *LEP* and *LEPR* will affect appetite control, food intake, and energy homeostasis [10]. Another gene, *MC4R*, is among the first to be associated with human obesity, as mutations in the *MC4R* gene represent the most common monogenic cause of early onset and severe obesity. *MC4R* encodes the neural-specific G-protein coupled receptor for melanocortins, a group of small protein hormones derived from post-translational cleavage of the pro-opiomelanocortin gene product. Binding of melanocortins to *MC4R* can activate G proteins, increase cAMP pro-

duction in the cell, and affect energy homeostasis, food intake, and energy expenditure [18]. Genetic variants in the *MC4R* gene primarily affect the receptor activity for melanocortins, causing an inability to generate cAMP and subsequently perturbs energy expenditure and food intake to confer predisposition to obesity [19]. Another gene with a relatively better illustrated mechanism is *BDNF*, which encodes brain-derived neurotrophic factor, a key protein involved in neuronal plasticity and recently linked to central control of energy balance [20]. *BDNF* expression in the ventromedial hypothalamus has been found to be inhibited by nutrient restriction and stimulated by increased nutrient availability, and reduction of *BDNF* expression leads to excessive feeding and obesity [21]. Both insulin and leptin can stimulate the translation of a specific *BDNF* transcript that has a long 3'-untranslated region, which is determined to be primarily responsible for mediating the action of leptin to activate hypothalamic neurons and inhibit food intake [22].

For polygenic obesity, the candidate genes underlying the top consistent obesity loci shown in Table 2 are involved in diverse functions and pathways such as energy metabolism and homeostasis, eating behavior, neuronal functions, cell adhesion, and ion channels, thus implicating these processes to play causal roles in the development of obesity. Several candidate genes such as *MC4R* and *BDNF* overlap with the genes for monogenic obesity and thus highlight shared mechanisms between monogenic and polygenic obesity. Many of the GWAS genes for polygenic obesity, however, have not been associated with monogenic obesity to date.

FTO is the first gene to be identified as associated with common forms of obesity. Since its discovery in the first obesity GWAS in 2007, this locus has been the most significant and reproducible locus across studies and across obesity phenotypes. Most studies have identified SNP rs9939609 in the large first intron of the gene. It has been calculated that possessing one risk allele of this SNP results in a weight gain of 1.5 kg (3.3 pounds) and being homozygous causes

a 3.0 kg weight gain [23]. The same locus has also been linked to additional diseases such as type 2 diabetes, osteoporosis, melanoma, and attention deficit hyperactivity disorders, demonstrating its pleiotropic effect on common complex diseases. *FTO* encodes an alpha-ketoglutarate-dependent dioxygenase, an enzyme in humans that demethylates N6-methyladenosine and regulates gene expression. Numerous human and mouse studies attempting to address the underlying mechanisms have converged on the connection of the *FTO* risk allele to increased food intake, food preference for energy rich diets such as high fat and sugar, and impaired satiety, although the effect of *FTO* on energy expenditure has remained controversial [24,25]. Reduced *FTO* expression in the hypothalamus has been shown to decrease food intake, but unknown, non-hypothalamic processes are also implicated [26]. To further explore the mechanisms underlying the effect of *FTO* on food intake, Karra et al. conducted various experiments in humans, mice, and cell models and convincingly pinpointed ghrelin, the key hormone that regulates both homeostatic and reward-related feeding behavior, as one of the substrates of *FTO* [25]. Specifically, they found that overexpression of *FTO* was associated with decreased m6A ghrelin mRNA methylation and increased ghrelin expression, thus offering direct insights into the mechanisms underlying the obesity-prone eating behavior of the *FTO* risk allele carriers.

ENVIRONMENTAL RISKS OF OBESITY

Numerous studies have unanimously pointed to the same set of environmental factors — energy rich diets (increased energy intake) and sedentary living (decreased energy expenditure) — as the culprit leading to the current obesity epidemic [5,27]. More recently, environmental xenobiotic chemicals termed obesogens have also gained recognition as powerful obesity-inducing agents [28-30]. Although the risk conferred by obesogens relative to diet and physical activity is not yet clear, it is postu-

Table 3. Tissue-specific molecular pathways perturbed by environmental risk factors revealed from genome-wide transcriptomic analysis (not including candidate gene approaches).

Tissue	High fat diet	High sucrose diet	Physical activity	Obesogens
Liver	Glycolysis, Krebs cycle, beta oxidation, fatty acid synthesis and oxidation, cholesterol biosynthesis, oxidative phosphorylation, insulin signaling, lipid metabolism, adipogenesis, PPAR signaling [46-50]	Lipid metabolism, amino acid metabolism, steroid metabolism, transcription, cell cycle, apoptosis, signal transduction, redox control, immune response [31]	Lipid metabolism, lipogenesis, defense response, stress response, detoxification, protein turnover, intracellular signaling, immunity, cellular architecture [51-53]	
Adipose	Inflammatory response, response to external stimulus, immune system, lipid metabolism, fatty acid synthesis and transport, PPAR signaling, leukocyte activation, Toll-like receptor signaling, cytokine-cytokine receptor interaction, mitochondrial biogenesis [32,49,54]		Metabolic process, lipid metabolism, inflammation, immune response, stress response, mitochondrial transport [52,55]	PPAR signaling, fatty acid metabolism, response to superoxide, glucose metabolism, response to hormone stimulus, response to glucocorticoid stimulus, pyruvate metabolism, fat differentiation, adipokine signaling [40]
Muscle	Glycolysis, Krebs cycle, beta oxidation, fatty acid synthesis, mitochondrial oxidative phosphorylation, mitochondrial biogenesis [46,56,57]		Lipid and carbohydrate metabolism, energy homeostasis, defense, inflammation, mitochondrion, oxidative phosphorylation, sarcoplasm, laminin complex, cytoskeleton, skeletal muscle plasticity, muscle cell proliferation and differentiation, calcium signaling, growth factors, cytokine signaling, ubiquitin proteasome [52,58-61]	
Gastrointestinal tract	Immunity, lipid and fatty acid metabolism, signal transduction, olfaction [62,63]			
Hypothalamus	Transcription, neuropeptide signaling, cell adhesion [64]			

lated that exposure to environmental obesogens, especially during early development, can exacerbate the obesity-inducing effects of energy-rich diets and lack of physical activity.

To investigate the mechanisms underlying the effect of these environmental risk factors on the development of obesity and related pathological conditions, numerous genomic studies have been conducted in the past few years using either candidate gene approaches or global analysis. Here, we focus on the results from global analysis of the transcriptome, as this type of analysis can objectively and systematically identify genomic changes associated with environmental perturbations to provide an unbiased, systems-level view of the general mechanisms related to human pathophysiology. A majority of these studies utilize DNA microarray technologies to profile the expression changes of >20,000 genes in individual tissues of animal models (primarily mouse and rat) and humans. The findings from these studies are summarized in Table 3. We also provide a brief overview of relevant studies of the epigenome that is postulated to transmit the environmental perturbations to transcriptomic alterations.

Genomic Alterations Associated with High Caloric Diets

Foods, as the fuel and energy supply, are perhaps the most powerful modulator for the body system. The global shift toward modern diets with high levels of saturated fats and sugars (primarily sucrose and fructose) tips the metabolic balance toward elevated energy storage in the adipose tissue, leading to increased fat mass and adiposity. Although it appears as a simple process at first glance, the intrinsic complexity of various important components that determine food intake and energy consumption, including eating behavior, food preference, digestion and absorption, nutrient metabolism, and basal metabolic rate, makes a comprehensive understanding of the molecular impact of food on the body a challenging task.

As shown in Table 3, numerous studies have investigated the gene expression

changes induced by high fat diet (HFD) feeding in various metabolically related tissues including liver, adipose, muscle, and hypothalamus. Large-scale gene expression changes in hundreds of genes have been identified for each tissue type. Perturbations in metabolic pathways such as lipid metabolism, oxidative phosphorylation, and peroxisome proliferator activating receptor (PPAR) signaling are observed in the peripheral, metabolically active tissues. Inflammatory and immune response-related pathways are also prominent in adipose tissue. In the hypothalamus, genes involved in neuropeptide signaling, cell adhesion, and transcription are altered. Similar studies of high sugar diet are also emerging, although scarce at the present time. For instance, Nojima et al. investigated liver transcriptome changes in a diabetes mouse model fed with a high sucrose diet and found changes in many pathways such as lipid/amino acid/steroid metabolism, cell cycle, transcription, apoptosis, and immune response in the liver [31]. Overall, these transcriptomic changes indicate that both HFD and high sugar diets affect metabolism and immune response, as well as many tissue-specific processes, all of which may play a role in the pathogenesis of obesity.

As dietary perturbations are not expected to change DNA sequences, what might be the triggering mechanism for the observed extensive transcriptome changes in the body? A logical source of transcriptional variations that are non-genetic (i.e., absence of DNA sequence changes) in nature is epigenetics, which involves multiple mechanisms such as DNA methylation, histone modification, chromatin remodeling, and microRNAs. An increasing number of studies have been conducted to investigate these potential epigenetic mechanisms that may sense the dietary signals and transmit the signals to gene expression alternations. Corroborating with such contentions, changes in the DNA methylation and histone modification status of various candidate gene regions have been observed. For instance, Toll-like receptor genes [32] and feeding-related leptin gene [33] exhibit HFD-induced methyl-

tion changes; histone modifications have been implicated for the hepatic phosphoenolpyruvate carboxykinase gene [34] and the p16 (INK4a) promoter region [34]. At the current time, genome-scale systemic analysis of the epigenome affected by energy-rich diets in individual tissues is still in its infancy and awaits further development.

Genomic Alterations Associated with Physical Activity

The connection between the lack of physical activity and obesity development is primarily viewed as the result of low energy expenditure that subsequently favors energy storage. Genomic studies of physical activity mainly focus on the effect of exercise or physical training on skeletal muscle, liver, and adipose (Table 3). As seen in the case of diets, physical activity induced changes in metabolic processes (primarily lipid metabolism) and inflammatory pathways across the three peripheral tissues. In skeletal muscle, the direct target tissue of most exercise training, muscle-related processes such as muscle proliferation and differentiation and calcium signaling are altered.

Similar to diets, there is increasing evidence supporting physical activity as an epigenetic regulator by affecting DNA methylation and histone modifications of candidate gene regions in various tissues including the brain (hypothalamus and hippocampus), muscle, and adipose [35-37]. Two recent genome-wide DNA methylation screening studies in human tissues have opened a new paradigm for the epigenomic research of environmental risks. Nitert et al. investigated the genome-wide DNA methylation patterns of the skeletal muscle of diabetes-prone individuals and those with no family history of diabetes, with or without exercise. They identified hundreds of genes involved in retinol metabolism, calcium-signaling pathway, starch and sucrose metabolism, insulin-signaling pathway, amino acid metabolism, and glycolysis/gluconeogenesis that demonstrated exercise-induced changes in DNA methylation status [38]. In another study, Ronn et al. explored the global DNA methylation changes in human

adipose tissue after 6 months of exercise and identified 17,975 individual DNA methylation sites in 7,663 unique genes that showed differential DNA methylation after exercise [39]. Many of these genes are known to be involved in obesity and diabetes. Moreover, both studies found significant correlations between DNA methylation status and gene expression levels, showing that the large-scale exercise-related transcriptomic alterations partially result from their modified epigenetic states.

Genomic Alterations Associated with Xenobiotic Obesogens

Besides diet and physical activity, obesogens have recently emerged as a third major category of environmental risk for obesity. Obesogens, a term first coined by Bruce Blumberg, are xenobiotic chemicals in the environment that directly or indirectly affect fat accumulation by disrupting adipogenesis and energy balance, subsequently causing obesity and its associated diseases [28,30]. To date, various types of chemicals including pesticides (e.g., dichlorodiphenyl-trichloroethane [DDT]), plasticizers (e.g., phthalates), organotins (e.g., tributyltin [TBT]), endocrine disruptors (e.g., bisphenol A [BPA]), and pharmaceutical agents (e.g., pioglitazone, an antidiabetic drug, as well as antidepressant and antipsychotic drugs) have been categorized as obesogens. Among these, TBT and BPA are two well-studied obesogens, and TBT has been used as a model obesogen for mechanistic studies. There are a number of ways through which obesogens stimulate the development of obesity. Most commonly, they increase the number of adipocytes by altering the basal metabolic rate, through the modification of metabolism to favor energy storage, and by interfering with the endocrine system to control appetite and satiety [28-30]. Both early-life and chronic exposure to obesogens has been linked to increased susceptibility to obesity, and in utero and early-postnatal exposure has particularly been shown to increase the sensitivity of offspring to other obesity-inducing risks such as high caloric diets.

Various molecular targets of obesogens have been proposed and tested to explain the obesity-promoting activities of obesogens, including PPAR signaling, sex hormone receptors, and glucocorticoids. However, a majority of the studies focus on candidate pathways or genes, and a systems-level, comprehensive understanding of the molecular impact of obesogens is in need. One recent study by Pereira-Fernandes et al. moved in this direction. By utilizing microarrays to study the transcriptome of an adipocyte cell line 3T3-L1 under TBT treatment, they identified a large number of pathways, including both the expected, known pathways (e.g., PPAR signaling, response to hormone stimulus, response to glucocorticoid stimulus, and fat cell differentiation), and relatively less reported processes (e.g., response to superoxide, glucose metabolism, pyruvate metabolism, and adipokine signaling) to be involved in the TBT action (Table 3) [40]. Additional studies at such a global level on additional cell and tissue types and on other obesogens will undoubtedly offer more comprehensive views of the molecular actions of obesogens.

TOWARD A BETTER UNDERSTANDING OF OBESITY VIA INTEGRATIVE GENOMICS

The genomics studies summarized above primarily explore the molecular mechanisms of individual genetic and environmental risks one at a time. These studies offer relatively clean pictures of what's happening in the body at the molecular level when a single type of perturbation is introduced. However, in reality, many genes and pathways together contribute to disease development, and both genetic and environmental risks act simultaneously through complex interactions to impact human health. In the past few years, integrative genomics strategies that aim to capture gene-gene interactions and gene-by-environment interactions have been carried out to enhance our understanding of obesity.

Through the efforts to explore gene-gene interactions, network biology has emerged as a powerful tool for studying the

complex mechanisms of obesity and its comorbidities [41]. Network biology aims to reconstruct and elucidate the regulatory relationships among genes by integrating genome-scale genetic, transcriptomic, and other types of molecular data. The global view of gene-gene interactions revealed by the gene network architecture is critical for pinpointing key regulatory events and regulatory/signaling cascades involved in physiological and pathological conditions. For instance, Chen et al. and Emilsson et al. studied liver and adipose tissues of humans and mice and reconstructed tissue-specific gene networks. They identified a specific part of the gene network that is conserved between tissues and between species, highly enriched for inflammatory genes and highly correlated with obesity and its related diseases such as CAD and T2D [42,43]. In another study, Yang et al. explored the webs of genes perturbed by obesity-causing genes in mice and identified a liver gene network that is highly relevant to lipid metabolism and fat cell differentiation to be part of the mechanism underpinning obesity [44]. In a bold departure from previous studies that either focus on genetic or environmental factors, Parks et al. recently conducted a study that incorporated both genetic variations (by using >100 inbred and recombinant inbred mouse strains) and environmental perturbation (by using high fat and high sucrose diets) [17]. The integration of both genetic and environmental variability in the study design allows close simulation of conditions in natural human populations and enables the capture of gene-by-environment interactions. They found strong evidence supporting genetic determination of obesity set point in response to high fat and high sucrose diets, that is, the dietary response is pre-determined by genetic background and obesity is the result of the specific combinations of genetic makeup with environmental challenge. Future integrative genomic studies on the same sets of mice from a network perspective will undoubtedly reveal critical insights into the molecular mechanisms underlying the complex gene-environment interactions that trigger obesity onset.

CONCLUSIONS AND FUTURE DIRECTIONS

The current obesity epidemic is obviously one of the most pressing health concerns in the modern society. Through recent systems biology studies that examine multiple levels of genomic information (i.e., genetics, epigenome, and transcriptome), multiple obesity-related tissues, and both genetic and environmental risks, we have gained substantial understanding of the molecular underpinnings of obesity and revealed unique challenges. From the genetics angle, recent GWAS have revealed >50 genetic loci that are significantly associated with obesity, several of which are involved in neuronal control of energy homeostasis and food intake. However, exactly how the majority of the novel genetic loci lead to obesity onset remains poorly understood and demands high-throughput, in-depth mechanistic studies. From the environmental angle, studies of environmental risks including high caloric diets, physical activity, and obesogens revealed large-scale transcriptomic and epigenomic changes, with major impact on metabolic pathways, inflammatory processes, as well as many tissue-specific mechanisms.

Despite the significant progresses, it is important to recognize the intrinsic limitations of each of the genomic approaches, as summarized and discussed in detail in our recent review article [41]. For example, GWAS suffer from limited statistical power due to severe multiple testing, are subject to noise, and lack functional and mechanistic insights. Transcriptomic and epigenomic studies, on the other hand, are correlative in nature and cannot directly provide causal inference. Network approaches are modeling techniques that rely heavily on various statistical and mathematical assumptions and the predictions require experimental validation.

In addition to addressing the technical limitations discussed above through advanced systems biology approaches [41], the intrinsic complexity of metabolic regulation that involves coordination of multiple biological processes in multiple metabolically

related cells, tissues, and organ systems demands more coordinated, systems-level investigations across the body. Interestingly, although prevailing evidence from the genetic signals points to a central role of the brain in controlling obesity phenotypes, the transcriptomic and epigenomic studies have mostly focused on the peripheral tissues and ignored the brain. Given the importance of the brain, especially hypothalamus, in regulating feeding behavior and energy homeostasis, it is critical to guide future efforts to include individual brain regions or even specific neurons. In addition, the majority of the current efforts focus on generating lists of genetic loci, epigenomic changes, and gene expression alterations without in-depth exploration of how multiple levels of genomic alterations interact to determine the states of key biological processes involved in obesity development. It is, therefore, pivotal to conduct future investigations of regulatory relationships across multiple levels of molecular information within and between multiple cell and tissue types. Moreover, the mechanistic studies reviewed here focus on the host genome. Considering that microorganisms living in the body outnumber our own cells and they play critical roles in modulating multiple host functions such as digestion, absorption, and immune response, coupled with the fact that diet is the major force shaping the composition of gut microbiome, it is highly plausible that the microbes also participate in the pathogenesis of diet-induced obesity interactions. Indeed, a number of high profile studies revealed a causal role of the microbiome [45]. Future studies to investigate which microbiota families have the ability to either trigger or inhibit the development of human obesity are necessary to explore their therapeutic potential.

As complex as obesity is, it is not surprising that most of the current therapies that aim to alleviate individual risk factors, genes, or pathways have proven ineffective for the prevention or treatment of obesity. The future of developing effective preventive and therapeutic strategies, in our opinion, relies on highly comprehensive,

systems-level maps of obesity mechanisms. These maps should clearly illustrate specific genes and pathways in specific tissues that are perturbed by specific risk factors. Furthermore, they should elucidate whether there are shared mechanisms between different risk factors and what types of molecular alterations are specific to individual risk factors. With the maps in hand, novel therapeutic strategies can then be developed and tested to evaluate their capacity to normalize the systems-level molecular changes and to reverse the pathological courses of obesity.

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