CHAPTER

9

Animal Models of Metabolic Syndrome

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OUTLINE

1 INTRODUCTION AND OVERVIEW

The metabolic syndrome (MetS) is a suite of metabolic complications including central obesity, hypertension, insulin resistance, impaired fasting glucose, dyslipidemia, and inflammation that, in some combination, increases an individual's risk of developing type-2 diabetes (T2D) and/or cardiovascular disease (CVD). Each of these metabolic complications is rising worldwide due to factors such as increasing population size, population aging, urbanization, excess caloric intake, and decreased physical activity. Prevalence of MetS currently exceeds 20% in the United States (for a review, see Eckel et al., 2005 and references within). While aspects of most expert definitions of MetS overlap, there is no single internationally accepted clinical definition because diagnostic criteria used to identify individual risk factors vary among populations, Table 9.1 (Einhorn et al., 2003; Grundy et al., 2005; International Diabetes Federation (IDF), 2006; Parikh and Mohan, 2012; The European Group for the study of Insulin Resistance (EGIR), 2002; World Health Organization (WHO), 1999). For example, intraabdominal fat deposits can lead to severe insulin resistance and T2D in some South Asian populations at body mass indexes that are not considered obese in Western populations (Spiegel and Hawkins, 2012). Variations in diagnostic criteria make it difficult to compare results of studies across populations, and worldwide prevalence of MetS per se is not well quantified. The lack of a single accepted definition has led some investigators to favor keeping individual metabolic complications separate for clinical management. However, others believe that identifying individuals with an aggregation of metabolic complications provides information that

Animal Models for the Study of Human Disease.

TABLE 9.1 Multiple Expert Definitions of the Metabolic Syndrome (MetS)

Group	Diagnostic criteria
American Association of Clinical Endocrinologists (AACE)	Based on clinical judgment, some combination of the following: • Central obesity • High blood pressure • Hypertriglyceridemia • Impaired glucose tolerance • Inflammation • Low HDL-C
American Heart Association/National Cholesterol Education Program Adult Treatment Panel III (AHA/NCEP ATP III)	Three or more of the following: • Central obesity • High blood pressure • High fasting glucose • Hypertriglyceridemia • Low HDL-C
European Group for the Study of Insulin Resistance (EGIR)	Elevated plasma insulin plus two or more of the following: • Central obesity • High blood pressure • High fasting glucose • Hypertriglyceridemia
International Diabetes Federation (IDF)	Central obesity plus two or more of the following: • High blood pressure • High fasting glucose • Hypertriglyceridemia • Low HDL-C
World Health Organization (WHO)	Glucose intolerance, impaired glucose tolerance or diabetes and/or insulin resistance plus two or more of the following: • Central obesity • High blood pressure • Hypertriglyceridemia • Microalbuminuria

should guide clinical management (Eckel et al., 2005; Grundy, 2008; Kahn et al., 2005; Simmons et al., 2010).

Although clinical definitions of MetS are disputed, the frequent clustering of certain metabolic complications has been a recognized phenomenon since the 1920s (Kylin, 1923). Over time, this clustering has been referred as *syndrome X*, *the deadly quartet*, and the *insulin resistance syndrome* and represents a premorbid condition that is a substantial biomedical and public health challenge. Knowing how and why multiple metabolic complications cluster in some individuals and not in others will greatly improve clinical prevention, management and therapy of TD2 and CVD. To this end, research using animal models has provided key insights into MetS etiology and pathophysiology with the goal of uncovering potential therapeutic targets.

Animal models provide complex biological systems that can be controlled, manipulated, sampled, and studied under conditions and at scales that are not ethical or practical for human studies. While some physiological features of MetS components vary among species, the units of research translation are the underlying genes, biological processes, and/or physiological pathways that can alter normal metabolic phenotypes (Kraja et al., 2008). MetS is complex, involving nonlinear interactions among multiple organs such as adipose tissue, brain, gut, liver, pancreas, and skeletal muscle. Animal models provide an opportunity to examine interorgan cross talk in a whole organism and to directly sample relevant tissues. Even when an animal model does not faithfully mimic the human disorder, novel biological insights can be gained from an in vivo approach. For example, the mouse- and rat-based characterizations of the genes encoding the hormone leptin, *Lep*, and the leptin receptor, *Lepr*, identified mutations affecting lipid metabolism that result in extreme obesity in rodents (Chung et al., 1998; Cool et al., 2006; Kurtz et al., 1989; Varga et al., 2009). In humans, mutations in the orthologous genes LEP and LEPR represent extremely rare cases of obesity (Bergman et al., 2006; Gesta et al., 2007). Yet this animal-based research was essential to further research characterizing leptin metabolism and to identify the hypothalamic pathways controlling signals of satiety and the neural pathways in the brain's striatal region that control perceptions of reward associated with food intake (Belgardt et al., 2009; Elmquist et al., 1999; Konner et al., 2009).

This chapter provides an overview of animal models of MetS that are used to understand etiology and pathophysiology to inform potential therapies. Research aimed at understanding MetS etiology can identify and protect at risk individuals. Research aimed at understanding pathophysiology can shed light on how the clustering of different MetS components increases an individual's risk of developing T2D and CVD. It is important to note that these two broad categories —etiology and pathophysiology—are not mutually exclusive. Rather each informs the other and each is used to develop further research efforts aimed at testing therapeutic strategies to guide appropriate clinical interventions. Animal models of MetS tend to focus on specific metabolic components although, as is seen in humans diagnosed with MetS, individual metabolic complications will often cluster in any given model. For example, obesity and insulin resistance generally coincide in most rodent models, and increased adiposity is associated with dyslipidemia and insulin resistance in both nonhuman primate and porcine models.

2 CHOOSING AN ANIMAL MODEL OF MetS

Rodents, especially mice, are the most commonly used animal model for studying MetS, particularly for genetic studies, because they are relatively easy to

breed and maintain, have highly standardized phenotyping protocols, and high-coverage whole-genome sequences are available for the most commonly studied inbred strains. Table 9.2 lists the most frequently used mouse metabolic phenotyping tests and the data collected for each test. When studying MetS, multiple tests are performed on the same animal to study correlations among different phenotypes that mimic the clustering of metabolic complications in humans. An exciting new technology for assaying multiple measurements simultaneously in rodents is the PhenoMaster system (TSE Systems, Germany). The system uses intrahome-cage technology, and is capable of indirect calorimetry, as well as measurement of food consumption, fluid intake, and activity.

TABLE 9.2 Common Mouse Phenotyping Tests for MetS

Phenotyping test	Data collected
Euglycemic-hyperinsulinemic clamp	Insulin action and glucose metabolism
Intraperitoneal insulin sensitivity test (IPIST)	Insulin resistance, glucose tolerance, glucose disposal
Intraperitoneal glucose tolerance test (IPGTT)	Insulin secretion, glucose tolerance, glucose disposal
Oral glucose tolerance test (OGTT)	Insulin secretion, glucose tolerance, glucose disposal
Fasted bleed (4–6 h)	Glucose, insulin, blood biochemistry, other hormones (e.g., leptin, glucagon, peptide-YY)
Metabolic caging	Food intake, water intake, urine and feces production, urine biochemistry and glucose
Noninvasive intestinal fat absorption	Fecal fat absorption
Echocardiography	Myocardial performance
Cardiac telemetry	Blood pressure, heart rate, pulse pressure, activity
Indirect calorimetry	Metabolic rate (oxygen consumption, carbon dioxide production, respiratory quotient)
Meal pattern analysis	Food intake
Lipid profiles	Triglycerides, total cholesterol, phospholipids, free fatty acids
Fast protein liquid chromatography	Lipoproteins, lipids, apolipoproteins
Quantitative magnetic resonance	Fat mass, lean mass, water content
Tail cuff	Blood pressure
Running wheels	Energy expenditure, circadian activity
Measuring animal	Body weight, body length, fatpad and organ weights (at necropsy)

Several obese, diabetic, and hypertensive mouse strains are well characterized and have been used for decades of biomedical research. Further, transgenic and knockout mouse resources are commercially available (these are discussed in Section 4.1). Other species, including nonhuman primates and larger mammals, such as dogs, pigs, and sheep have also made important contributions to understanding MetS. In some cases these other species may be more appropriate animal models than rodents (Varga et al., 2009). For example, rodent fat depots are not directly translatable to humans (Pond and Mattacks, 1987). Thus the most common measurements used in human anthropometric studies (e.g., waist circumference) are not equivalent in mouse studies of obesity and adiposity. Additionally, there are important biochemical distinctions in adipose tissue between humans and mice. For example, levels of the adipokine adipsin are low in obese mice, but high in obese humans. High levels of the adipokine resistin impairs glucose tolerance in mice, but does not appear to do so in humans (Arner, 2005). Further, mouse lipoprotein profiles are composed mainly of high-density lipoprotein (HDL), which is atheroprotective, whereas humans carry mostly low-density lipoprotein (LDL). Pigs have similar LDL levels to humans, develop atherosclerotic plaques at the same sites as humans (in the aorta and carotid artery), and have similar hemodynamic parameters (Kalt et al., 2008; Turk and Laughlin, 2004). Pigs are frequently used to model CVD and atherosclerosis because their cardiovascular system is morphologically and functionally similar to humans (Litten-Brown et al., 2010). Certain breeds of pigs (namely the Ossabaw minipigs and domestic Piebalds) develop obesity, hypertension, and insulin resistance that fit some definitions of MetS in humans (Spurlock and Gabler, 2008).

Precise criteria to diagnose T2D in mice are not established, however, many aspects of blood glucose control are similar between mice and humans, so mice are frequently used to model glucose homeostasis and regulation of glucose metabolism. Notably, nonhuman primates develop T2D with similar pathological features as humans, including increased plasma triglyceride levels and total cholesterol concentrations (Wagner et al., 2006). Increased levels of adiposity in both baboons and rhesus macaques is associated with insulin resistance and dyslipidemia, similar to humans diagnosed with MetS (Comuzzie et al., 2003). Further, obesity and its associated metabolic complications have been extensively studied in dogs, since domestic dogs have experienced their own obesity "epidemic." There exists a wealth of pathological data available for many different breeds, and dogs exhibit variation in metabolic traits similar to that seen among human populations. Thus dog represents a potentially fruitful large animal model for testing biomedical hypotheses because of the

abundant physiological information available through veterinary data (Edney and Smith, 1986). Additionally, canine models provide a resource for longitudinal data collection that is not possible in smaller animal models (Ionut et al., 2010; Zheng et al., 2010).

Larger mammals may represent more physiologically faithful models of the individual metabolic complications comprising MetS. However, the expense, housing requirements, longer lifespan, relatively less standardized phenotyping protocols, and species-specific ethical considerations, particularly with nonhuman primates, make large scale studies using these animal models impractical. Thus when choosing an animal model of MetS, one must consider its relevant strengths and, when possible, integrate multiple lines of evidence. For example, results identified through discovery research using a rodent model can inform, be integrated with, and ideally validated by, small-scale follow-up studies using a larger mammalian system, which can then be a bridge toward designing relevant human studies.

3 ANIMAL MODELS OF MetS ETIOLOGY

Understanding MetS etiology is important for implementing prevention strategies, as well as for prescribing appropriate therapeutic interventions. The rising prevalence of metabolic complications among human populations is environmental in origin yet there is clearly an important genetic component reflected in variations in prevalence between the sexes and among ethnic groups (Eckel et al., 2005). Given the same environment, some individuals will develop metabolic complications while others will not, indicating that standing genetic variation modifies the effects of the environment on phenotype. In some cases, but not others, metabolic complications will cluster resulting in MetS. For example, common complications that cluster with obesity (and in some combinations define MetS, as discussed earlier) include insulin resistance, dyslipidemia, and increased blood pressure. Data from 1994 to 2004 of National Health and Nutrition Examination Survey (NHANES) found that approximately 32% of obese adults were "metabolically healthy" based on measures of blood pressure, homeostasis model assessment of insulin resistance (HOMA-IR), and plasma triglycerides and HDL-cholesterol concentrations (Wildman et al., 2008). Heritability estimates for individual MetS components are up to 70% for body mass index (BMI), 50%–90% for T2D, 22%–62% for systolic blood pressure, 20%–66% for diastolic blood pressure, 8%–72% for total cholesterol, and 19%–72% for total triglycerides (Bogardus, 2009; Clee and Attie, 2007; Elder et al., 2009; Permutt et al., 2005; Song et al., 2006; Walley et al., 2009). Clearly much phenotypic variability in these metabolic parameters can be attributed to heritable genetic variation, and animal models are fundamental in discovering the genetic underpinnings of these parameters and of their relationships to each other. Animal models of MetS etiology allow both genetic and environmental factors to be controlled for, manipulated, and monitored in study populations of known origin (Lawson and Cheverud, 2010). This facilitates the discovery of genetic mechanisms, the testing of environmental influences, and the investigation of how these two factors—genetics and environment—sometimes interact and result in MetS.

4 GENETIC FACTORS

4.1 Common Rodent Genetic Models

Genetic studies using classic rodent models of obesity, such as the ob/ob and db/db mice and the Zucker fa/fa rats have identified single genes, namely leptin, *Lep* (ob/ob and Zucker fa/fa rats), and the leptin receptor, *Lepr* (db/db), with major variants leading to extreme obesity and other metabolic complications. Leptin is an adipose tissue derived protein hormone that binds to, and decreases the activity of, neuropeptide Y neurons. This signals satiety, and mutated forms of leptin results in an inability to feel sated leading to hyperphagia and obesity (for a comprehensive review of leptin biology, see Havel, 2004). In addition to extreme obesity, each of these animals to some degree are hyperinsulinemic, insulin resistant, and exhibit defective thermogenesis (Chung et al., 1998; Cool et al., 2006; Kurtz et al., 1989). The leptin-deficient mouse model, $Lep^{ob/ob}$, arose from a spontaneous mutation at the Jackson Laboratory (Ingalls et al., 1950). These mice are obese by 4 weeks of age, and can weigh up to 4 times that of their littermate controls on a standard chow diet. In addition to hyperphagia, reduced energy expenditure and extreme obesity, $Lep^{ob/ob}$ mice have elevated serum cholesterol levels. However, this elevation is in HDL-rather than in LDL-cholesterol, so they are actually protected from developing diet-induced atherosclerosis (Nishina et al., 1994). An additional metabolic abnormality in $Lep^{ob/ob}$ mice involves disregulation of the hypothalamic-pituitary-adrenal axis, of which leptin is an important regulating hormone (Malendowicz et al., 2007). A complication with this model is that $Lep^{ob/ob}$ are infertile, which impedes collecting appropriate samples sizes (Kennedy et al., 2010). Leptin receptor-deficient mice, $LepR^{db/db}$, have nearly identical metabolic profiles and hypothalamic-pituitary-adrenal axis and reproduction problems as $Lep^{ob/ob}$ mice. The significant difference between the two models is that $LepR^{db/db}$ mice have pronounced concentrations of circulating leptin while the $Lep^{ob/ob}$ mice have none at all. Thus the $LepR^{db/db}$ model is frequently used to study

how leptin concentrations affect different cell types in metabolic studies (Surmi et al., 2008). Finally, the leptin receptor-deficient obese Zucker fa/fa rat model is significantly hyperphagic compared to its lean littermates by as early as 17-days old (Kava et al., 1990). These rats are hyperlipidemic and hyperinsulinemic, but are relatively normoglycemic. They are frequently used to study adipose tissue in obesity and the physiological effects of disregulation of leptin signaling (Miranville et al., 2012; Pico et al., 2002).

The Agouti lethal yellow mouse model, $A^{y/a}$, has several spontaneous mutations affecting expression of the agouti protein, transcribed by the agouti gene, *A*. The agouti protein acts as an antagonist of the melanocortin-signaling pathway, which mediates leptin action. These mice display variation in coat colors and develop adult onset obesity and insulin resistance due to hyperphagia and hypoactivity. Obese $A^{y/a}$ mice are hypertensive but they do not form atherosclerotic lesions on high fat diets (Burgueno et al., 2007). It is easier to obtain offspring and appropriate sample sizes from $A^{y/a}$ than from either $Lep^{ob/ob}$ or $LepR^{db/db}$ because $A^{y/a}$ remain fertile until approximately 4 months of age (Kennedy et al., 2010). Other commonly used single-gene rodent models of obesity and insulin resistance include the fat/fat (variants responsible for the phenotype have been characterized in carboxypeptidase E, *Cpe*) and tub/tub (variants responsible for the phenotype have been characterized in the tubby candidate gene, *Tub*) mice (Carroll et al., 2004; Coleman and Eicher, 1990; Naggert et al., 1995).

The low-density lipoprotein receptor-deficient mouse, $LdlR^{-/-}$, is a model of hyperlipidemia that has elevated atherogenic LDL lipoprotein levels similar to that seen in humans with hypercholesterolemia (Ishibashi et al., 1993). These mice will become obese and develop insulin resistance in response to high fat diets (Wu et al., 2006). The apolipoprotein E-deficient mouse, $apoE^{-/-}$, is another frequently used model that develops severe hyperlipidemia, but generally does not become obese and insulin resistant, even on a high fat diet (Hofmann et al., 2008). APOE is a lipoprotein ligand that is recognized by multiple receptors in the liver. Mutant forms of APOE result in elevated very low-density lipoprotein (VLDL). To better approximate the clustering of metabolic complications of MetS, $A^{y/a}$, $Lep^{ob/ob}$, and $LepR^{db/db}$ mice can be crossed with $LdlR^{-/-}$ or $apoE^{-/-}$ mice (Hasty et al., 2001).

When designing an experiment using these classic monogenic models it is important to consider strain background (Coleman and Hummel, 1973). Each strain has a unique combination of alleles and therefore of disease susceptible loci. Different mouse strains vary for different phenotypic traits and when considering complex, polygenic traits, such as those comprising MetS, genetic background—that is, strain effects—can confound results. This is because of epistatic and/or compensatory interactions among loci. For example, when the Lep^{ob} mutation is bred into a BTBR (black and tan, brachyuric strain) background, the $Lep^{ob/ob}$ mice develop severe diabetes (Clee et al., 2005). However, in the C57BL/6J (the most widely used of all inbred strains and the *Mus musculus* reference genome strain) background, $Lep^{ob/ob}$ mice present as mildly hyperglycemic between 8 and 12 weeks of age, but then glucose levels return to near normal (Coleman and Hummel, 1973). In contrast to other strains, BALB $Lep^{ob/ob}$ mice are fertile (Clee and Attie, 2007). FVB (friend virus B strain) $LepR^{db/db}$ mice have more severe hyperinsulinemia and hyperglycemia than C57BL/6J $LepR^{db/db}$ mice (Chua et al., 2002). $A^{y/a}$ mice are generally studied on C57BL/6J or KK (originally generated from wild-derived ddY mice in Japan) backgrounds, and the MetS phenotype is more extreme in the KK strain, with KK $A^{y/a}$ animals exhibiting age-onset obesity, hypertension, insulin resistance, and diabetic nephropathy (Okazaki et al., 2002).

The models described previously represent some of the most commonly used monogenic rodent models of metabolic complications. However, single-gene mutations account for a very small percentage of the overall heritable variation of any MetS component, and account for a negligible percentage of MetS cases in human populations (Bogardus, 2009). Polygenic rodent models have also been developed that include, but are not limited to, the NZO, TallyHo, and KK inbred mouse strains and the OM, WOKW, and SHR rats (Bielschowsky and Goodall, 1970; Clee and Attie, 2007; Dulin and Wyse, 1970; Fisler et al., 1993; Kloting et al., 2006; Noda et al., 2010; Song et al., 2006). Each of these models is frequently used to study the genetic inheritance of individual MetS components and how these components correlate. NZO, the New Zealand obese strain, is a spontaneous model of polygenic obesity and insulin resistance. It is frequently used as a model of MetS because in addition to being obese, animals are hyperinsulinemic, show reduced insulin-stimulated glucose uptake in muscle and adipose tissues, have high serum triglyceride levels, and present with elevated blood pressure. TallyHo is a naturally occurring model of obesity and diabetes that also displays hyperlipidemia. KK mice (of which there are many substrains) are obese, hyperleptinemic, severely hyperinsulinemic, and animals display insulin resistance in both adipose and muscle tissues. OM, the Osborne–Mendel rat, is susceptible to dietary obesity and develops cardiac hypertrophy and hyperinsulinemia (Fitzgerald et al., 2001). WOKW, the Wistar Ottawa Karlsburg W rat, is obese and exhibits decreased insulin sensitivity and insulin-stimulated glucose uptake in adipose tissue. WOKW animals also display dislipidemia and hyperleptinemia and are frequently crossed with disease-resistant rat strains for studying

MetS (Kovacs et al., 2000). SHR, the spontaneously hypertensive rat, is the most frequently used animal model of blood pressure and hypertension, but it is commonly used to study MetS because these animals also display insulin resistance, hypertriglyceridemia, and obesity (van den Brandt et al., 2000). Recently, a new rat model of MetS, the UCD-T2DM, was generated by researchers at the University of California-Davis by crossing the obese Sprague-Dawly rat with lean Zucker diabetic fatty rats. The UCD-T2DM rat develops polygenic obesity with insulin resistance, impaired glucose tolerance and beta-cell decomposition (Cummings et al., 2008).

Polygenic models may be better suited than monogenic models for studying the genetic underpinnings of MetS etiology, because most of the metabolic complications comprising the individual MetS components are the result of many interacting genes of individually small effects. The Jackson Labs provides a comprehensive catalog of mouse models of MetS components including both monogenic and polygenic models for CVD, T2D, and obesity (http://www.jax.org). Their catalog provides information on strain history, phenotypes, health and husbandry, as well as their susceptibility to diseases. Further, the Jackson Labs provides an online version of the book "Biology of the Laboratory Mouse," which provides information on mouse biology, as well as husbandry (http://www.informatics.jax.org/greenbook/). Additionally, the Mouse Phenome Database provides phenotypic information across mouse strains. The Rat Genome Database (http://rgd.mcw.edu/) provides genotypic and phenotypic information on rat models, and provides multiple tools for researchers to mine rat-generated genomic and phenotypic data. These resources include valuable information for designing and executing rodent model studies, which are not only aimed at understanding MetS etiology, using the aforementioned described monogenic or polygenic models, but also aimed at identifying the genetic underpinnings of MetS components through targeted candidate gene approaches or through genetic mapping studies that identify quantitative trait loci (QTL) and novel genes associated with variation in metabolic parameters.

Recently, the number of whole genome sequences for individual mouse strains has expanded immensely. The Wellcome Trust's Mouse Genomes Project has made the complete genomes of 36 inbred strains available for the scientific community's use (http://www.sanger.ac.uk/science/data/mouse-genomes-project). Their database includes raw sequence data in addition to characterized polymorphisms, such as SNPs, indels, and structural variants. Additionally, whole genome sequences for the FVB/NJ (Wong et al., 2012), LG/J, and SM/J (Nikolskiy et al., 2015) inbred strains are available, including strain specific variants relative to the reference C57BL/6J strain. Further, the Mouse Diversity Array measures ~600,000 genotypes and has been applied to several hundred laboratory strains (Yang et al., 2009). These data significantly improve researchers' ability to relate genotype to phenotype and elucidate underlying molecular mechanisms (Keane et al., 2011).

4.2 Candidate Gene Approaches: Testing Biological Hypotheses

Candidate gene approaches can be used to test hypotheses about the genetic basis of a trait, based on a priori knowledge of that trait and some biological evidence that a focal gene of interest affects phenotype. Candidate genes are usually studied in animal models by targeted manipulation using genetic engineering technologies in embryonic stem cells to knockout (to remove or disrupt transcription of the gene of interest) or to knock-in (to mutate or to insert a variant of the gene of interest), and then to monitor any phenotypic consequences in mice heterozygous and/or homozygous for the targeted gene. Knockout (KO) models can provide key insights into target gene actions, and knock-in (KI) mice are useful for assessing subtle effects of mutations on protein structure or function. KO and KI mice are produced by incorporating the modified embryonic stem cells into the germ line, and then by interbreeding animals that develop with one copy of the targeted gene in their cells to generate animals that are homozygous for the manipulated gene. A key problem with genetic manipulation approaches is that manipulation of the targeted gene may result in embryonic death, preventing study of its effects on offspring or adult phenotype. Another problem is that genetic manipulations can induce compensatory changes, where other mechanisms take over the targeted gene's action and the targeted gene appears to have little phenotypic effect, even if it is important. Still, targeted manipulation of candidate genes is a powerful way to test biological hypotheses because mutations can be selectively targeted to specific tissues, cells, and/or particular developmental stages allowing physiological, developmental and/or behavioral complications to be studied. Manipulation of the mouse genome has been possible for over 20 years, and there are many resources available for planning and executing such experiments (Capecchi, 1989a,b; Nadeau et al., 2001). For example, The International Mouse Phenotying Consortium (http://www.mousephenotype.org/) is working to mutate every protein-coding gene in the mouse, and Mouse Genome Informatics (http://www.informatics.jax.org/) provides a collection of phenotypes and mutagenesis community resources (Collins et al., 2007).

Recently, CRISPR/Cas9 technology has allowed for generation of null, conditional, precisely mutated, reporter, or tagged alleles in mice (Jinek et al., 2012; Singh et al., 2015). This gene-editing technique is being used

to generate new KO mice, via a CRISPR (clustered regularly interspersed short palindromic repeats) mediated nonhomologous end joining approach. This approach utilizes single guide RNAs, which are directly injected into embryos along with Cas9 mRNA. This results in mice bearing frame-shifting mutations (insertion/deletions) that disrupt target genes. KI mice use CRISPR mediated homology directed repair. This approach utilizes a single-stranded oligonucleotide or a plasmid template, which is coinjected with single stranded RNAs and Cas9 mRNA. Generation of Cre-recombinase-dependent Cas9 allows tissue-specific or conditional gene editing (Yang et al., 2013). The Jackson Labs has generated close to 200 CRISPR/Cas9 mediated mouse models on multiple genetic backgrounds (www.jax.org/mouse-search).

4.3 Thrifty Genes

One hypothesis proposed to explain the genetic underpinnings of MetS, and which has motivated many candidate gene studies, is the "thrifty genotype hypothesis". The thrifty genotype hypothesis proposes that for most of evolutionary history, high-caloric foods were rare relative to the energy that was required to acquire them (Neel, 1962). Genetic variation that was efficient at stowing away excess calories in adipose tissue may have led to survival and fertility advantages that were advanced by natural selection over time. This hypothesis assumes that these "thrifty" genetic variants predispose an individual to metabolic complications in an environment where high-caloric foods are easily obtained. Candidate gene approaches testing the thrifty genotype hypothesis focus on genes involved in physiological processes necessary for energy balance, storage, and nutrition partitioning.

For example, the peroxisome proliferator-activated receptors (PPARs) are lipid-activated nuclear receptors that are necessary for multiple physiological processes important for energy balance (Lodhi and Semenkovich, 2014; Wang, 2010). These include fatty-acid catabolism (PPARα), thermoregulation and insulin homeostasis (PPARδ), and lipid metabolism and glucose homeostasis (PPARγ) (Barbier et al., 2002; Grimaldi, 2005; Wahli et al., 1995). *Pparα*$^{-/-}$ mice have decreased expression of fatty-acid oxidation genes and have a fatty liver phenotype indicating that *Pparα* regulates hepatic lipid catabolism (Akiyama et al., 2001; Aoyama et al., 1998; Sugden et al., 2002). *Pparδ* adipose-specific knockout mice have compromised thermoregulation. Analysis of *Pparδ* deficient brown fat cells generated from mice containing floxed [a method of conditional targeting wherein one or more of a candidate gene's exons are flanked by *lox P* sites, allowing the gene to be manipulated by Cre recombinase action (reviewed in Brault et al., 2007)] *Pparδ* alleles show downregulated expression of fat-burning genes, indicating that *Pparδ* is important in brown fat metabolism (Pan et al., 2009). Additionally, *Pparδ* skeletal muscle-specific knockout mice have increased weight and insulin resistance relative to controls (Schuler et al., 2006). *Pparγ* adipose-specific knockout mice show lipodystrophy, high serum free-fatty acids and triglyceride levels, and decreased leptin and adiponectin levels (He et al., 2003). *Pparγ* muscle-specific knockout mice show increased adiposity and insulin resistance (Hevener et al., 2003; Norris et al., 2003). These studies have demonstrated that the PPARs play a substantial role in energy balance and metabolism, and that disruption of normal PPAR transcription can contribute to multiple metabolic complications clustering as MetS. These genes have been implicated in human association studies for both obesity and T2D (Kunej et al., 2012). However, more research is required to understand the detailed molecular mechanisms by which the PPARs interact with each other and with other genes in their transcriptional pathways.

Another candidate gene studied to test the thrifty gene hypothesis is the melanocortin-4 receptor, MC4R. The central melanocortin system mediates leptin action and plays an important role in energy homeostasis (Cone, 2005). Variations in MC4R cause obesity in humans (Marti et al., 2003; Willer et al., 2009). *Mc4-R*$^{-/-}$ mice develop the obese, hyperglycemic, and hyperinsulinemic phenotypes that are typical of MetS; however, their triglycerides appear to be normal and they tend to be hypotensive (Tallam et al., 2005). The obesity in these mice is behavioral, resulting from hyperphagia, but the hyperinsulinemia is only partially due to obesity as young *Mc4-R*$^{-/-}$ animals have elevated circulating insulin levels prior to becoming obese (Fan et al., 2000). A final example of this candidate gene approach tested the thrombosponin receptor, CD36. CD36 first identified as associated with insulin sensitivity and hypertension in SHR rats discussed earlier (Aitman et al., 1999; Pravenec et al., 2001). Insulin sensitivity affects the body's ability to use stored fat for energy and reduced insulin sensitivity or insulin resistance is a precursor for T2D. Further studies in *Cd36* knockout mice revealed that this gene plays an important role in lipid processing, in insulin action, and atherogenesis (Drover et al., 2005; Goudriaan et al., 2005; Varga et al., 2009). Follow-up studies in human populations confirm that mutations affecting CD36 expression play a role in MetS risk (Griffin et al., 2001; Ma et al., 2004; Miyaoka et al., 2001).

Mouse models focused on testing biological hypotheses about candidate genes have advanced the study of MetS etiology by identifying a large number of physiologically plausible genes and pathways. However, as discussed earlier, strain background is important for assessing phenotypic consequences of candidate gene effects. For example, 85% of C57BL/6J mice heterozygous

for the insulin receptor knockout (*InsR*$^{+/-}$) and heterozygous for the insulin receptor substrate-1 (*Irs-1*$^{+/-}$) develop apparent diabetes by 6 months of age. However, only 64% of DBA mice and only 2% of 129Sv mice with the same mutations do (Kulkarni et al., 2003). Thus strain background must be considered along with the use of appropriate controls when designing candidate gene experiments.

4.4 Candidate Gene Approaches: Translating Human GWAS Results

Related to the candidate gene approach to test specific hypotheses about thrifty genes is the use of mouse models to directly test hypotheses about variants that were identified in human genome-wide association studies (GWAS). The idea is to translate variants that are significantly associated with common diseases in human studies to an animal model, with the goal of identifying the disease-causing gene within a GWAS linkage block. Variants identified in GWAS are part of linkage blocks, or sequences that are nonrandomly inherited together, and the disease-associated variant is usually not obvious and can often be found in noncoding sequence far from annotated genes (Wellcome Trust Case Control Consortium, 2007). An example of this candidate gene approach used mouse models to test the fat mass and obesity associated FTO locus. Multiple GWAS identified FTO as associated with T2D (although the association with T2D was lost once body mass index was controlled for) (Cox and Church, 2011; Dina et al., 2007; Frayling et al., 2007; Scuteri et al., 2007). *Fto* knockout mice showed a lean phenotype, reduced adipose tissue, and increased energy expenditure, despite being hyperphagic relative to controls. These animals also exhibited increased postnatal lethality and growth retardation (Fischer et al., 2009). An experiment that incorporated a missense mutation into the gene, causing loss of *fto* demethylase function, resulted in mice with reduced adipose tissue but without the additional phenotypic complications of the knockout (Church et al., 2009). Finally, over expression of the *fto* gene in mice resulted in extreme obesity due to hyperphagia (Church et al., 2010). Targeted manipulation of *fto* in these mouse models suggests FTO plays a role in energy homeostasis. This supports the GWAS results indicating that certain variants in human populations put an individual at risk for developing obesity and other obesity-related metabolic complications of MetS.

Another focused candidate approach targeted a linkage peak (containing multiple genes) on human chromosome 3 that is associated with a combined obesity-insulin factor in multiple human studies (Kraja et al., 2012). The researchers integrated the human linkage results with results from a mouse genetic mapping study that identified a significant association with serum insulin and glucose levels in the syntenic region of mouse chromosome 6. The researchers then performed a targeted association analysis in the focal human chromosome 3 region. By using the mouse results to "protect" the focal region in the human data from the extreme burden of multiple tests correction (essentially using the mouse results as an a priori hypothesis), novel genetic associations with multiple MetS components were identified.

The National Center of Biotechnology Information (NCBI) provides a database of genotypes and phenotypes (dbGaP) that both archives and distributes results of GWAS and other human genotype to phenotype studies, including many studies focused on metabolic complications (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap). As discussed in Section 1, the exact same variant is unlikely to affect humans and other species in the exact same way, but the units of research translation are the underlying genes, biological processes, and/or physiological pathways the genes interact in that can alter normal metabolic phenotypes. An assumption of this candidate approach is that these units of research translation have a similar function in humans and other species with respect to the disease process. Developing animal models that target variants underlying GWAS loci represents a powerful translational tool for identifying potential causal genes and elucidating how they function in disease and/or disease risk. Additionally, because human/mouse homology is well defined, using mouse results to protect homologous regions in these archived human data for correlated phenotypes will increase statistical power and identify new variants in candidate regions associated with disease and/or disease risk. Incorporating the candidate gene approach with GWAS results is a powerful method of identifying potentially relevant variants associated with MetS and rodent, especially mouse, models have proven invaluable tools for characterizing function and deconstructing molecular mechanisms (Fig. 9.1). Other genetic studies using animal models aim to identify novel MetS associated variants through hypothesis-free genetic mapping studies.

4.5 Identifying Quantitative Trait Loci and Novel Genes

Genetic mapping studies in animal models allow large numbers of offspring to be generated from relatively few founders of known genomic background. When the founders crossed differ in the trait of interest, for example, in levels of adiposity, phenotypic variation in the offspring can be correlated with genotypic variation in markers scored in the same individuals to identify QTL. Common markers used in QTL mapping are single-nucleotide polymorphisms, SNPs, but other

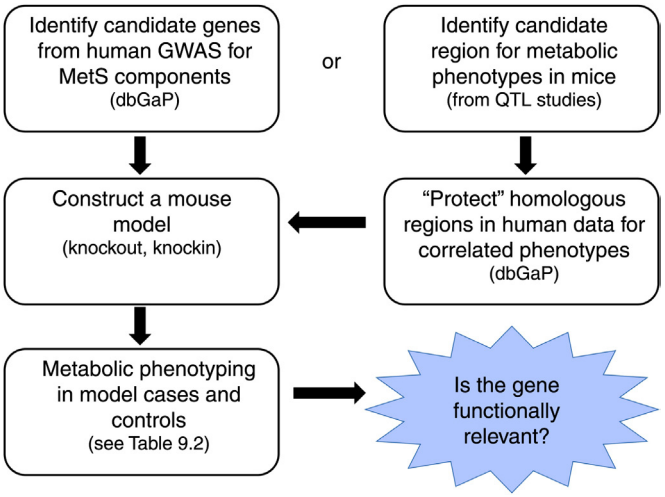

FIGURE 9.1 A candidate gene approach for translating human GWAS results. Associations identified in human studies can be directly tested in a mouse model to determine if the candidate gene affects the phenotype. Alternatively, mouse QTL regions can be used to mine GWAS results for correlated phenotypes and protect human syntenic regions from strict genome-wide multiple tests corrections. Candidate genes that are significant in the protected region (but that may have only been suggestive at the genome-wide level) can then be tested for functional relevance by going back to the mouse.

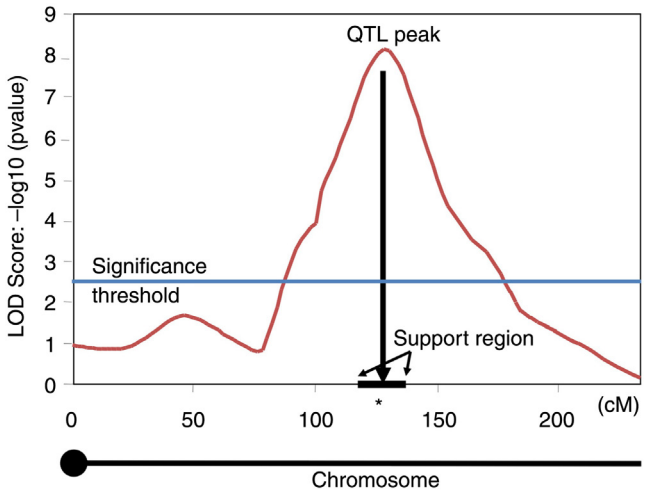

FIGURE 9.2 An example QTL. LOD scores (limit of detection, *y*-axis) are plotted along a chromosome (illustrated here in centiMorgans, *x*-axis) and represent the strength of a genomic association with phenotypic variance in a population. A higher LOD score indicates a lower probability that the association is by chance. A significance threshold is determined based on the number of genomic markers tested. The highest LOD score indicates the peak of the QTL and a support interval is defined (generally a 1-LOD drop from the peak position) to identify a physical region of the genome that can be interrogated for candidate genes. Note the cartoon chromosome refers to a generic mouse chromosome. All mouse chromosomes are acrocentric.

markers that vary within the experimental population, for example, microsatellites, can be used. In QTL studies, smaller sample sizes are required to find associations that can explain more than 50% of the heritable variation of a trait. In humans, thousands of individuals are required and findings typically explain a very small portion of the heritable variance. QTL represent physical locations on a chromosome in which genetic variants associate with phenotypic variation that was measured in the experimental population, illustrated in Fig. 9.2. As with candidate gene approaches, most QTL associated with MetS components have been identified and characterized in rodent, especially mouse, models (reviewed in: Lawson and Cheverud, 2010).

For rodent QTL mapping studies, the general experimental design is to first cross two inbred strains that are phenotypically distinct for the trait of interest (appropriately distinct strains can be identified using the Mouse Phenome Database and the Jackson Labs catalog as discussed earlier). The F_1 offspring are then either bred to each other to generate an F_2 intercross population, or bred back to one or both parental strains to generate a backcross population. An intercross between two genetically identical F_1 animals will result in F_2 animals with recombination on both transmitted chromosomes. An F_2 intercross, by having all possible combinations of genotypes represented at each locus, can provide information on both additive and dominance genetic effects. One-half of the chromosomes in backcross offspring will be recombinants from the F_1 parents. Backcross offspring are either heterozygote or homozygote for one parental allele at each locus, and can therefore provide information on alleles acting in a dominant fashion. In addition to using backcross or intercross experimental populations, QTL are commonly mapped in recombinant inbred (RI) strains, chromosome substitution strains or outbred stocks (for detailed discussion on these types of mice, refer to the Jackson Labs' "Biology of the Laboratory Mouse" online reference: http://www.informatics.jax.org/greenbook/). In a QTL study, mapping resolution (the power to localize an association) is a function of the number of recombination events that occur between genotyped markers. For example, an F_{16} Advanced Intercross population has 8 times the recombination of an F_2 intercross and QTL support intervals will be much smaller, containing 10s rather than 100s of genes to interrogate, as illustrated in Fig. 9.3 (Lawson and Cheverud, 2010).

Mouse Genome Informatics (http://www.informatics.jax.org/) archives and maintains a database of mouse QTL under their Genes and Markers Query form. This resource can be used to determine if intervals identified in a QTL mapping study replicate (have been identified in mapping studies using other strains of mice), lending higher confidence to the region. There are currently 326 QTL associated with obesity, 212 with T2D, 340 with serum lipid traits, and 33 with hypertension listed in the database (Lawson and Cheverud, 2010). For studying

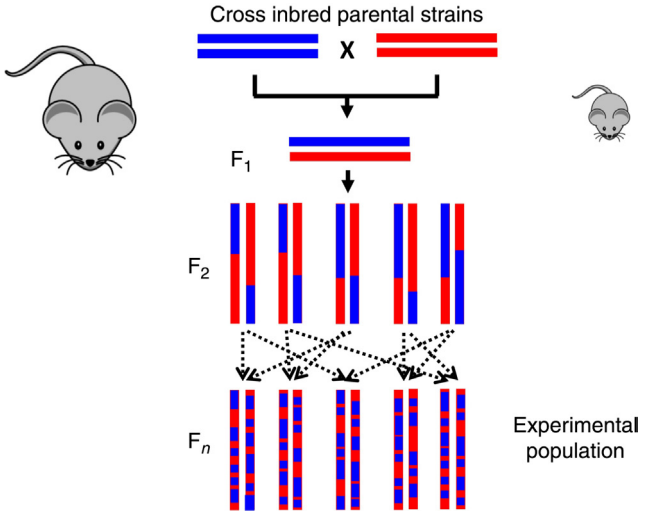

FIGURE 9.3 Recombination increases the power to identify candidate genes. When designing a QTL mapping study, the F1 offspring will be genetically identical. Brother-sister mating in the F1 will result in recombination and QTL can be identified in an F2 population. However, support intervals can be enormous, composed of hundreds of genes. Further interbreeding will increase recombination in every subsequent generation, which will narrow QTL support intervals and reduce the number of candidate genes to interrogate.

MetS, the most relevant QTL will be those associated with variation in multiple metabolic components.

For practical reasons the mouse is the animal model of choice for QTL mapping, but other studies have identified QTL associated with MetS components using pedigreed populations of baboons and pigs. The Southwest National Primate Research Center (SNPRC) at the Texas Biomedical Research Institute (San Antonio, TX) houses a large captive, pedigreed and genotyped baboon population (http://txbiomed.org/primate-research-center/). Baboons are used extensively in biomedical research and there are many physiological and genetic similarities between old-world monkeys and humans that make baboons an ideal model for MetS. SNPRC collects multiple metabolic data on their pedigreed colony (which is a mixture of yellow, *Papio hamadrayas cynocephalus*, and olive, *Papio hamadrayas anubis*, baboons) including measures of body composition (e.g., fat mass, fat-free mass, and waist circumference) and blood serum parameters. Approximately 10% of these animals become obese despite the population's uniform environment. Studies have found that increased adiposity in these baboons is significantly associated with dyslipidemia and insulin resistance, indicating this population is a good model for MetS (Comuzzie et al., 2003). Similar clustering of metabolic complications is also observed in studies of the rhesus macaque, another old-world primate frequently used in biomedical research (Bodkin et al., 1993). The SNPRC baboons have been used to quantify heritabilities of multiple metabolic traits (Cai et al., 2004; Cole et al., 2003; Comuzzie et al., 2003). Research integrating the rich phenotype and genotype data collected in this pedigreed population have identified QTL associated with metabolic traits, such as HDL cholesterol (Cox et al., 2007), LDL cholesterol (Kammerer et al., 2002), adipocyte volume (Bose et al., 2010), and cholecytokinin, a major satiety signaling protein (Voruganti et al., 2007).

Pigs have been studied for fat and meat production in agriculture for decades, and pigs are becoming more frequently used animal models in biomedical research. The Ossabaw minipig and the Pietrain domestic pig are particularly promising porcine models for MetS research because obesity, insulin resistance and hypertension cluster in these two breeds. Combining metabolic phenotypes with genotyped markers in pig mapping studies has identified QTL for lipid metabolism in a Duroc x Pietrain intercross (Uddin et al., 2011), for obesity in a Meishan x Large White intercross (Bidanel et al., 2001), and for serum glucose and lipid levels in a White Duroc x Erhualian intercross (Chen et al., 2009). The Animal QTLdb (http://www.animalgenome.org/QTLdb/app) has a searchable pig QTL database that stores published pig QTL results and phenotypic parameters for different breeds, including phenotypes that are biomedically relevant to MetS (fat and adipose traits are listed as "meat quality traits") (Hu et al., 2005).

QTL mapping is powerful in that it requires no a priori knowledge of genes that may be involved in the trait. This is important for multifactorial conditions, such as MetS, which have complex etiologies and likely result from interactions of many genes of individually small effects. QTL mapping can thus lead to discovery of novel genes and genetic variants associating with phenotypic variation that can subsequently become targets of focused candidate gene studies. The UCSC Genome Browser has multiple mammalian genomes available to query, including low-coverage assemblies, such as the pig (*Sus scrofa*). Genes located in a QTL interval submitted to the browser can be identified (http://genome.ucsc.edu/index.html) and interrogated. The human genomic position homologous to the QTL region identified in an animal model study can be evaluated using NCBI Homology tools (http://www.ncbi.nlm.nih.gov/guide/homology/). Once a QTL is identified in an animal model mapping study, genes within the support interval can be interrogated using public databases, such as the National Center For Biotechnology Information: Entrez Gene (http://www.ncbi.nlm.nih.gov/gene) and PubMed (http://www.ncbi.nlm.nih.gov/pubmed) to identify and rank order the most plausible candidate quantitative trait genes for further interrogation of function (using methods discussed in Sections 4.2 and 4.4).

4.6 GWAS in Model Organisms

GWAS in model organisms can deconstruct the genetic underpinnings of metabolic disorders with improved power of detection and resolution over QTL mapping. GWAS are now feasible in mice given the breadth of variants characterized across phenotypically diverse strains as discussed earlier in Section 4.1. New strains, such as those generated by the collaborative cross, as well as commercially available outbred strains, such as the diversity outbred mice, provide gene-level resolution for mapping complex traits (Churchill et al., 2004; Svenson et al., 2012). GWAS in mice have identified loci involved in MetS components including lipids and blood pressure (Bennett et al., 2010; Zhang et al., 2012). Incorporation of additional phenotypic information, for example, of metabolites, has allowed researchers to place GWAS associated genes into specific metabolic pathways and identify correlations among metabolic parameters. For example, researchers were able to establish that insulin resistance and plasma arginine levels are related in mice (Parks et al., 2015). Identifying such correlations could help establish biomarkers for the complex relationships among MetS components.

A proposed approach for using GWAS in dogs is to identify loci associated with obese and lean dogs within breeds prone to obesity, followed by crossbreed comparison of variants (Switonski and Mankowska, 2013). In swine, there is a spectrum of fatness and body size phenotypes bred for agriculture. Selective genotyping of animals at the far ends of the phenotypic spectrum allows a reduction of sample size and an increase in cost efficiency (Fowler et al., 2013). As dogs and pigs are both appreciated models for human disease, any advancement in our understanding of their genetic susceptibility to MetS is beneficial to medical research.

4.7 Identifying Epigenetic Signatures Associated with MetS Components

Imprinting is defined as the unequal expression of an allele depending on its parent-of-origin. Over 80 imprinted genes have been identified in humans, and ≈30% of these genes overlap with genes demonstrated to be imprinted in mice (Herrera et al., 2011). Bioinformatic studies predict that several hundred more genes are likely to be imprinted in both species (Luedi et al., 2005, 2007). Most direct observations of imprinting have been carried out through analysis of monoallelic expression in reciprocal matings of inbred mouse strains. Imprinted genes are known to be involved in metabolic functions (Rampersaud et al., 2008), and failures in imprinting can result in metabolic complications by altering expression of growth and cellular differentiation factors. Genomic imprinting failures have been associated with rare genetic syndromes having extreme forms of obesity (e.g., Prader–Willi syndrome) and mapping studies have identified imprinted genes (e.g., *GNAS*) affecting metabolic complications, such as obesity and insulin resistance in both humans and mice (Butler, 2009; Dong et al., 2005). QTL mapping studies in mice have identified loci having parent-of-origin effects on multiple MetS components, and some of these loci may be imprinted (Cheverud et al., 2011; Lawson et al., 2011a,b; Lawson et al., 2010). A striking result from these murine mapping studies is that parent-of-origin effects on metabolic traits is complex: in addition to the better characterized paternal and maternal patterns, polar and bi-polar dominance patterns were frequently observed. Polar dominance occurs when there are no additive genetic effects, so the two homozygote trait values are the same, yet there is a difference in the trait values between the two heterozygotes such that one class of heterozygote is in line with the homozygotes and the other is not, depending on parent of origin. In bi-polar dominance, again there is no significant difference between the two reciprocal homozygotes, but a pronounced difference occurs between the two reciprocal heterozygotes such that one class of heterozygote has the highest trait value of any other genotype class and the other class of heterozygote has the lowest trait value of any other genotype class as illustrated in Fig. 9.4 (for a detailed explanation of complex parent-of-origin patterns, including mathematical derivation, see Wolf et al., 2008). This complex pattern is rarely documented in human studies due to lack of sufficient genotypic information or statistical power, but

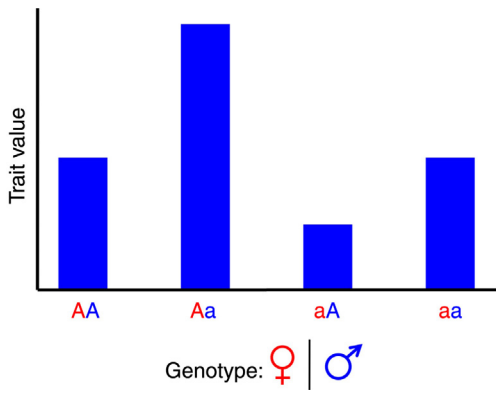

FIGURE 9.4 An example of a bi-polar dominance parent-of-origin effect In this example, trait value is the *y*-axis (trait value can be weight, cholesterol level, etc.) and the genotype classes are depicted on the *x*-axis. Parent-of-origin of alleles is known with *red* indicating a maternally derived allele and *blue* indicating a paternally derived allele. At this locus, there are no additive genetic effects (no difference in trait value between individuals belonging to the two homozygote classes), but there is a difference between the two reciprocal heterozygotes such that one class has the lowest trait value of any other genotypic class and the other has the highest trait value. Thus the same allele can be both protective and a potential risk factor depending upon parent-of-origin.

it does occur. For example, a study using the Icelandic genealogy database showed that the same genetic variant increases T2D risk when paternally inherited but decreases T2D risk when maternally inherited (Kong et al., 2009). This is consistent with a bipolar dominance parent-of-origin effect.

Epigenetic modifications, cell-specific changes in DNA chemistry that do not alter the DNA sequence itself, can affect gene expression and may underlie some of the parent-of-origin effects associated with MetS components found in mouse mapping studies. Commonly studied molecular epigenetic signatures include DNA methylation, histone modification, and small noncoding RNA interference. Tissue and T2D disease-stage specific altered methylation patterns were found in the Zucker diabetic fatty rat model relative to controls (Williams and Schalinske, 2012). A genome-wide methylation analysis in pig adipose and muscle tissues identified methylation patterns that segregated by breed (the researchers compared methylomes among three breeds: Landrace, Rongchang, and Tibetian), by sex, by tissue, and by fat depot (Li et al., 2012a). This work also identified regions that were "differentially methylated," meaning one allele is methylated while the other allele is not. This pattern is suggestive of parent-of-origin methylation, which may affect expression of genes in a parent-of-origin manner, and is a signature of imprinting (Harris et al., 2010). An exciting direction for future research is to combine results identified in animal QTL mapping studies with whole-genome epigenetic profiling.

4.8 Mouse ENCODE

The Mouse ENCODE Consortium was developed as a counterpart to the human Encyclopedia of DNA Elements (ENCODE). Using similar technologies and pipelines, Mouse ENCODE maps functional elements of the mouse genome, such as transcription, transcription factor binding, chromatin modifications, and replication domains. These data were mapped in a wide range of mouse tissues and cell types, mostly generated from C57BL/6J and are available to the research community at www.mouseencode.org.

The public availability of these data sets has significantly impacted comparative studies of mouse and human genomes. Studies have revealed conservation of some basic regulatory systems between humans and mice, as well as high levels of divergence in gene expression and regulation (Yue et al., 2014). Washington University in St. Louis houses an epigenome browser with which researchers can explore these existing datasets, or upload their own data to visualize a candidate region's genomic context (http://vizhub.wustl.edu/). Identification of patterns of epigenetic effects in animal models can be translated to human studies and will provide a framework for clarifying the relationship between DNA sequence variation and metabolic complications. This is a step toward development of better therapeutic strategies for MetS.

5 ENVIRONMENTAL FACTORS

5.1 Fetal Programming

Maternal developmental environment has been shown to affect not only fetal and early postnatal growth, but also adult susceptibility to MetS (Barker, 2007). Compromised nutrition during fetal and early postnatal life increases susceptibility to metabolic complications later in life because of "fetal programming" during critical periods of development and a mismatch between early and adult nutritional environments. The precise mechanism(s) through which this phenomenon occurs is unknown, but recent work suggests that epigenetic modifications of DNA affect many molecular processes related to intrauterine growth and development (Aagaard-Tillery et al., 2008; Heerwagen et al., 2010). Research in guinea pigs found that mothers fed moderately restricted diets throughout their pregnancies (70% of ad libitum intake/kg body wt.) gave birth to small pups and their adult male offspring were hyperinsulinemic (Kind et al., 2003). A similar study in guinea pigs found that mildly restricting maternal diet (85% of ad libitum) increased both total cholesterol and LDL cholesterol levels in male offspring (Kind et al., 1999). Maternal undernutrition is associated with increased arterial blood pressure in offspring that persists into adulthood in both sheep and rat models (McMillen and Robinson, 2005). Offspring of rat mothers fed restricted protein diets (50% less protein than standard chow-fed controls) had poor pancreatic beta-cell proliferative capacity at birth. When the low protein diet was maintained, weanlings, pancreatic islet morphology, and insulin content was found to be reduced (Bertram and Hanson, 2001). Recent rodent models have shown that maternal high fat diet, or overnutrition, also increases offspring susceptibility to developing adult metabolic complications (Liang et al., 2009; Morris and Chen, 2009; Odaka et al., 2010). When mothers consume a high fat diet their offspring tend toward obesity as adults, regardless of offspring diets. However, other research suggests that it is maternal adiposity and not dietary fat consumed that affects offspring susceptibility to adult metabolic complications (White et al., 2009). In sheep, maternal obesity and overnutrition results in metabolic complications in adult offspring (Long et al., 2011). Further, ovine models suggest that overnutrition affects adult offspring risk in two stages: first during the periconceptional period and second in late pregnancy (George et al., 2010). Studies

in nonhuman primates support findings from rodents and sheep. In baboons, preweaning overnutrition resulted in permanently increased adiposity in females (Lewis et al., 1986). In macaques, maternal high fat diet predisposed adult offspring to adult nonalcoholic fatty liver disease, which is highly correlated with MetS (McCurdy et al., 2009). Additionally in macaques, maternal overnutrition before and during pregnancy resulted in increased postnatal adiposity in offspring (Grayson et al., 2010). Thus the pattern of susceptibility to adult metabolic disease resulting from fetal programming is U-shaped, with both maternal undernutrition and overnutrition affecting offspring MetS risk (Taylor and Poston, 2007). Work in the rhesus macaque also showed that offspring of high fat fed mothers had increased proopiomelanocortin mRNA expression and decreased agouti-related protein mRNA expression, suggesting that maternal overnutrition during pregnancy may activate proinflammatory cytokines that could alter melanocortin activity (Grayson et al., 2010). Evidence in rodent models suggest molecular epigenetic mechanisms, such as those discussed in Section 4.7, underlie at least some of these effects (Lillycrop et al., 2005; Vickers et al., 2005). Identifying variation in epigenetic marks that associate with variation in maternal effects on fetal programming is a fruitful avenue for further research.

Maternal effects on fetal programming are generally considered environmental because they are not a function of the offspring's genome. Rather they result from the maternally produced developmental environment. Nearly all studies in mouse model systems have focused exclusively on maternal environmental effects using genetically uniform C57BL/6J mothers and offspring. But maternal genetic variations are also responsible for substantial variability in the fetal and neonatal developmental environment (although the effect is environmental with respect to the offspring). Murine maternal effect QTL associated with early offspring growth in a cross-fostered F_3 generation of a LG/J x SM/J intercross showed that variation in maternal genotype accounted for >30% of among litter offspring variation (Wolf et al., 2002). Another mouse study measured maternal genetic effects on multiple MetS components and found that variation in maternal genotype accounted for up to 10% of adult offspring phenotypic variation (Jarvis et al., 2005).

5.2 Nutrition

Animal models are ideal for studying the effects of nutrition on MetS etiology because commercial diets are readily available, allowing dietary composition to be precisely monitored. Further, specialized high fat and high sucrose diets have been developed that mimic the human "Western" diet hypothesized to underlie the past 30 years' rising rates of metabolic disease. In mouse, strains have been characterized by whether they become obese on a Western diet [referred to as diet-induced obesity (DIO) strains] or not (referred to as dietary-resistant strains). Variation in high fat dietary response has been quantified in 43 inbred strains for 10 phenotypic traits, including metabolic parameters in blood serum levels and adiposity (Svenson et al., 2007). Another study quantified variation in macronutrient diet self-selection across different inbred mouse strains (Smith et al., 2000). This study is interesting in that it shows that individual preference for certain macronutrients vary according to genetic background. Consistent with rodent results, dietary studies in nonhuman primates show that some species become obese and/or develop other metabolic complications while other species do not. For example, squirrel monkeys fed high fat, high sucrose diets became obese, while cebus monkeys fed the same diet did not (Ausman et al., 1981). This supports results from rodent models indicating that genetic background is an important factor of dietary risk. A recent study in rhesus macaques provided with a 500 mL/day 15% fructose-sweetened beverage showed that animals developed multiple MetS components including central obesity, dyslipidemia, inflammation, and insulin resistance. A subset of these animals developed overt T2D (Bremer et al., 2011), indicating that rhesus macaque is a good model for studying variation in dietary response and MetS. Further, the rhesus macaque genome has been sequenced at relatively high coverage, providing an opportunity to study gene by dietary–environment interactions in a nonhuman primate model of MetS (Gibbs et al., 2007). An exciting avenue of research uses animal models to study dietary influences on gut microbiota and the metabolic consequences of upsetting gut microbial diversity. Studies in rodents indicate that dietary environment can lead to bacterial disruptions that influence energy extraction from food, fat storage, serum lipid levels, and insulin resistance. This suggests that the gut microbiome is an important link between dietary environment and MetS (for a review, see Tilg and Kaser, 2011 and references within). However, much of this research has been conducted in rodent knockout or germ-free models, so the translational application to human subjects remains to be explored.

5.3 Gene by Dietary Environment Interactions

As discussed previously, standing genetic variation can modify the effects of the environment on expression of phenotype. Characterizing gene by environment, particularly gene by dietary environment, interactions are essential to understand MetS etiology and for identifying potential risk factors in the context of personalized medicine. These interactions are challenging to parse

from human studies where environmental factors can be difficult to accurately measure or, in the case of GWAS, can be computationally prohibitive to analyze. However, some studies have successfully characterized gene by diet interactions in human populations and there is an effort to archive these results as they pertain to metabolic traits (Lee et al., 2011). Animal models can dissect gene by environment interactions and the most frequently studied model is the mouse, using the targeted or discovery methods discussed earlier in Sections 4.2 and 4.4, but with an added dietary parameter in the model (Lawson and Cheverud, 2010). As discussed earlier, genetic background (strain or species) needs to be taken into account when designing such studies.

For example, a targeted knockout approach demonstrated that high fat fed *Ppar*$\alpha^{-/-}$ mice accumulated significantly higher lipid concentrations in their livers relative to wild-type controls. Interestingly, *Ppar*$\alpha^{-/-}$ mice that were fasted for 24 h developed additional metabolic complications including hypoketonemia, hypoglycemia, impaired thermoregulation, and increased serum free fatty acid (FFA) levels. This result indicates that *Ppar*α plays a role in regulating the fatty acid oxidation response to fasting (Kersten et al., 1999; Leone et al., 1999). QTL have been identified in crosses between inbred mouse strains fed with high fat diets (e.g., Taylor et al., 2001; West et al., 1995; York et al., 1996). However, generally these studies do not map variation in metabolic traits in high fat versus low fat diets, which is necessary to characterize the gene by diet interaction. Recently, gene by diet interactions (high fat vs. low fat isocaloric diets) were characterized for multiple MetS components in studies of an F_{16} generation of an Advanced Intercross between the LG/J and SM/J inbred mouse strains (Cheverud et al., 2011; Lawson et al., 2010, 2011a,b). A major finding from these studies was that interactions are not consistent between the sexes or among the traits studied: for adiposity and serum lipid levels, high fat fed females were the most affected cohort and for diabetes-related traits, high fat fed males were the most affected cohort. These studies also demonstrated that if context was not taken into account in the mapping model (context referring to the sex and/or dietary environment), genetic associations are missed. For example, if a genetic effect is found only in high fat fed females, the lack of genetic effects in the other sex by diet cohorts will wash the association out if the entire population is pooled together in the analysis. Additionally, if the additive genetic effects between two cohorts in a population are of opposite directions (the homozygote genotypes change ranks in different environments), the association will be washed out if the two cohorts are pooled (Fig. 9.5). This result has important implications for human studies.

Gene-by-dietary-environment interactions that are identified in rodent models have potential to inform human studies and potentially increase their power to identify patterns of interactions in populations of human subjects (using methods described in Sections 4.2 and 4.4). The idea is to identify biomarkers with dietary effects on metabolic parameters that may be context-dependent and that are relevant to the clinical reality of MetS in a nutritional genomics context. The promise of nutritional genomics is that it can provide personalized dietary recommendations based on an individual's genetic make-up at loci found to interact with diet. Personalized dietary recommendations aimed at prevention and therapy represent a practical clinical translation of animal model research to public health, particularly for MetS components, such as dyslipidemia or insulin resistance that could be delayed or even prevented through dietary modifications (Bouchard and Ordovas, 2012).

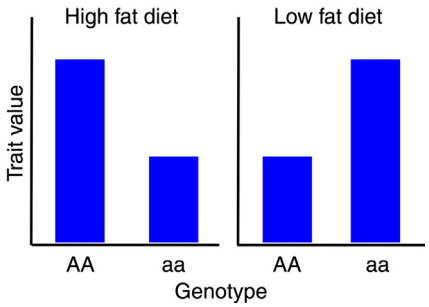

FIGURE 9.5 An example of a gene-by-diet interaction with homozygote genotypes changing rank order in different dietary environments. This figure illustrates that genetic associations can be missed if environment is not taken into account. In this example, trait value is the *y*-axis and homozygote genotypes are depicted on the *x*-axis. On a high fat diet, individuals with the AA genotype at the marker locus have a higher trait value than individuals with the aa genotype. The opposite is true for individuals on a low fat diet. If individuals of both diets are pooled together in an analysis, the opposite genetic effects of the two cohorts will wash each other out, despite a clear genetic association with the trait and gene by diet interaction. This result has been found in mouse models of MetS (Lawson et al., 2011a) and has implications for human studies.

6 ANIMAL MODELS OF MetS PATHOPHYSIOLOGY

Understanding MetS pathophysiology in animal models can shed light on why some obese individuals develop metabolic complications while others do not. Further, animal models may also shed light on why some lean individuals develop multiple MetS components in the absence of obesity. Most animal models focused on pathophysiology attempt to understand the link between obesity and insulin resistance, which is an important risk factor for developing T2D and CVD. Physiological studies generally focus on the roles of adipose tissue, endocrine function, and fatty acid metabolism in metabolic complications, although each of these

factors is interrelated. Recently, the role of autophagy in obesity has been garnering attention. This is important because some fat depots (visceral adipose tissue) are correlated with increased risk of developing insulin resistance whereas others (subcutaneous adipose tissue) are actually correlated with decreased risk (reviewed in: Hardy et al., 2012), yet the mechanisms underlying these correlations are unknown. Animal models that test mechanistic hypotheses are the first step toward gaining new biological insight into MetS. Physiological studies inform genetic studies (and are also informed by genetic studies), and candidate gene studies can be designed based on physiological results with the idea that identifying molecular mechanisms underlying metabolic complications can lead to the discovery of novel biomarkers and the development of better therapeutic strategies for MetS.

6.1 Adipose Tissue: Hormones, Remodeling and Inflammation

The role of leptin in MetS is discussed earlier in Section 4.1. Adiponectin is another adipose tissue derived protein hormone that was discovered shortly after leptin was characterized in a mouse model (Havel and Bremer, 2010). Adiponectin has been shown to be a key factor linking visceral adipose tissue to MetS. It is the most abundant protein secreted by adipocytes, and high circulating levels of adiponectin is correlated with increased insulin sensitivity and resistance to metabolic complications (Scherer, 2006). Low circulating adiponectin levels is correlated with insulin resistance in rhesus monkeys (Hotta et al., 2001) and in rodent models (Cummings et al., 2008; Nawrocki et al., 2006). A recent study used RNA-sequencing to profile genes expressed in liver in both adiponectin knockout and wild-type mice (Liu et al., 2012). This study found differential expression in genes involved in several glucose and lipid pathways that are fruitful targets for focused candidate gene studies aimed at identifying functional pathways regulated by adiponectin.

Adipose tissue remodeling and inflammation is likely involved in the pathogenesis of MetS. The accumulation of large adipocytes is associated with increasing rates of adipocyte death, inflammation, and insulin resistance. Macrophages localize to the dead adipocytes and form crown-like structures that envelope and ingest the adipocyte and its lipid droplet (Cinti et al., 2005). This clearance of dead adipocytes by macrophages is an initial remodeling event that promotes proinflammatory activation of macrophages, and results in a bimodal distribution of both large and small adipocytes. DIO mice show marked increases in adipocyte size, death, and macrophage content (Strissel et al., 2007). This is positively correlated with insulin resistance, dylipidemia, and non-alcoholic fatty liver disease (Ferrante, 2007). Mice with loss of function mutations in monocyte chemoattractant protein-1 (*Mcp1*) and its receptor (*Ccr2*) have decreased adipose macrophage content and appear to be protected from high fat diet induced insulin resistance (Kanda et al., 2006; Weisberg et al., 2006). Further, mice overexpressing *Mcp1* have increased levels of adipose tissue macrophages and increased insulin resistance. At baseline, mouse adipose tissue macrophages show low expression of macrophage differentiation markers. Overnutrition results in a macrophage population "shift," with increasing levels of monocytes being recruited from circulation into the adipose tissue (Lumeng et al., 2007), indicating that DIO results in adipose tissue remodeling and remodeling-associated inflammatory response. Expression studies in rodent models are illuminating some of the genes involved in the intercellular communication among adipose tissue cell types, including TNF, SFRP5, and WNT5a (among others). A promising avenue of research using animal models is aimed at inhibiting the infiltration of monocytes into adipose tissue and potentially ameliorating the inflammatory response and preventing insulin resistance (Ouchi et al., 2011; Suganami and Ogawa, 2010 for a review of macrophages in adipose tissue remodeling and inflammation). It is worth noting that adiponectin is considered an antiinflammatory adipokine, and circulating levels decrease as inflammation increases. Recent pharmaceutical based research in mice has found that inhibiting *Mcp1* expression both reduces inflammation and increases adiponectin levels in adipose tissue (Vinolo et al., 2012).

6.2 Free Fatty Acid Metabolism

Altered FFA metabolism is an important factor in the pathogenesis of insulin resistant glucose metabolism, dyslipidemia, and possibly inflammation associated with obesity in MetS (Boden, 2006; Shulman, 2000). Excess plasma FFA concentration impairs the ability of insulin to stimulate muscle glucose uptake and suppresses hepatic glucose production (Ferrannini et al., 1983). Increased FFA delivery to the liver increases hepatic VLDL-triglyceride production and plasma triglyceride concentrations (Lewis et al., 1995). This increases the transfer of triglycerides from VLDL to HDL, which leads to increased HDL clearance and results in decreased plasma HDL concentrations (Hopkins and Barter, 1986). Visceral adiposity is associated with lipid accumulation in the liver and insulin resistance. It has been hypothesized that FFAs released during lipolysis of visceral adipose tissue causes insulin resistance because these fatty acids enter the portal vein and are directly delivered to the liver, leading to hepatic insulin resistance, triglyceride accumulation and increased secretion of atherogenic lipoproteins (Despres and Lemieux, 2006).

This so-called "Portal Theory" of MetS has been tested in canine models at both molecular and physiological levels. Dog intraabdominal fat depots are easily measured using magnetic resonance imaging, and invasive procedures, such as portal vein cannulation and omentectomy (surgical removal of the greater omentum and its constituent visceral adipose tissue) are easier to perform in larger mammals than in rodents, although recently a rat model has been developed with a chronically implanted portal vein catheter to quantify pulsatile insulin secretion (Matveyenko et al., 2008). As is observed in human populations, dogs naturally exhibit wide variance in fat deposition and increased levels of visceral adiposity is highly correlated with insulin resistance in dogs (Bergman et al., 2006, 2007; Kim et al., 2003). Differential expression of several important genes involved in FFA metabolism (LPL, HSL, and PPARγ) in both visceral fat and liver tissue was observed between high fat fed dogs and controls. Liver insulin receptor binding was decreased by 50% in the high fat fed dogs, providing circumstantial molecular evidence supporting the portal theory (Kabir et al., 2005). Omentectomy in a dog model has been demonstrated to improve insulin sensitivity, supporting the association between visceral fat and insulin resistance, however, the FFA-portal vein link between visceral fat and insulin resistance was not supported in this model (Lottati et al., 2009). Further, isotope tracer data from human subjects suggests that only 20% of FFAs delivered to the liver derive from visceral fat, indicating that it is unlikely that the delivery of fatty acids via the portal vein underlies the strong correlation between visceral fat and insulin resistance (Nielsen et al., 2004). Thus more research is required to test the FFAs hypothesis in the context of visceral fat and insulin resistance, and animal models will be integral to understanding this connection in the context of MetS pathophysiology.

6.3 Gastrointestinal Hormones

Hormones produced by the gastrointestinal tract are essential to glucose and lipid metabolism. These hormones are involved in short-term regulation of satiety and potentially in long-term regulation of adiposity and energy homeostasis (for a comprehensive review of endocrine regulation of energy balance, see Havel and Bremer, 2010; Suzuki et al., 2011). Animal models have been critical to understanding the roles of hormone action in MetS, and to characterizing receptor, and signaling pathways that can be manipulated for therapeutic intervention. Ghrelin is a peptide hormone produced by, and secreted from, endocrine cells of the stomach and proximal small intestine. It increases hunger and potentially stimulates food intake, although high circulating ghrelin concentrations are negatively correlated with body weight (Tschop et al., 2001). Nevertheless, direct administration of ghrelin has been found to increase food intake resulting in weight gain in a rat model (Tschop et al., 2000). Glucose-dependent insulinotropic polypeptide (GIP) is a hormone secreted by K-cells in the duodenum and proximal jejunum in response to carbohydrate ingestion. GIP receptors are located in pancreatic islets, brain, adrenal, and adipose tissues. GIP augments glucose-stimulated insulin secretion (D'Alessio et al., 2001). Ablating GIP producing K-cells in a mouse model appears to protect animals from dietary obesity, and administering a GIP receptor antagonist in high fat fed mice has been reported to reverse obesity and insulin resistance (Althage et al., 2008; McClean et al., 2007). Glucagon-like peptide-1 (GLP-1) is produced by L-cells in the ileum. Administering GLP-1 receptor antagonist has been shown to improve glucose tolerance in a baboon model (D'Alessio et al., 1996). GLP-1 is also produced in neurons in the hypothalamus. Administering GLP-1 directly into the brains of rats has been shown to inhibit feeding and result in weight loss (Tang-Christensen et al., 1996). Oxyntomodulin (OXM) is cosecreted with GLP-1 from L-cells in the ileum. Administration of OXM inhibits food intake in rats, likely by increasing energy expenditure (Dakin et al., 2002). Peptide-YY (PYY) is produced by L-cells in the ileum and colon. PYY concentrations rise after feeding, peak after 1–2 h, and remain elevated for several hours longer. Higher levels of PYY are secreted after fat ingestion than after either carbohydrate or protein ingestion (Suzuki et al., 2011). It has been observed in a mouse model that PYY administration inhibits food intake, promoting weight loss and fat oxidation (Adams et al., 2006), although this result has not been observed in other mouse studies (Tschop et al., 2004). These gastrointestinal hormones all converge on major signaling pathways in the brain, namely the arcuate nucleus of the hypothalamus and the dorsal motor nucleus of the vagus nerve in the brainstem. Each represents promising therapeutic targets for appetite regulation in the context of MetS. The fundamental knowledge derived from animal models of endocrine regulation can set the stage for planning appropriate and safe therapeutic manipulations of these signaling pathways in human subjects.

6.4 Autophagy

Autophagy is an intracellular process wherein cell cytoplasm constituents and structures are isolated by a phagophore, segregated from the cytoplasm into an autophagosome, which then fuses with a lysosome to form an autolysosome whose contents are degraded and recycled for use in the cell. Autophagy is a response to stress but it also plays a role in normal cell remodeling (Mizushima and Klionsky, 2007). Autophagy is inhibited by mTOR activation in the presence of insulin-like and

other growth factors (Jung et al., 2010). It is induced by a reduction in mTOR signaling, activating a protein serine/threonine kinase complex. Autophagy has recently been associated with metabolic function, including regulation of lipid metabolism (Singh et al., 2009) and obesity (Goldman et al., 2010; Zhang et al., 2009). Inhibiting autophagy leads to an increase in circulating triglyceride levels and a decrease in triglyceride breakdown, suggesting that accumulating lipids decreases autophagy, potentially leading to a deleterious positive feedback loop in the cell's physiology (Singh et al., 2009). Key genes involved in this process include: ATG1, ATG7, BCN1, LC3, and class III P13K among others. Recently, an adipose tissue specific *Atg7* knockout mouse model found that adipose-specific $Atg7^{-/-}$ animals weigh less and have reduced reproductive fat depots relative to wild-type controls (Zhang et al., 2009). The knockouts consumed food at the same rate as wild-type mice regardless of diet, yet they failed to develop obesity when on a high fat diet. Additionally, they were more active than wild-type controls. $Atg7^{-/-}$ animals exhibited lower serum triglyceride, cholesterol, FFA, and leptin levels, but normal adiponectin and glucose levels with decreased serum insulin levels and increased insulin sensitivity. At the cellular level, adipogenesis was less efficient in $Atg7^{-/-}$ primary mouse embryonic fibroblasts than in wild-type controls. Histological analysis showed that $Atg7^{-/-}$ adipocytes were smaller and often contained multiple small lipid droplets rather than the single large droplet found in wild-type cells. A recent study in an Ossabaw pig model found that inhibited myocardial autophagy was associated with development of MetS (Li et al., 2012b). Better understanding the pathophysiology and the molecular underpinnings of autophagy in metabolic traits in animal models could facilitate the exploration of this cellular process in human obese subjects with the idea that exploitation of this pathway has therapeutic potential for MetS.

7 CONCLUSIONS

To prescribe the right therapies for MetS, one needs to be able to predict phenotype from genotype to some degree. Animal models have advanced our understanding of the genotype-metabolic function relationship through testing hypotheses about genetic underpinnings and molecular pathophysiologies that lead to the clustering of metabolic complications putting an individual at risk of developing T2D and/or CVD. Bioinformatic tools and archived genomic and phenotypic data for different animal model systems make it possible to design and execute focused studies using the species or strains that most appropriately mimic the clinical reality of MetS. Exciting advances in genomics make it possible to explore both genetic and epigenetic modes of gene regulation and to understand how genes modify environmental effects on phenotypic variation. Characterization of molecular physiological pathways in animal models—especially direct characterizations in disease-relevant tissues—can illuminate potential therapeutic targets. Patterns identified in animal models can be translated to studies of human subjects for the improvement of human health.

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