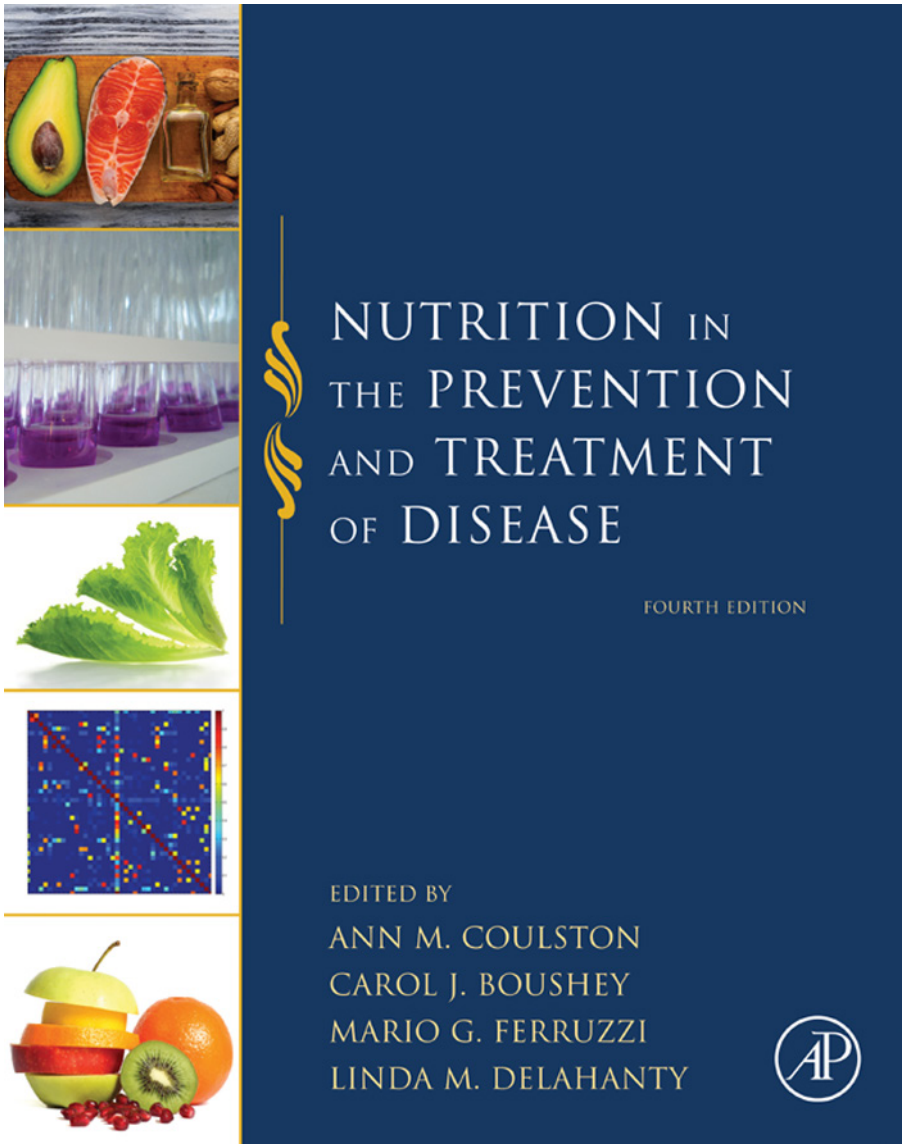

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Chapter 21

Genetics of Nonsyndromic Human Obesity, With Suggestions for New Studies From Work in Mouse Models

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I INTRODUCTION

Complex and incompletely defined interactions between environment and genetics determine each individual's height and weight, as well as other human quantitative traits. The result is a population in which individuals vary widely for height and weight, but no one factor can be identified as controlling either trait in most people. In humans, long-term adult weight is relatively stable, as evidenced by the difficulty of sustaining intentional weight loss and the almost automatic return to previous weight following brief periods of overeating. This drive to constancy of body weight is due to both behavioral and physiological alterations that accompany weight change. Convincing evidence of the biological basis of the regulation of body fat stores comes from the identification of dozens of rare single-gene Mendelian mutations and syndromes that result in spontaneous massive obesity or in adipose tissue atrophy.

Most human obesity, however, is not due to mutations in single genes that have overwhelming effects, but is inherited as a complex, multigenic, quantitative trait influenced by many genetic and environmental variables. There are likely to be interactions among genes and between genes and environmental factors such that some alleles of one gene will not cause obesity unless specific alleles of another gene or environmental pressures are also present. Dietary effects on parents and parental genetics, independent of progeny genotype, also exert powerful but indirect effects on obesity. Genetic heterogeneity, where similar phenotypes are caused by more than one gene, and incomplete penetrance of the trait, where not all people with the gene develop the phenotype, also make dissection of complex phenotypes difficult. Expression of an obesity gene may also be age- or gender dependent. Thus, identification of all the genes promoting human obesity has not been, and will never be, a trivial task.

This chapter is not an exhaustive compendium of all things of genetics and obesity. This chapter does not include discussion of congenital lipodystrophies [1] nor rare genetic syndromes that include obesity in the phenotype, such as Bardet–Biedl and Prader–Willi, as these do not seem to contribute significantly to common causes of obesity and are reviewed elsewhere [2,3]. The present chapter is focused on human genetics, thus we do not plan to discuss the many effects of gut microbiota on metabolism and obesity [4]. The present chapter will discuss both monogenic and multigenic obesity, some of the techniques used to discover them, and general principles derived from these studies. We will list and discuss the most important known obesity genes, but we will not attempt to provide an exhaustive catalog of obesity genes (see [5,6]). Genetics is a rapidly progressing field, and knowledge of the genetic basis for obesity is expanding exponentially. Therefore, the reader should use this chapter to understand the most common genes and mechanisms, general ideas for finding more human obesity genes based on what has already been demonstrated in mice, and an appreciation of the wide variety of mechanisms by which genetics influences obesity.

II THE BIG PICTURE—HOW MUCH OBESITY IS DUE TO GENETICS

Genetic epidemiology of human obesity is the study of the relationships of the various factors determining the

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frequency and distribution of obesity in the population. Such studies of obesity are limited in that they do not examine genetic variations and rarely directly measure the amount or location of body fat. However, genetic epidemiology studies do provide information as to whether there is a genetic basis for the trait, whether a major gene is involved in the population, whether inheritance is maternal or paternal, the relative importance of genes and shared or nonshared environment, and whether expression of the trait is gender or age dependent. Genetic epidemiology studies of human obesity employ a variety of designs and statistical methods, each giving somewhat different estimates for heritability of obesity. For a discussion of genetic epidemiology methods employed in the study of obesity, see [7].

The heritability estimates for human obesity are derived from a large number of studies of adoptees, twins, families, or communities. Population or family studies tend to have lower, and twin studies to have higher, heritability for body mass index (BMI). Heritability of BMI has been estimated from adoption studies to be as low as 10% and from twin studies to be as high as 85% [7,8] (Fig. 21.1). In a pediatric twin study, genetic influences contributed 75–80% in the percent of body fat [11]. The heritability for BMI in a study of childhood obesity in a Hispanic population was 40% and, in that study, heritability of diet and physical activity phenotypes ranged from 32% to 69% [12]. By using data from all types of studies, it is estimated that 40–70% of the within population variation in obesity is due to genetic variation [10] (Fig. 21.1). Most studies indicate that familial environment has only a minor impact on obesity.

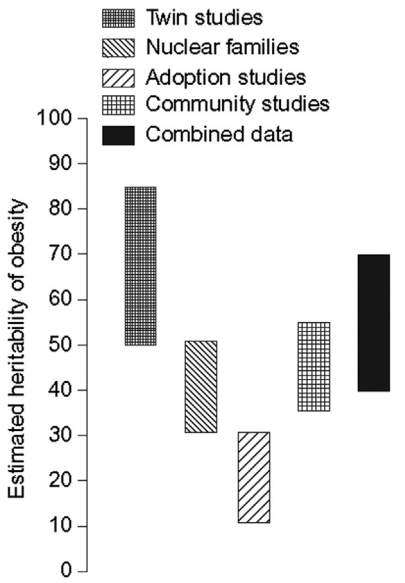

FIGURE 21.1 Heritability of obesity as determined by different study types. *Data for studies of twins, nuclear families, and adoption studies are taken from C. Bouchard, L. Perusse, T. Rice, D.C. Rao, The genetics of human obesity, in: G.A. Bray, C. Bouchard, W.P.T. James (Eds.), Handbook of Obesity, Marcel Dekker, New York, NY, 1998. Data for community-based studies are taken from A. Herbert, N.P. Gerry, M.B. Mcqueen, I.M. Heid, A. Pfeufer, T. Illig, et al., A common genetic variant is associated with adult and childhood obesity. Science 312 (2006) 279–283. Range of heritability estimated from all study types is taken from A.G. Comuzzie, D.B. Allison, The search for human obesity genes. Science 280 (1998) 1374–1377.*

III WHY FINDING OBESITY GENES MATTERS

During 2011–14, in the United States the prevalence of obesity in adults aged 20 years and over was 36% and in youths was 17% [13]. According to the World Health Organization in 2014 of the world population more than 1.9 billion adults were overweight (39%) and of these over 600 million were obese (13%). A total of 42 million children under the age of 5 years were overweight or obese in 2013 (http://www.who.int/mediacentre/factsheets). Obesity rates worldwide are predicted to rise to between 42% and 51% of the adult population by 2030 [14]. If obesity rates were to remain at 2010 levels the savings in medical expenditures over the next two decades could approach $550 billion.

Obesity is not just a financial burden. Sometimes the problems caused by obesity are social, such as discrimination, sometimes obesity influences quality of life by, for example, limiting physical activity, and sometimes obesity is associated with diseases that shorten lifespan, such as heart disease, type 2 diabetes, hypertension, and cancer. Additional obesity comorbidities include arthritis, limitations on mobility, sleep apnea, gallstones, and kidney disease. Until recently there was no way to determine if obesity caused these comorbidities or if they were simply correlated with obesity. Mendelian randomization, a study design that incorporates genetic information into traditional epidemiological methods, now provides a method to determine if genes simultaneously cause both obesity and comorbidity [15]. Causal relations then mean that treatment for obesity becomes even more urgent and that treatment can target specific genetic pathways that cause both obesity and comorbidity.

Studies of people who have lost weight by diet or bariatric surgery prove reduced mortality and improved quality of life. A person's genes influence weight gain, weight loss, and health consequences of obesity. Finding obesity genes may provide tools to improve health of people worldwide.

IV THE SEARCH FOR OBESITY GENES

A Lessons for Human Obesity From Genetic Studies in Mice

Although many decades ago genetic epidemiology studies provided evidence that obesity is highly genetic, there

was no understanding of the molecular basis for obesity until the identification of genes that cause Mendelian forms of obesity in mice. Five genes were known for many decades to cause monogenic obesity syndromes in mice. Positional cloning of the mouse obesity genes, *Lep*^{ob}, *Lepr*^{db}, *Tub*, *Cpe*^{fat}, and *A*^{y}, from naturally occurring mutant models between 1992 and 1996 led to an explosion of knowledge of the genetic causes of obesity [16]. When the third edition of this chapter [17] was published, human orthologs of three mouse obesity genes were known to cause obesity in humans and a fourth mouse obesity gene identified a pathway that caused human obesity. Subsequent studies have now demonstrated that human versions of all five mouse Mendelian obesity genes act in the brain to either directly cause obesity or identify a pathway that causes obesity. These mouse monogenic obesity genes in most instances are recessive and their human orthologs are expected to rarely cause obesity in the human population.

Mouse models of obesity provide information that often replicates causes of human obesity. Several hundred different knockout and transgenic mice have been developed where absence or replacement of a single-gene affects obesity or its phenotypes (for a listing of knockout and transgenic mouse models of obesity and related phenotypes see [6]). These are all possible human obesity genes. These and other genetic studies in mice also show that separate genes control (a) body weight, (b) BMI, (c) sizes of individual fat depots, and (d) responses of individual fat depots to dieting and exercise. Feeding different diets to mice revealed that some mice resist weight gain on diets that make other strains obese, indicating gene−diet interactions. These diet responsive genes are mostly separate from genes for spontaneous obesity on healthy chow diets. Human genetic studies have extensively investigated BMI, have produced smaller studies of overall fatness, but have produced virtually no data on genetics of individual fat depots, and despite many underpowered efforts, almost no significant results on gene−diet interactions.

Mice are valuable in the study of parental effects. Parents may exert indirect effects where female genotype influences progeny phenotype independent of progeny genotype [18,19] possibly by influencing milk composition or quantity, or quality of maternal care. Diets fed to male or female parents may influence weight and health of progeny through epigenetic effects that are heritable changes not due to changes in the underlying DNA sequence. Although similar maternal diet effects are well known in people, the paternal effects in mice have been a surprise as they occur through sperm. Recent evidence from mice suggests that RNA found in sperm can cause obesity and metabolic disorders in progeny of males fed high-fat diets [20]. No comparable studies exist for humans.

Although studies directly in humans have now successfully identified almost 200 obesity genes, taken altogether these account for a limited fraction of heritability and for only a few traits, such as BMI and overall fat distribution. The studies in mice and rats strongly suggest that work to find genes with similar effects on fat depots, diet, and parental effects in humans will identify entire new classes of human obesity genes. Doing so will likely increase the total heritability of obesity that can be explained in humans. Human geneticists have either not explored at all, or only begun to explore, these fundamental aspects of obesity that have been reproducibly demonstrated in mice and rats.

B Identification of Human Obesity Genes by Sequencing

Several approaches are currently used to find new human obesity genes, the most direct of which is to sequence DNA. One can sequence all DNA (whole genome sequencing), or only parts, such as the exome, the protein coding portion of the gene [21]. One recent paper used whole genome sequencing of Sardinians to identify genes influencing height, inflammatory markers, and lipids [22]. Although whole exome sequencing in obesity has been reported [23], no whole genome sequencing studies for obesity have yet been published.

Whether sequencing whole genome or whole exome, most investigators look for genes with mutations that obviously alter function such as stop codons, insertions, or deletions. One of the primary limitations of this approach is that missense mutations that substitute one amino acid for another in genes not previously known to cause obesity tend to be ignored, despite the fact that missense mutations can alter protein function. The practical problem is that each person has many thousands of missense variants and investigators cannot directly test functional effects of all to determine which of these are causal for obesity. Much effort is being devoted to methods to predict which missense mutations in protein coding regions will have functional effects on proteins, but at present there is no substitute for direct studies showing that a missense mutation alters protein function. And since many alleles that cause obesity are not in protein coding regions, ability to predict functional effects of these alleles ranges from nonexistent for alleles far from any gene to sometimes useful predictions for alleles in obvious gene promoter regions. Once again, there remains no convincing substitute for determining direct functional effects.

One exception is that some missense mutations in known obesity genes can be labeled as putative obesity causing. The most common results from sequencing are identification of novel mutations in known obesity genes,

for example, *LEP* [24,25], *LEPR* [24], and *MC4R* [25]. In ideal cases, investigators can show that missense mutations present in obese people will alter the function of a protein. For instance, missense mutations in *MC4R* may alter binding of α-melanocyte-stimulating hormone (α-MSH), localization on the cell surface, or production of cGMP on binding of α-MSH. Currently, mutations have been identified by sequencing in human orthologs of the known obesity genes *LEP* [24,25], *LEPR* [24], *MC4R* [25], *CPE* [26], *TUB* [27], and *PCSK1* [28]. New papers reporting discovery of mutations in these genes occur regularly, so we will not attempt to provide a comprehensive list.

Selected sequencing papers are presented in Table 21.1. Saeed and colleagues found that 30% of severe obesity in children in consanguineous or inbred families was due to variants in *LEP*, *LEPR*, or *MC4R* [24]. Philippe et al. [28] sequenced coding regions of 34 obesity genes in 201 individuals, including 126 who were obese. They report discovery of a nonsense loss of function mutation in *PCSK1* that causes a dominantly inherited familial obesity in a single three-generation pedigree. These investigators [28] also report finding another missense mutation in *PCSK1* and a missense mutation in *POMC* that were previously identified as putative obesity mutations but which are not associated with obesity in their study. This emphasizes the necessity for functional studies of missense mutations for putative obesity genes. Tan et al. [29] provide experimental evidence that exome sequencing did not identify all the obesity-causing mutations in *MC4R*, confirming prior hypotheses that whole genome sequencing will be needed to more completely catalog obesity-causing alleles.

TABLE 21.1 Sequencing Studies of Human Obesity

Gene	Protein Coded	Population	Method (Ref.)	Functional effects of variants confirmed	Comments
Mutations Identified by Sequencing Targeted at Known Obesity Genes					
LEP	Leptin	22 probands from consanguineous families	Targeted sequencing [24]	No	30% of severe obesity in children of consanguineous families due to *LEP*, *LEPR*, or *MC4R*
LEPR	Leptin receptor				
MC4R	Melanocortin receptor 4				
CPE	Carboxypeptidase E	1 individual from consanguineous family	Whole exome sequencing [26]	Yes	Obesity, intellectual disability, abnormal glucose, hypogonadism
TUB	Tubby bipartite transcription factor	1 individual from consanguineous family	Exome sequencing [27]	Yes	Frameshift mutation of *TUB* likely cause of obesity and retinal degeneration in humans Incomplete penetrance
PCSK1	Proprotein convertase subtilisin/kexin type 1	General population 206 of whom 126 obese	Sequenced coding regions [28]	No	One 3-generation family with dominantly inherited obesity
MC4R	Melanocortin receptor 4	267 obese children	Sequenced promoter region of *MC4R* [29]	Yes	Found novel promoter polymorphism that greatly reduced transcriptional activity in 1 child
Novel Obesity Genes Identified by Sequencing					
COA3	Cytochrome C oxidase assembly factor 3	1 obese adult	Whole exome sequencing [30]	Yes	Subject with exercise intolerance, obesity, neuropathy
DYRK1B	Duel specificity tyrosine-phosphorylation-regulated kinase 1B	3 multigenerational families 300 obese	Linkage analysis and whole exome sequencing [31]	Yes	Missense mutation associated with increase of BMI from 23 to 33. Separate variants associated with central obesity and metabolic syndrome

Several recent papers identify novel obesity genes and provide functional data demonstrating that the identified mutations have causal roles. Ostergaard et al. [30] studied a single subject with exercise intolerance, obesity, and neuropathy using whole exome sequencing and found compound heterozygous mutations in cytochrome c oxidase assembly factor 3 (*COA3*). *COA3* is an autosomal gene that is localized to mitochondria with expression highest in metabolically active tissues such as brain, liver, heart, kidney, and small intestine. Thus, effects of this mutation may be tissue specific which may explain the mild phenotype. Keramati et al. [31] used linkage analysis and whole exome sequencing to identify the gene *DYRK1B* as the cause of autosomal dominant coronary heart disease and metabolic syndrome in three multigenerational families. Initial studies identified significant logarithm of the odds (LOD) scores for linkage between markers in *DYRK1B* to BMI, blood pressure, and type 2 diabetes. Whole exome sequencing then identified a missense mutation in *DYRK1B* located in the LOD peak. In the families this mutation is associated with an increase of BMI from 23 to 33. The investigators screened 300 additional obese people and identified a separate variant that was also associated with central obesity. Subsequent letters to the editor by other groups confirmed that yet other missense mutations in *DYRK1B* influence metabolic syndrome.

Sometimes whole exome sequencing does not identify a mutation in a specific gene that causes Mendelian obesity but does identify susceptibility genes that increase risk. Pima Indians have one of the highest incidences of obesity and type 2 diabetes in the United States. They have been participants in, and subjects of, a long running study aimed at discovering if there is a genetic basis for this high incidence of obesity. Whole exome sequencing of 177 Pima Indians identified 31,441 coding variants, none of which had genome-wide significant association with adiposity or measures of type 2 diabetes [32]. A total of 345 of these variants that were predicted to have functional effects were genotyped in additional Pima Indians. *CYB5A* and *RNF10* showed significant association with adiposity and type 2 diabetes but the effects on type 2 diabetes were eliminated when the data were adjusted for BMI. Individuals with the risk allele of *CYB5A* were about 1 BMI unit heavier, while those with the risk allele for *RNF10* were about 3 BMI units heavier. Although these genes are risk factors for obesity, they cannot presently be considered as genes causing Mendelian forms of nonsyndromic obesity.

A small number of papers report the identification of novel genes through whole exome sequencing with possible causal mutations for obesity but do not demonstrate that the mutations identified influence function of the candidate obesity gene. Nevertheless, the utility of the sequence-based approach is likely to grow rapidly because costs are dropping and software for analyzing the results is improving [21]. Several low cost for-profit and nonprofit vendors already offer sequence-based diagnosis to primary care physicians with difficult to diagnose cases. However, most exome sequencing studies do not identify causal genes, and those that do, identify causal genes in only a fraction of the obese people studied. Thus, methods to determine causality of specific mutations remain essential. As discussed in Section IV-A, Lessons for Human Obesity from Genetic Studies in Mice, mouse genetic models often have phenotypes similar to those observed in humans with mutations in orthologous genes. Thus, one method to determine causality is to make the corresponding mutation in mice and then evaluate phenotype. Availability of clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 technology means that genetically engineered mice can be made and phenotyped much more quickly than was possible with traditional knockout or transgenic methods [33]. Characterizing the genetic basis of obesity will likely require much more than just exome sequencing.

C Genome-Wide Association Studies—Finding Most of the Common Disease Variants

Starting in 2005, human genetics entered a new era with the introduction of genome-wide association studies (GWAS) that examine many genetic variants in different individuals to see if any variant is associated with a trait. In one of the GWAS several hundred thousand single nucleotide polymorphism (SNP) markers, spread throughout the genome, are used to identify chromosomal regions influencing traits anywhere. GWAS owe their existence to several converging discoveries; sequencing of the human genome, identification of millions of naturally occurring SNPs, and discovery of technologies for determining which allele a person has for hundreds of thousands of SNPs in a single experiment.

One of the key analytical features of GWAS is that investigators do not need to use SNPs that cause disease; they only need to use SNPs that are near the disease-causing allele. More specifically, they need to be in linkage disequilibrium, or close, to the causal alleles. GWAS examine SNPs throughout the genomes of individuals to identify associations between those markers and diseases or specific traits, often comparing genomes of cases (disease) with controls (no disease). GWAS SNP panels are efficient at finding common variants that cause common diseases, but they cannot find rare disease-causing variants. The SNPs used are themselves ones where the minor alleles are relatively common, for instance many

have frequencies of 1−5%, meaning that there cannot be a unique association of any one common SNP with a rare allele. Rare allele discovery requires other methods, such as sequencing. Whole genome association studies have several other disadvantages. In some cases SNPs associated with obesity are located in introns of one gene but appear to act by influencing expression of an adjacent gene. In other cases SNPs associated with obesity are not located within a gene but are between genes.

Many GWAS have been performed for obesity [34,35]. The first obesity locus identified by GWAS was the fat mass and obesity-associated (*FTO*) gene [36], which has significant effects on feeding and on adipose tissue [37]. Although explaining only 1−3% of the variance in BMI, *FTO* polymorphisms have been found in multiple studies of populations worldwide.

The studies with the largest number of patients and providing higher statistical power are meta-analyses from the Genetic Investigation of ANthropomorphic Traits (GIANT) consortium that published several papers in 2015. One paper examined GWAS for BMI in adult men and women [38], while another examined GWAS for waist-to-hip ratio (WHR) after adjustment for BMI [39], and yet another examined the data stratified by gender and age [40].

The BMI study [38] identified 97 genome-wide SNPs, 56 of which were novel and 41 SNPs that had previously been significantly associated with BMI. Table 21.2 lists 13 of the 41 loci with significant association with BMI in multiple GWAS and highlights five of these genes that are components of the leptin-melanocortin pathway. When the data were stratified by gender and age, there was a larger effect in younger rather than older subjects at most of the loci [40]. There were no gender differences.

Estimation of overall heritability explained was conducted in two ways. First, just using genome-wide significant SNPs it was found that about 4% of heritability in BMI was explained. However, using all SNPs in the entire GWAS it was estimated that about 20% of heritability was explained. Of course, this would include some false positives, but may be a better estimate because it includes all the genes with very small effects on BMI.

A total of 35 of the BMI significant SNPs are also identified as associated with other diseases in the National Human Genome Research Institute (NHGRI) GWAS catalog. These include genes that are associated with cardiovascular disease, schizophrenia, smoking, irritable bowel syndrome, and Alzheimer's disease. As we will discuss later, these SNPs and traits are candidates for having causal relationships with BMI in Mendelian randomization studies.

Genes consistently and strongly associated with common obesity (Table 21.2), as measured by BMI in GWAS, include *FTO* (found only in GWAS), *MC4R* (identified as causing single-gene obesity, from sequencing studies, and from GWAS), *TMEM18*, *SEC16B*, and *TFAP2B* (all identified in GWAS), *BDNF* (identified as causing single-gene obesity and from GWAS), *NEGR1* and *FAIM2* (identified from GWAS), *SH2B1* (identified as causing single-gene obesity and from GWAS), and *GIPR* (identified from GWAS) [38,41,43,44].

The *FTO* was first identified through GWAS [45] and consistently shown to be associated with common human obesity across populations and ethnic groups [46]. The function of the FTO protein is unknown although mouse studies suggest it is a 2-oxoglutarate-dependent oxygenase that catalyzes nucleic acid demethylation [47]. *FTO* was recently found to interact with promoters of *IRX3* and *IRX5* that are involved in early neural development and may play a role in adipocyte, especially brown adipocyte, development [48]. *FTO* is highly expressed in the hypothalamus, a primary site for regulation of energy balance and satiety [45]. Risk alleles of *FTO* are associated with increased food intake, increased hunger, and reduced satiety [42], as well as with increased protein intake [49,50]. *FTO* variants interact with fat and carbohydrate intakes to affect BMI [51]. Individuals carrying homozygous *FTO* obesity predisposing alleles may lose more weight through diet or lifestyle intervention than noncarriers [52]. *FTO* variants have also been examined for interaction with physical activity, but the studies to date are not consistent [53].

MC4R, *BDNF*, *SH2B1*, *POMC*, and *TUB*, as components of the melanocortin pathway, contribute to single-gene obesity. However, variants of these genes also contribute to common obesity as measured by BMI in GWAS. Transmembrane protein 18 gene (*TMEM18*) expression levels are related to phenotypes of obesity and glucose metabolism [43,54]. *TMEM18* is widely expressed in the body, both centrally and in adipose tissue. However, its function in energy metabolism is not yet known. *NEGR1* codes for a neuronal growth-promoting factor which may be involved in synaptogenesis, neurite outgrowth, and cell-cell recognition/adhesion [43], and the gene is expressed in the hypothalamus and in peripheral adipose tissue and muscle. The gastric inhibitory polypeptide receptor (*GIPR*) gene codes for a receptor for an appetite-linked hormone, GIP, which is produced in the alimentary tract and mediates enhanced release of insulin from the pancreas. *GIPR* is also expressed in the hypothalamus and adipocytes.

Statistically significant SNPs are rarely causal for common diseases. Locke et al. [38] sought to determine if the significant SNPs were in linkage disequilibrium (close to) coding variants. They found coding variants predicted to have damaging effects on protein function in five genes in linkage disequilibrium with BMI SNPs; *ZNF142*, *STK36*, *TRIM66*, *BDNF*, and *GIPR*. Further study of these variants is needed to determine if they are

TABLE 21.2 Selected BMI Loci Identified in Multiple GWAS (Loci Listed in the Sequence of Strength of Association with BMI) [38]

Notable Gene	Gene Name	Chr	Function	Reference
FTO[a,c]	Fat mass and obesity associated	16	Catalyzes demethylation of RNA. Increased hypothalamic *FTO* expression associated with regulation of energy intake.	[38,41,42]
MC4R[a,b,c]	Melanocortin 4 receptor	18	MC4 protein binds α-MSH and is involved in regulation of feeding behavior and metabolism.	[38,41]
TMEM18[b,c]	Transmembrane protein 18	2	Transcription repressor. Cell migration modulator that enhances the glioma-specific ability of neuronal stem cells.	[38,41,43,44]
SEC16B[b,c]	SEC16 homolog B, endoplasmic reticulum export factor	1	Required for organization of transitional endoplasmic reticulum sites and protein export.	[38,41,44]
TFAP2B[c]	Transcription factor AP-2 beta	6	Transcription factor thought to stimulate cell proliferation and suppress terminal differentiation of specific cell types during embryogenesis.	[38,41]
BDNF[a,c]	Brain-derived neurotrophic factor	11	Helps support growth and differentiation of new neurons and synapses and support the survival of existing neurons.	[38,44]
NEGR1[b,c]	Neuronal growth regulator 1	1	May function as a trans-neural growth-promoting factor.	[38,43,44]
FAIM2[c]	Fas apoptotic inhibitory molecule 2	12	Protects cells from Fas-induced apoptosis.	[38,41]
POMC[a]	Proopiomelanocortin	2	Polypeptide hormone precursor that undergoes extensive, tissue specific, posttranslational processing to produce biologically active peptides, including α-MSH, important in regulation of appetite.	[38]
SH2B1[a]	SH2B adapter protein 1	16	The protein mediates activation of various kinases including *LEP* signaling and other genes of the leptin-melanocortin pathway.	[38,43]
GIPR	Gastric inhibitory polypeptide receptor	19	Stimulates insulin release in the presence of elevated glucose.	[38,43]
POC5	POC5 centriolar protein	5	Essential for the assembly of the distal half of centrioles, required for centriole elongation.	[38,43]
LINGO2	Leucine-rich repeat and Ig domain containing 2	9	unknown	[38,43]
TUB[a]	Tubby bipartite transcription factor	11	Plays a role in obesity and sensorineural degradation	[38]

[a]*Loci associated with genes involved in the leptin-melanocortin pathway. Each of these genes has variants that can cause single-gene obesity in humans.*
[b]*SNPs located near these genes have stronger association with BMI in younger versus older adults.*
[c]*SNPs located near these genes are significantly associated with BMI in children and adolescents.*

causal for the BMI effects. The authors also identified many genes where the BMI SNPs were associated with mRNA levels for adjacent genes, consistent with hypothesis that some of the SNPs influence BMI by altering mRNA levels.

The WHR GWAS, which focused on finding genes for upper versus lower body fat distribution rather than fat mass or mass of individual fat depots, showed several novel features [55]. Shungin et al. [55] found a total of 49 significant SNPs for WHR and another 19 associated with waist or hip circumference measures. Twenty of the SNPs showed strong gender dependence with 19 having stronger effects in women and only a one having stronger effects in men. When stratified for both gender and age, only gender differences were apparent in the data [40]. An additional GWAS paper focusing on adiposity or fat depots is consistent with the GIANT consortium findings. Sung et al. [56] identified multiple SNPs in several genes with gender-specific effects on visceral and subcutaneous adipose tissue.

Alternative measures of obesity may produce different GWAS results. Lu et al. [57] measured percent body fat in 100,716 people by either bioelectric impedance or dual-energy x-ray absorptiometry (DEXA) and found 12 genome-wide statistically significant SNP loci. Seven loci had larger effects on percent body fat than BMI. Five had larger effects on BMI than percent fat. None of the genes was significant for WHR adjusted for BMI. Thus, GWAS contrasting results for BMI and WHR adjusted for BMI, or examining percent body fat or different adipose depots, show different genes for weight and for individual fat depots and substantial genetic differences between males and females. The results are strongly consistent with mouse studies showing that body weight or BMI only partially overlap with percent fat and fat pad genetics.

Other GWAS examined children and various ethnic groups. Studies of children found that most genes for BMI are common with adults and only a few are child specific [41,44]. The results could mean that there are some different genetic controls between adult and childhood obesity, or they could mean that both adult and childhood obesity studies were underpowered and would find the same genes if enough people were studied. Also, most genes in other ethnic groups were the same as those observed in Caucasians; genes that were different may or may not indicate true ethnically different obesity pathways.

GWAS led to the development of genetic risk scores (GRS), multilocus profiles calculated by summing up the number of risk alleles for elevated BMI and obesity. GRS are very useful for studying effects of genetics on response to diet and exercise. They have also been used for the technique of Mendelian randomization, which determines if obesity has a causal effect on correlated comorbidities.

D Mendelian Randomization or Genetic Correlations and Causal Relationships

Obesity is correlated with many diseases, for example, hypertension, type 2 diabetes, cardiovascular disease, serum triglycerides, and more. Until recently there was no method to determine if obesity caused these other diseases, or if one or more other diseases caused obesity. Several methods have just recently become available to identify causal relationships between correlated complex traits. One method looks for genetic correlations. The method does not require individual genotypes, genome-wide significant SNPs, nor even measuring multiple traits for the same individuals. Thus, genetic correlations can be measured for large numbers of traits. Bulik-Sullivan et al. [58] estimated genetic correlations between 24 traits, including BMI. They report positive genetic correlations between BMI and type 2 diabetes, coronary artery disease, and serum triglycerides. They report statistically significant negative correlations between BMI and HDL cholesterol, age at menarche, height, and years of education. These results, and limitations, are quite similar to those observed using the technique of Mendelian randomization. Note that they did not have data on fat mass or fat distribution so comparisons of genetic correlations of BMI with fat mass or fat distribution were not possible.

Mendelian randomization combines genetics with traditional epidemiologic methods to provide causal information about correlated phenotypes, without conducting a randomized controlled trial. The Mendelian randomization approach limits both confounding and reverse causality errors, but assumes there is no linkage disequilibrium or pleiotropy where one gene has a primary effect on more than one phenotype.

A number of recent studies used Mendelian randomization to determine if obesity, as measured by BMI, is causal for disease. Using GRS generated from BMI risk alleles, Todd et al. [59] found evidence that obesity is causal for diabetic kidney disease in type 1 diabetes and Cole et al. [60] found evidence that obesity is a causal risk factor for coronary artery disease. On the other hand, Davies et al. [61] found little evidence that genetically determined BMI and height had influence on prostate cancer risk, but were associated with increased mortality in low-grade disease. Furthermore, Nordestgaard et al. [62] concluded that, although high coffee intake was correlated observationally with low risk of obesity, metabolic syndrome, and type 2 diabetes, there was no evidence to support a causal relationship.

Observational studies suggest that a leptin surge in the perinatal period may program the long-term risk of obesity. A study by Allard et al. [63] using DNA methylation levels near the *LEP* locus in a Mendelian randomization study supports causality between maternal hyperglycemia and epigenetic regulation of leptin in the newborn.

We expect to see more studies using Mendelian randomization to determine causal relations between obesity and disease and between environmental factors and obesity.

E The Problem of Missing Heritability

As much as 70% of any one person's risk for being obese may be heritable, that is, genetic. Yet, to date, less than 10% of that heritability has been identified. The gene most strongly associated with common human obesity, the fat mass and obesity-associated gene (*FTO*), only

contributes about 1% of the variance in BMI [36]. The problem of missing heritability is true not only for obesity but also for most common genetically complex traits and diseases. For example, height is estimated to be 80–90% heritable, yet large-scale population studies identify less than 10% of height's heritability [64].

This missing heritability may be due to the presence of rare variants, variants with low penetrance, copy number variants, epigenetic tags, numerous variants with small effects, or may be due to overestimation of heritability due to epistasis (gene–gene interaction), none of which are readily identified by GWAS.

Rare variants are likely to be missed by the GWAS approach despite the fact that they may have larger effects than common variants [65–67]. Rare variants with large effects seem to be more common in people with early onset or more severe obesity [68,69]. Indeed, sequence-based studies have already found that cohorts with extreme phenotypes of obesity are enriched with highly penetrant but rare alleles. It is likely that sequencing will continue to discover rare variants.

Copy number variation (CNV) is when the number of copies of a particular gene varies among individuals. CNVs comprise more total nucleotide content than SNPs and may encompass one or more partial or entire genes. Yet, in current SNP analytic methods homozygous (A/A), hemizygous (A/O), and duplicative (A/A/A) tend to be lumped. One study of CNVs showed that deletion at the 16p11.2 locus resulted in altered satiety response and subsequent obesity in children whereas duplication at that locus was associated with leanness [70]. Taking account of the structural dimension of the genome might recover some of the missing heritability for many traits [71,72].

Epigenetics refers to changes in gene expression that are not the result of DNA sequence, but can be the result of chemical modifications of DNA or of proteins that bind DNA. Common epigenetic tags are those resulting from DNA methylation or deacetylation of DNA-binding histones. These often occur as the result of early life environment. Evidence now suggests that epigenetic changes are important contributors to inheritance of obesity phenotypes. Since standard sequencing or GWAS techniques do not measure epigenetic tags, they may be a major component of missing heritability. Epigenetics will be discussed in more detail in Section V-A, Epigenetics.

Estimates of heritability assume that there are no gene–gene interactions (epistasis), a phenomenon found in all animal models of obesity. Epistasis is where a gene (or genes) masks or amplifies the effects of another gene (or genes). A classic example of the effect of gene–gene interactions on a complex trait comes from mouse studies. The severity of diabetes in both *Lep*^{ob} (leptin) and *Lepr*^{db} (leptin receptor) mutant mice is determined by the genetic background upon which the mutation is expressed. There are many different inbred mouse strains. These strains differ from each other at millions of places through their genomes. Thus, if the same mutation is moved from one strain to another by breeding, then one can determine if all these other variants influence the phenotypes produced by the mutation. Both *Lep*^{ob} and *Lepr*^{db} mutations in C57BL/6 strain mice result in hyperinsulinemia and obesity, whereas these mutations in C57BLKs strain mice result in severe diabetes and early death [73]. This means that genes other than *Lep*^{ob} and *Lepr*^{db} have dramatic effects on the phenotype that is observed. Characterization of epistasis influencing polygenic obesity in the BSB mouse model, produced by breeding C57BL/6J with *Mus spretus* (a different mouse species) F1 × C57BL/6J, found interaction between genes on different chromosomes that contributed significant variation to obesity and related phenotypes [74]. Each BSB mouse is genetically unique and weights, fat distribution, and fat mass vary widely from mouse to mouse. Quantitative trait locus mapping demonstrated both direct genetic effects and epistatic effects [74].

Gene–gene interactions are likely a universal phenomenon in common human diseases and may be more important in determining the phenotype than the independent main effects of any one susceptibility gene [75,76]. Zuk et al. [77] argue that a high proportion of heritability for certain traits could be due to genetic interactions. Gene–gene interactions are difficult to identify using traditional genetic studies in humans and studies searching for interactions of any gene with all genes are lacking for human obesity. Instead, investigators have performed more limited studies searching for epistasis of pairs of specific genes chosen by investigators. Gene–gene interaction effects have been shown on BMI and waist circumference [78], extreme obesity [79], abdominal fat [80], and on immune dysfunction in obesity [81]. Gene–gene interactions among variants of the β-adrenergic receptor genes (*ADRB1*, *ADRB2*, and *ADRB3*) contribute to longitudinal weight changes in African and Caucasian American subjects [82]. Epistasis affecting obesity was also found in African derived populations in Brazil where interactions between *LEPR* and *ADRB2* polymorphisms as well as a third-order effect between *LEPR*, *ADRB2*, and *INSIG2* were found [83]. In the study of Feitosoa et al., blood lipid profile and dietary habits were found to have confounding effects in the analysis [78]. It is possible to have both gene–gene and gene–environment interactions affecting the same pathway.

Zuk et al. [77] describe a method for estimating heritability not inflated by genetic interactions, but the method requires isolated populations.

V GENE–ENVIRONMENT INTERACTIONS

A Epigenetics

Epigenetics is the study of mitotic and/or meiotic changes due to environmental factors that switch genes on and off without changes in the DNA sequence [84]. Common epigenetic changes result from DNA methylation or histone deacetylation and often occur as the result of environmental exposure in utero, in the early neonatal period or early in life [85]. Epigenetic processes include genome imprinting, gene silencing, and noncoding microRNA, among other effects. Technically, the term epigenetics applies to only those changes that are stably inherited. However, the term epigenetics is also commonly used to describe processes that have not been shown to be heritable but that effect the development of the organism. There is increasing evidence that epigenetics is a mediator of gene–environment interactions underlying the development of obesity and comorbidities [86–90].

Nutrition and activity levels can both affect metabolism through epigenetic gene regulation. Studies that show epigenetic changes associated with weight regulation include studies of the Dutch winter famine that occurred in 1944 showing that children conceived during famine were small and underweight with increased risk for obesity and type 2 diabetes as adults [91,92]. In the Chinese famine of 1958–61, only females developed obesity in later life [93]. DNA isolated from individuals decades after the famine showed abnormal DNA methylation [94]. Studies of identical twins with discordant BMIs identified DNA methylation and expression differences in subcutaneous adipose tissue that distinguish one twin from the other, differences that tended to increase with diverging life experience suggesting that the difference in obesity is epigenetically regulated [95].

Epigenetic studies of human obesity are just beginning. A large-scale epigenome-wide study found significant associations between DNA methylation and BMI and waist circumference in European Americans, which was then replicated in two independent populations including both European and African Americans [96].

Studies showing inheritance of epigenetic changes have been done in rodents. Increased maternal energy intakes affect the epigenetic changes of rats [97]. Feeding a high-fat diet to female mice resulted in increased growth and insulin insensitivity in the progeny [98]. Wei et al. [99] found that prediabetes in male mice increased susceptibility to diabetes in progeny through gamete methylation changes. Mice with the Agouti viable yellow obesity mutation given dietary methyl group supplements have epigenetic changes that prevent passage of obesity to subsequent generations through the agouti viable yellow allele [100]. Integrating mouse to human approaches will be essential to the understanding of the epigenetic contribution to the current obesity epidemic.

B Genetic Effects on Weight Gain or Loss Due to Diet or Exercise

Why some people in modern societies become obese, despite considerable effort and expense to avoid this condition, whereas others stay lean without such effort, appears to have a genetic basis [101,102]. Chronic overfeeding studies by Sims and colleagues beginning in the 1960s showed interindividual differences in weight gain [103,104]. More recently, Bouchard and colleagues determined the response to changes in energy balance by submitting pairs of monozygotic twins either to positive energy balance induced by overeating [105] or to negative energy balance induced by exercise training [106]. Significant intrapair resemblance was observed for changes in body composition and was particularly striking for changes in regional fat distribution and amount of visceral fat. One explanation for these differences is that some twin pairs were better oxidizers of lipid, as evidenced by reduced respiratory quotient, during the submaximal work than were the other twin pairs [106].

Recent epigenome-wide association studies may help explain some of these interindividual differences in body weight response to diet or exercise [107,108]. Young men with low birth weight, suggesting in utero undernutrition, were compared to men of normal birth weight both on a control diet and after 5 days of a high-fat diet. There was no difference in skeletal muscle DNA methylation between low and normal birth weight cohorts on the control diet. However, after the high-fat diet the normal weight group had widespread skeletal muscle DNA methylation, whereas the low birth weight group had few methylation changes [108]. In obese adolescents DNA regions were differentially methylated consistent with weight loss response to diet [109].

Several papers now report DNA methylation changes as a result of acute [110] or chronic [107] exercise training including altered DNA methylation patterns in adipose tissue of healthy young men in candidate genes for obesity including *FTO*, *GRB14*, and *TUB* [107]. A meta-analysis of 10 studies found that individuals carrying homozygous *FTO* obesity predisposing allele lose more weight through diet or lifestyle intervention than noncarriers [52]. These studies are consistent with the hypothesis that exercise may modify DNA methylation, and thus gene expression, for many genes that influence BMI, fat mass, or fat distribution.

Other studies examined genes in the lipolysis pathway for influence on weight loss success but results are not consistent (see [111] for review).

C Genetic Effects on Weight Loss Due to Bariatric Surgery

Several studies with small numbers of subjects report on weight loss in individuals with *MC4R* mutations following bariatric surgery. Patients with *MC4R* mutations are able to lose as much weight as those without such mutations with bariatric surgery in children [112], adolescents [113], and adults [114]. GWAS-type studies with small numbers of subjects are just beginning to look at SNPs associated with weight loss success following bariatric surgery and need confirmation. At the present time, obesity GRS does not predict weight loss results following bariatric surgery [115].

VI GENETIC PATHWAYS OF OBESITY

The hypothalamus is of great importance in obesity as it integrates peripheral hormonal and neuronal signals of satiety and nutritional status, senses nutrients, controls glucose homeostasis and peripheral lipid metabolism, and functions to control whole body energy balance. Much of this control is through the leptin-melanocortin pathway in the hypothalamus. Recent studies also implicate adipose tissue as important in obesity, both as an active endocrine organ [116,117] and in regard to body fat distribution [55].

A The Leptin-Melanocortin Pathway in the Hypothalamus

The first five causal human obesity genes were identified using the mouse models Lep^{ob}, $Lepr^{db}$, *Tub*, Cpe^{fat}, and A^{y}. Once these genes were identified in mice, geneticists began searching for obese humans with mutations in these same genes. Study of the yellow obese A^{y} mouse led to the discovery of the leptin-melanocortin pathway, the primary pathway in the brain which functions in the regulation of body weight (Table 21.3). Searching in highly

TABLE 21.3 Single Gene Mutations Causing Uncomplicated Obesity in Humans and Confirmed in Mouse Models

Gene Name	Chromosomal Transmission	Function of Gene Product Relative to Obesity	Mouse Ortholog
Components of the Leptin-Melanocortin Pathway of the Hypothalamus			
Leptin (*LEP*) [118]	7q31.3 Recessive	Hormone secreted from adipocytes that plays a critical role in regulation of body weight by inhibiting food intake and stimulating energy expenditure. Deficiency causes hyperphagia, early onset obesity, hypogonadotropic hypogonadism, and altered carbohydrate metabolism.	Lep^{ob} cloned from the ob/ob mouse [154]
Leptin receptor (*LEPR*) [119]	1p31 Recessive	Receptor for the hormone leptin. LEPR deficiency causes same phenotype as LEP deficiency.	$Lepr^{db}$ cloned from the db/db mouse [120]
SH2B adaptor protein 1 (*SH2B1*) (155,156)	16p11.2 Recessive	Adaptor protein enhances intracellular leptin signaling in the brain. Loss of function mutation results in hyperphagia, childhood onset obesity, disproportionate leptin resistance, and reduced height as adult.	*Sh2b1* No obesity identified in homozygous null mice.
Proopiomelanocortin (*POMC*) [121]	2p23.3 Recessive	Located in centrally projecting neurons that contain peptide products of proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART). POMC is a precursor protein that is ultimately cleaved into ACTH, α-MSH, β-MSH, γ-MSH, and β-endorphin. Mutation causes hyperphagia, early onset obesity, hypocortisolism, and skin and hair hypopigmentation.	*Pomc* [157]
Tubby bipartite transcription factor (*TUB*) [27]	11p15.5 Recessive	Functions as a membrane-bound transcription regulator in the hypothalamus that translocates to the nucleus in response to phosphoinositide hydrolysis. Deficiency results in hyperphagia, obesity, altered glucose metabolism, and sensorineural degradation.	*Tub* cloned from the tubby mouse [158]

(*Continued*)

TABLE 21.3 (Continued)

Gene Name	Chromosomal Transmission	Function of Gene Product Relative to Obesity	Mouse Ortholog
Carboxypeptidase E (*CPE*) [26]	4q32.3 Recessive	Involved in the synthesis of most neuropeptides and peptide hormones. Deficiency results in severe obesity, type 2 diabetes, intellectual disability, and hypogonadotrophic hypogonadism.	Cpe^{fat}, cloned from the fat mouse [159]
Melanocortin 4 receptor (*MC4R*) [122,123]	18q22 Dominant	The encoded protein is a membrane-bound receptor and member of the melanocortin receptor family, interacts with adrenocorticotropic and MSH hormones, and is mediated by G proteins. Defects in this gene are a cause of hyperphagia, early onset obesity, increased height, and fasting hyperinsulinemia. MC4R mutations cause the most common form of human monogenic obesity ~ 5%.	*Mc4r* [160]
Proprotein convertase subtilisin/kexin type 1 (*PCSK1*) [161]	5q15-q21 Recessive	PC1/3 is a neuroendocrine convertase encoded by *PCSK1* that cleaves POMC and also proinsulin to insulin. Mutation causes hyperphagia, early onset obesity, hypogonadism, and altered carbohydrate metabolism.	*Pcsk1* mutation in the HRS/J inbred mouse results in late onset obesity
Melanocortin 2 receptor assembly protein 2 (*MRAP2*) [162]	6q14.2 Recessive	Regulates energy homeostasis through signaling of MC4R and PKR1.	*Mrap2* knockout produces severe early obesity [163]
Components of the Paraventricular Pathway of the Hypothalamus			
Brain-derived neurotrophic factor (*BDNF*) [124]	11p13 Recessive	Plays a role in the growth, maturation, and maintenance of cells in the brain and is active where cell-to-cell communication occurs. BDNF protein is found in regions of the brain that control eating, drinking, and body weight. Mutation causes hyperphagia, early onset obesity and cognitive impairment.	*Bdnf* [164]
Neurotrophic tyrosine kinase, receptor, type 2 (*NTRK2*) ([165,125])	9q22.1 Recessive	Receptor for BDNF. Mutation results in early onset obesity, hyperphagia, and developmental delay.	*Ntrk2* knock-in results in adiposity [166]
Single-minded homolog 1 (*SIM1*) ([167,126])	6q16.3 Recessive	Transcription factor that is essential for the development of the PVN of the hypothalamus. Haploinsufficiency causes hyperphagia, early onset obesity, altered carbohydrate metabolism, dysmorphic features, and mental retardation.	*Sim1* heterozygous mutants exhibit hyperphagic obesity [168]

consanguineous families including obese individuals, a mutation in the leptin (*LEP*) gene was discovered [118] and confirmed in additional homozygous *LEP*-deficient patients [25]. As with the *LEP* gene, homozygous leptin receptor (*LEPR*) deficiencies were found in severely obese siblings [119] and in families where severe obesity segregated with mutations in *LEPR* [127,128]. Individuals homozygous for mutations in either *LEP* or *LEPR* are hyperphagic and gain weight rapidly in the first year of life [118,129] and have delayed puberty due to hypogonadotropic hypogonadism [128]. Heterozygotes for *LEP* and *LEPR* mutations have increased fat mass but are not morbidly obese.

Borman et al. [130] identified a homozygous mutation in *TUB* in a child of consanguineous marriage. The 11-year old was identified with mild obesity and a 2-year history of deteriorating vision. Functional studies demonstrated that the mutated protein is expressed at low levels in the retina. Alsters et al. [131] identified a homozygous mutation in carboxypeptidase E (*CPE*) in a severely obese woman from a consanguineous marriage. The proband had severe obesity, intellectual disability, abnormal

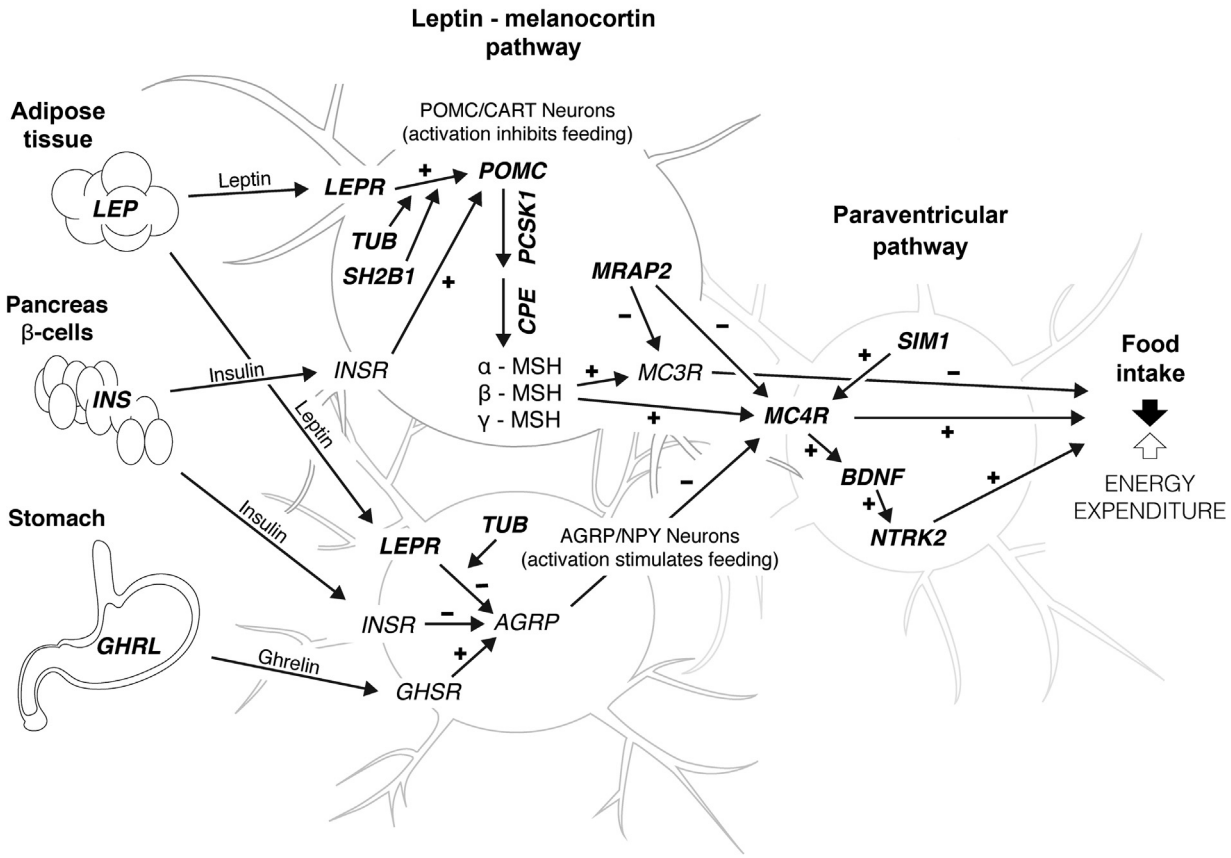

FIGURE 21.2 Simplified schematic of the genes involved in the leptin-melanocortin and paraventricular pathways in the hypothalamus. Mutations of genes in bold are known to cause monogenic obesity in humans. These pathways are essential components of the central control of energy homeostasis, propagating the signals that result in satiety and increased energy intake. Leptin is secreted from adipocytes, crosses the blood–brain barrier and activates leptin receptors. This activation, mediated by tubby bipartite transcription factor, stimulates POMC/CART neurons producing melanocortins that activate melanocortin 4 receptors in the paraventricular and ventromedial nuclei, resulting in satiety. *SIM1* is essential for development of the paraventricular nucleus. Brain-derived neurotrophic factor and its receptor, neurotrophic tyrosine kinase receptor, type 2, are part of the MC4R cascade leading to satiety. When stimulated by ghrelin receptors in the arcuate nucleus, agouti-related protein inhibits MC4R activity. AgRP, agouti-related protein; BDNF, brain-derived neurotrophic factor; CART, cocaine- and amphetamine-related transcript; CPE, carboxypeptidase E; GHSR, ghrelin receptor; INSR, insulin receptor; LEP, leptin; LEPR, leptin receptor; MC3R, melanocortin 3 receptor; MC4R, melanocortin 4 receptor; MRAP2, melanocortin 2 receptor assembly protein 2; α-MSH, α-melanocyte-stimulating hormone; β-MSH, β-melanocyte stimulating hormone; NPY, neuropeptide Y; NTRK2, neurotrophic tyrosine kinase receptor, type 2; PCSK1, proprotein convertase subtilisin/kexin-type 1; POMC, proopiomelanocortin; SIM1, single-minded homolog 1 of *Drosophila*. *Figure illustration by Venus Nguyen.*

glucose, and hypogonadotropic hypogonadism. The proband's symptoms closely match those of Cpe^{fat} mice.

To date, mutations in 12 genes, all components of the leptin-melanocortin or paraventricular pathways, have been reliably shown to cause spontaneous Mendelian increased BMI in humans; leptin (*LEP*), leptin receptor (*LEPR*), tubby bipartite transcription factor (*TUB*), SH2B adapter protein 1 (*SHR2B1*), proopiomelanocortin (*POMC*), proprotein convertase subtilisin/kexin type 1 (*PCSK1*), carboxypeptidase E (*CPE*), melanocortin 2 receptor assembly protein 2 (*MRAP2*), melanocortin 4 receptor (*MC4R*), brain-derived neurotrophic factor (*BDNF*), neurotrophic tyrosine kinase receptor type 2 (*NTRK2*), and single-minded homolog 1 (*SIM1*) (Table 21.3; Fig. 21.2). Mutations in these genes in humans are recessive, with the exception of *MC4R*, and therefore are rare, are associated with hyperphagia and severe obesity beginning in childhood, and may include developmental, endocrine, and behavioral disorders. (For a description of the most common single-gene obesity disorders and syndromes, see [132].)

Leptin (product of *LEP*) is secreted by adipocytes and its concentration in blood is proportional to fat mass. Leptin crosses the blood–brain barrier and activates leptin receptors (product of *LEPR*) on the surface of neurons in the arcuate nucleus of the hypothalamus. This activation, with the assist of the tubby bipartite transcription

factor (product of *TUB*) and SH2B adapter protein 1 (product of *SH3B1*), stimulates proprotein convertase (product of *PCSK1*) and carboxypeptidase E (product of *CPE*) to cleave proopiomelanocortin (product of *POMC*) into the melanocortins including α-MSH, the primary ligand for melanocortin receptors and activation of downstream signaling to regulate energy balance. (For reviews of the leptin-melanocortin pathway and downstream signaling in obesity see [6,133,134].) Activation of the agouti-related protein (AgRP)/neuropeptide Y (NPY) neurons in the hypothalamus stimulates feeding. Leptin binding inhibits the AgRP protein, thereby inhibiting feeding. Thus, leptin functions as an afferent signal in a negative feedback loop to maintain constancy of body fat stores.

Leptin acts through the leptin receptor, a single-transmembrane-domain receptor of the cytokine-receptor family [120]. The leptin receptor is found in many tissues in several alternatively spliced forms, raising the possibility that leptin affects many tissues in addition to the hypothalamus.

Leptin clearly has a broader physiological role than just the regulation of body fat stores. Leptin deficiency results in many of the abnormalities seen in starvation, including reduced body temperature, reduced activity, decreased immune function, and infertility. (For reviews of the physiological role of leptin see [135−137].) Leptin deficiency results in severe hyperphagia and early onset obesity. Replacement with human recombinant leptin in children with severe leptin deficiency normalizes food intake and body composition [136]. Studies of long-term replacement therapy in patients with congenital leptin deficiency show that leptin regulates many body functions including the endocrine system, energy balance, the adipoinsular axis, inflammation, and immunity [138].

Sequential cleavage of the precursor protein proopiomelanocortin (product of *POMC*) generates the melanocortin peptides adrenocorticotrophin (ACTH), the MSHs (α-, β- and δ-MSH), and the opioid-receptor ligand β-endorphin (for review see [139]). α-MSH plays a central role in the regulation of food intake by the activation of the brain melanocortin 4 receptor (product of *MC4R*). The dual role of α-MSH in regulating food intake and influencing hair pigmentation predicts that the phenotype associated with a defect in *POMC* function would include obesity, alteration in pigmentation (e.g., red hair and pale skin in Caucasians), and ACTH deficiency. The observations of these symptoms in two probands led to the identification of three separate mutations within their *POMC* genes [121]. Another *POMC* variant in a region encoding β-MSH results in severe early onset obesity, hyperphagia, and increased linear growth, a phenotype much like that seen with mutations in *MC4R* [140]. Heterozygosity for a *POMC* mutation having subtle effects on proopiomelanocortin expression and function was shown to influence susceptibility to obesity in a large family of Turkish origin [141].

A wide variety of hormones, enzymes, and receptors are initially synthesized as large inactive precursors. To release the active hormone, enzyme, or receptor, these precursors must undergo limited proteolysis by specific convertases. An example is the clipping of proopiomelanocortin by proprotein convertase subtilisin/kexin type 1, also known as prohormone convertase-1 (product of *PCSK1*). Mutations in *PCSK1* were found in individuals with extreme childhood obesity and elevated proinsulin and proopiomelanocortin concentrations but very low insulin levels (for review see [28]). Carboxypeptidase E (CPE) then removes a single basic amino acid from the C-terminus of many different hormones. For example, ACTH is produced from POMC by action of proteases, including PCSK1, then an intermediate product is produced by another protease, and finally α-MSH is produced when CPE removes a final C-terminal amino acid from this last intermediate. A recessive mutation of the gene producing carboxypeptidase E causes obesity in the Cpe^{fat} mouse. Since the human cases and the Cpe^{fat} mouse share similar phenotypes, it can be inferred that molecular defects in prohormone conversion represent a generic mechanism for obesity.

Several melanocortin receptors are highly expressed in the hypothalamus. Mutations in *MC4R* are found in various ethnic groups and cause the most common form of monogenic obesity in humans. The global presence of obesity-specific *MC4R* mutations is estimated to vary from 2% to 7% among population groups [139,142]. *MC4R*-linked obesity in humans is dominantly inherited with incomplete penetrance. Homozygotes have been observed in consanguineous families and have more severe phenotypes than heterozygotes. Subjects with *MC4R* deficiency are obese from an early age. Adrenal function is not impaired but severe hyperinsulinemia is present in the *MC4R*-deficient subjects. Sexual development and fertility are normal. Affected subjects are hyperphagic and have increased linear growth, similar to what occurs in heterozygous *Mc4r*-deficient mice. *MC4R*-deficient humans also have increased lean mass and bone mineral density and mild central hypothyroidism. Female haploinsufficiency carriers who have only a single functioning copy of *MC4R* are heavier then male carriers in their families, a pattern also seen in *Mc4r*-deficient mice. These data are strong evidence for dominantly inherited obesity, not associated with infertility, due to haploinsufficiency mutations in *MC4R*.

MC3R, while not known to cause single-gene obesity, acts as an autoreceptor indicating the tight regulation of the melanocortin system in energy balance. MC3R modifies energy balance by decreasing feed efficiency. Mutations in *MC3R* are not as common as in *MC4R* and

do not result in an autosomal dominant form of obesity, but may be important contributors to susceptibility to obesity. Two variants of the *MC3R* gene interacted with diet to affect weight loss success in an Italian clinic treating severe childhood obesity [143].

B The Paraventricular Pathway

Three genes important in downstream signaling of the melanocortin system have also been shown to cause single-gene obesities. Single-minded homolog 1, product of *SIM1*, is a regulator of neurogenesis and is essential to the development of the paraventricular nucleus of the hypothalamus [126]. Brain-derived neurotrophic factor, product of *BDNF*, and its receptor, neurotrophic tyrosine kinase receptor type 2, product of *NTRK2*, are involved in signaling in the ventromedial nucleus of the hypothalamus and contribute to memory and learning [125]. *BDNF*-deficient rodents are hyperphagic and obese. Case reports associate mutation in *BDNF* or *NTRK2* with massive obesity and impaired cognitive function [124].

C Genetic Pathways Involved in Common Obesity

Locke et al. [38], using data from all loci significantly associated with BMI in the GIANT Consortium GWAS meta-analysis, examined the data using pathway analysis. Biochemical analysis identified several gene sets with significant enrichment; neurotrophin signaling, general growth and patterning, basal cell carcinoma, acute myeloid leukemia, and hedgehog signaling. Pathway analysis showed that genes expressed in the nervous system were particularly enriched in the BMI GWAS, with genes expressed in the immune and hemic systems second most abundant. Genes for monogenic obesity, hypothalamic function, and energy homeostasis were frequently observed. Pathway analysis provided "strong support for a role of the central nervous system in obesity susceptibility and implicated new genes and pathways, including those related to synaptic function, glutamine signaling, insulin secretion/action, energy metabolism, lipid biology and adipogenesis" [38].

D Genetic Pathways Involved in Body Fat Distribution

Fewer studies have looked at body fat distribution by GWAS and, except for the GIANT study, sample sizes have been limiting. However, the GIANT consortium data showed that there was little or no overlap between genes associated with BMI and genes associated with WHR.

Pathway analysis also demonstrated that most WHR genes are expressed primarily in adipocytes and adipose tissue. Lack of evidence for association with brown adipose tissue and other adipose depots is likely due to absence of data for these traits. Using predefined gene sets Shungin et al. [55] observed enrichment for vascular endothelial growth factor (*VEGF*), phosphatase and tensin homolog (*PTEN*), insulin receptor (*INSR*), and peroxisome proliferator activated receptors (*PPAR*s). PPARs regulate expression of genes involved in, among other things, adipocyte differentiation, lipid metabolism, and energy balance. Pathway analysis implicated adipogenesis, angiogenesis, transcriptional regulation, and insulin resistance as processes affecting fat distribution. Of note, there was no overlap of these pathways with those identified for BMI.

VII CLINICAL IMPLICATIONS OF THE DISCOVERY OF OBESITY GENES

A Identification of Monogenic Causes of Obesity

Until recently, only the rare Mendelian syndromes, such as Prader–Willi and Bardet–Biedl, were known to cause heritable obesity. These disorders are easily recognized, both by a wide spectrum of phenotypes [132,144] and by the use of cytogenetics assays that are widely available. However, the Mendelian, nonsyndromic obesity disorders are not so easily diagnosed, because obesity is often the only apparent phenotype and clinical assays for known obesity gene mutations are rarely practical. It is estimated that 2–7% of morbidly obese patients have mutations in *MC4R* [122,123,145,146] and an unknown, but smaller, percent have mutations in other obesity genes, including *POMC* [147] and *NTRK2* [125]. Thus, only about 1 in 10 morbidly obese patient has a known mutation that explains the obesity, and molecular assays for the currently known Mendelian obesities would be negative in the majority of morbidly obese patients. Also, there are many known distinct mutations in each of these genes. Thus, no clinical laboratories yet provide diagnosis of these mutations, rather they have only been diagnosed by research laboratories. However, inability to make specific molecular diagnosis does not mean that one cannot identify people with increased risk for genetic obesity, and this may influence choices or approaches to treatment.

Several criteria can be used to estimate the probability that an individual's obesity has a genetic cause (Table 21.4). At the present time, due to the lack of data, these estimates do not produce any quantitative values revealing individual risk that obesity is monogenic, but rather just generic classification, such as likely genetic, uncertain, and likely not genetic.

TABLE 21.4 Suggestions to Evaluate Suspected Monogenic Etiology of Severe Obesity[a,b]

Phenotype	Phenotype Indicative of Genetic Etiology
Characteristic of All Genetic Obesities	
Family history	Having first-degree relatives with severe obesity
Age of onset	Normal birth weight but age of onset of obesity before age 10
Hyperphagia	Hyperphagia developing within first year of life
	Aggressive food-seeking behavior
Phenotype Associated with Specific Gene Mutation	
Very low leptin levels	Mutation in *LEP*
Hypogonadism, delayed puberty, lack of growth spurt	Mutation in *LEP* or *LEPR* or *PCSK1*
Disproportionate insulin resistance	Mutation in *SH2B1*
Developmental delay	Mutation in *SH2B1* or *SIM1*
Low ACTH or high proinsulin levels	Mutation in *POMC* or *PCSK1*
Frequent infections	Mutation in *POMC* (ACTH), *LEP* or *LEPR*
Defective prohormone processing	Mutation in *CPE* or *PCSK1*
Red hair segregating with obesity	Mutation in *POMC*
Severe hyperinsulinemia, acanthosis nigricans	Mutation in *MC4R*
Accelerated linear growth, increased bone mass	Mutation in *MC4R*
Delayed language skills, impaired short-term memory	Mutation in *NTRK2* or *BDNF*

[a]*Data adapted from ([169,170,155,161], [172], [171,128,132,148]).*
[b]*For a complete algorithm for the assessment of a severely obese individual, see [132].*
BDNF, brain-derived neurotrophic factor; *LEP*, leptin; *LEPR*, leptin receptor; *SH2B1*, SH2B adapter protein 1; *SIM1*, single-minded homolog 1; *MC4R*, melanocortin 4 receptor; *NTRK2*, neurotrophic tyrosine kinase receptor, type 2; *PCSK1*, proprotein convertase subtilisin/kexin-type 1; *CPE*, carboxypeptidase E; *POMC*, proopiomelanocortin.

Factors indicating a genetic basis for obesity are: (1) a family history of obesity is consistent with the presence of an obesity gene shared among family members; (2) early age of onset and extreme obesity indicate a genetic basis for obesity; and (3) children with single-gene obesity are normal weight at birth but severe early hyperphagia, often associated with aggressive food-seeking behavior, results in rapid weight gain, usually beginning in the first year of life. Severe obesity in children has been variously defined as a standard deviation score for BMI of more than 2.5 [148] or 3 [128] relative to the appropriate reference population. Extreme trait values are more likely to be genetic for many complex diseases, simply because extremes tend to result from the actions of severe mutations or from mutations in genes that have larger effects [149].

At present, a few diagnostic tools are available for the medical evaluation of patients suspected of having monogenic obesity. The only screening tests available are for those mutations that cause endocrine abnormalities. Serum leptin should be measured. Very low or very high serum leptin levels will indicate mutation in *LEP* or *LEPR*, respectively. However, lack of very high leptin levels cannot rule out homozygous mutations in *LEPR* [128]. A subset of obese individuals has inappropriately low leptin levels for their fat mass, suggesting a less severe defect in leptin regulation [150]. ACTH and proinsulin should be measured to indicate defects in *POMC* or in prohormone processing. Insulin should be measured to evaluate the appropriateness of the degree of hyperinsulinemia as this may indicate an *MC4R* mutation.

Physical appearance provides evidence of *POMC* mutations or the syndromic obesities. *POMC* defects can cause red hair and obesity [121], although most red hair results from mutations in melanocortin 1 receptor (*MC1R*) [151], which does not influence obesity. Thus, red hair is only informative when red hair, ACTH deficiency, and obesity cosegregate within a family.

Prader–Willi, Bardet–Biedl, and other syndromic obesities can be diagnosed by a variety of characteristic

phenotypes, such as small hands and feet, polydactyly, and mental retardation as well as by cytogenetic assays. Thus, one should rule out these diagnoses by phenotype determination and by absence of characteristic chromosomal abnormalities.

B Personalized Treatment Based on Genotype

At present the impact of genetics on diet effectiveness has been the subject of many papers, but all current studies have severe limitations. First, there are some large longitudinal or cohort studies that have reported statistically significant diet−genotype interactions. However, diet-based correlations have yet to provide evidence that stands the test of time. For example, correlations formed the basis for advice to avoid cholesterol and saturated fat, which have rarely been supported by randomized controlled trials. Second, all current randomized controlled trials are underpowered for genetics and thus find no or few significant results. Third, the underlying diet studies test too few diets for too short a time. Not even one large, well-powered study has examined diet−genotype interactions for diets that range from ketogenic to low carbohydrate or the typical U.S. diet to extreme low fat and vegan. Many basic questions are thus unanswered. For instance, does each person have one ideal diet for weight management or many possible equally healthy diets?

Matching diets to genotype is a goal for personalized medicine. Goals of personalized medicine are sometimes called P4; predictive, preventative, personalized, and participatory. The ability to calculate GRS is now well established but surprisingly, GRS may not predict weight gain or loss. Other components of P4 are not as advanced. Diet predictions based on questionnaire are flawed because diet questionnaires are unreliable. If people are resistant to trying new diets on their own, will they also resist when some professional or expert says "your obesity GRS means that you should be eating…?" One recent study reported that subjects told that they have higher genetic risk alleles of *FTO* had enhanced readiness to control weight but the knowledge of *FTO* status had no impact on behavior [152].

A 2016 NIH Working Group Report [153] on using genomic information to guide weight management pointed out that technologies are available for the fast characterization of the transcriptome, proteome, epigenome, and metabolome of an individual. But effective algorithms are yet to be developed to combine these data with classical medical and behavioral measures of the individual to personalize weight loss recommendations in the clinical setting.

Despite the ability to generate overwhelming amounts of genetic and other data for people, P4 recommendations for diet cannot be implemented. It is not known which of the many natural variants detected matter, nor do the diet studies needed to evaluate variants for causal effects on diet−genotype interactions exist. Thus, for a long foreseeable future, individuals will need to determine optimal diets by personally testing several different diets. The first step toward generalized discovery of personalized diets will require large highly powered randomized diet studies testing a full range of diets.

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