Struan F.A. Grant *Editor*

The Genetics of Obesity

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Chapter 1
Genetic Variation and Obesity Prior to the Era of Genome-Wide Association Studies

Shana E. McCormack

Abstract The purpose of this chapter is to present a comprehensive review of the evidence for how genetic variation contributes to common obesity (which we have chosen to define as affecting >1 % of obese individuals, likely non-syndromic in etiology, and related to diet and inactivity) in the general population prior to the era of genome wide association studies (GWAS). Twin and adoption studies demonstrate that the tendency to be obese is highly heritable, and also suggest that the cumulative effect of gene–environment interactions on body mass index (BMI) seems to increase with age. Before GWAS, one common approach to dissecting the role of genetic variability in common obesity was to investigate candidate genes. Some of the genes implicated in monogenic or syndromic obesity by traditional linkage analysis, for example, *MC4R*, have milder variants that appear to be important in common obesity as well. By 2004, over 600 candidate genes or chromosomal regions had been implicated in the pathogenesis of obesity, and 18 of these had multiple lines of supporting evidence. Despite this, much of the heritability in obesity remained to be explained. Next-generation sequencing technology should produce additional insights that extended these seminal investigations but despite this, much of the so-called missing heritability identified prior to the era of GWAS persists.

The global prevalence of obesity has risen rapidly, in particular during the latter part of the twentieth century and the beginning of the twenty-first century. Obesity became a World Health Organization priority after its significant contribution to cardiovascular disease burden as well as all-cause mortality from other conditions, including cancer, came to be appreciated [1]. In the USA, the harbinger of the global

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epidemic, obesity prevalence nearly tripled, from 12 to 33 % in men and 17 to 37 % in women between 1971 and 2006 [2].

Part of the impetus to pursue studies related to the genetics of obesity is an attempt to explain its rising prevalence, and in particular, the rising frequency of extreme and young-onset obesity phenotypes [3]. Nuclear genetic variation alone is unlikely to account for such a recent and rapid change in phenotype; a complex variety of interacting environmental, nutritional, microbial, epigenetic, behavioral, sociocultural, economic, and other factors likely contribute as well [4]. Genetics, however, may help to explain why some individuals are more vulnerable to similar so-called obesogenic environmental influences than others. An understanding of differential genetic susceptibility may inform our search for modifiable or even reversible obesity risk factors, and motivate individualized, targeted prevention efforts.

Prior to the era of genome-wide association studies (GWAS), which will be described in greater detail in subsequent chapters, these efforts took several forms. The purpose of this chapter is to present a comprehensive review of the evidence for how genetic variation contributes to common obesity (which we have chosen to define as affecting >1 % of obese individuals, likely diet-induced, and apparently non-syndromic) in the general population prior to the era of GWAS.

First, twin and adoption studies are two strategies to produce heritability estimates for obesity, and the successes and limitations of this work are considered here. Next, racial and ethnic differences in susceptibility to obesity, along with related traits (body composition and resting energy expenditure) may be related to inherited factors, and some of this is discussed as well. In addition, for individuals who have obesity along with other unique clinical features (e.g., intellectual impairment, dysmorphism, visual or hearing anomalies) and similarly affected family members, an inherited syndrome may be present, and traditional linkage analyses have been used to implicate a particular chromosomal region in these patients. In some cases, the relevant gene(s) and function(s) have been characterized (e.g., *MC4R* and Bardet–Biedl syndrome); the reader is referred to the chapters on monogenic and syndromic obesity for a more detailed discussion of these. For some of these genes where mutations cause severe obesity phenotypes, milder variants seem to play a role in common obesity, and we present some of this evidence here, and in the Table 1.1 below.

Some of the identified genes belong to biological pathways whose integrated function affects the propensity to develop obesity, including, for example, the hypothalamic regulation of appetite and energy balance. As the nature of these pathways has been more fully elucidated in model systems, new candidate genes have been put forth and their contribution to common obesity has been investigated, and some of these findings are reviewed, as well as summarized in the Table 1.1. By 2004, over 600 candidate genes or chromosomal regions had been implicated in the pathogenesis of obesity, and 18 of these had multiple lines of supporting evidence [5]. The advent of next-generation sequencing technology should build on these seminal investigations, and we conclude this chapter by outlining some of the most pressing questions in the field for which, it was hoped, the new techniques would provide much-needed answers.

Table 1.1 Identification of selected candidate genes, and investigation of their contribution to common obesity prior to GWAS

Candidate gene	Mechanism	OMIM #	Role in common obesity (>1 %) demonstrated prior to era of GWAS (or after, where noted, in candidate approach)
Monogenic, obesity-predominant (see text)			
Leptin	Secreted by adipocytes in white adipose tissue [32]; circulating levels higher in obesity [39]	614962	Pre-GWAS studies suggested association, particularly in 5′ region, with common obesity [44] that may be sex-dependent [45]
Leptin receptor	Hypothalamic receptor for leptin [38]	614963	Mutations may be present in up to 3 % of children with severe, early-onset obesity [42], but consistent results for variation in larger populations was lacking
Melanocortin 4 receptor (*MC4R*)	Hypothalamic G-protein-coupled receptor [49]	155541	Most variation in severe obesity explained by a single locus prior to GWAS [54]
Pro-opiomelanocortin (*POMC*)	Hypothalamic preprohormone produces α-MSH, a ligand for MC4R [56]	609734	Variants at *POMC* locus affect BMI in Europeans [59] and Hispanic Americans [60], and influences waist-hip ratio in the normal range [113]
Proprotein convertase subtilisin/kexin type 1 (*PCSK1*)	Prohormone convertase, cleaves POMC into signaling ligands [56]	600955	Post-GWAS studies demonstrate a role in common obesity [63]
Syndromic (selected)			
Prader–Willi Syndrome (deletion of paternally imprinted *SNRPN*, necedin, possibly others 15q11-q13)	Disorder of imprinting/maternal heterodisomy [114]	176270, 182279 (SNRPN), 602117 (necedin)	Possible association with copy number variation at this locus in later candidate study [115]; necedin candidate study did not disclose an association [116]
Bardet–Biedl (at least 18 implicated loci)	Disorder of ciliary function [117], hypothalamic appetite dysregulation [118]	209900, many implicated genes	Possible evidence in isolated population [119], relationship of MKKS and common obesity was investigated, no clear evidence [120], possible evidence in weight loss [121]
Alstrom syndrome (*Alms1*) [122, 123]	Ciliary dysgenesis is implicated [124]	203800	Early association studies did not identify a role for variation in common obesity [125]

(continued)

Table 1.1 (continued)

Candidate gene	Mechanism	OMIM #	Role in common obesity (>1 %) demonstrated prior to era of GWAS (or after, where noted, in candidate approach)
Associations by other candidate approaches			
Hypothalamic			
AGRP (agouti-related protein)	Cell-signaling protein, antagonist at melanocortin-3 and melanocortin-4 receptors [126]; plasma levels related to obesity [127]	602311	Variation may be related to age-dependent onset of obesity [65]; possible association of a variant with reduced fat mass [66]
Brain-derived natriuretic factor (*BDNF*)	Neuronal growth factor [128] downstream of MC4R [68]	113505	Association with disordered eating (including bulimia) has been identified [73]
Syndecans 1,3 (Sdc 1,3)	Membrane-bound heparin sulfate proteoglycans, feeding in mice [67, 129]	186355, 186357	Variation associated with obesity in Koreans [130]
Single-minded, drosophila, homolog (*Sim1*)	Sim1 haploinsufficiency appear to cause hypothalamic obesity in mice [74]	603128	Candidate approach suggests a possible association [76]; association in the Pima Indians where risk allele is major allele [77]
Cocaine- and amphetamine-regulated transcript (*CART*)	Anorectic peptide activated by leptin [78]; a single family [79] where mutation co-segregated with phenotype	602606	Initial study negative, e.g., [80]; sequencing demonstrated possible association [81]
Mitochondrial			
Uncoupling proteins (nuclear-encoded)	Directing energy to either ATP production or thermogenesis [131]	113730, 602044	REE (racial differences) [22, 23] Lifetime weight gain [132, 133]
Enteroendocrine			
Ghrelin/obestatin (*GHRL*); growth hormone secretagogue receptor (*GHSR*)	Growth hormone, secretagogue, role in energy homeostasis [134], may be a role for rare variants [84]; Circulating levels role in long-term weight loss [83]	605353, 601898	Initial work suggesting variation contributing to common obesity not confirmed [85]; later work demonstrates an association of variation in the *GHSR* and BMI [86]

Peptide YY (*PYY*)	Enteroendocrine hormone [88]; rare variant with altered function segregated with obesity in a family [89]	600781	*PYY* haplotype related to circulating levels and metabolic traits [90]; possible association with obesity in young African-American adults [91]
Fat and glucose utilization			
Peroxisome proliferator activated receptor (PPARs), peroxisome proliferator activated receptor gamma coactivator 1 beta (*PPARGC1B*)	Lipid sensors, may control adipocyte expansion in relation to BMI [93]	601487, 608886	Association of Pro12Ala variant in *PPAR2* with obesity in Caucasians [94] and the general population [95], initial observations in *PPARGC1B* also [96]
Nuclear receptor subfamily 0, group B, member 2 (*NR0B2*)	Orphan nuclear receptor [97], initial association with HNF4-α caused exploration of DM association	604630	Variation associated with obesity in Japanese [98], but less often in Danish [99]
Ectonucleotide pyrophosphatase/ phosphodiesterase type 1 (*ENPP1*)	Plasma membrane glycoprotein [135] associated with generalized arterial calcification of infancy, rickets [101, 102]	173335	Ethnic susceptibility to insulin resistance [100], many early studies focus on this aspect of its pathophysiology
Beta-adrenergic receptors (*ADRBs*)	Beta-adrenergic receptor [103]	109690, 109691	Polymorphisms are frequent, with functional consequences [104], including resistance to catecholamine-induced lipolysis [106]

Evidence for a Genetic Component to Obesity

The obesity epidemic led to a renewed interest in the pathogenesis of obesity. An excess of readily available, highly processed, nutritionally dense foods along with reduced physical activity and increased time spent sitting are all implicated in persistent positive energy balance that leads to overweight [6, 7]. In light of this, the role of genetics remained to be explained in the face of such a rapid change in the prevalence of obesity, especially extreme phenotypes.

One line of thinking invoked the existence of the "thrifty phenotype" originally proposed by Neel in 1962 [8] and cited with increasing frequently as the obesity epidemic progressed e.g., [9]. According to this argument, in prehistoric times there would have been positive selection for traits conferring the ability to store energy efficiently in periods of limited food availability. Alternate views exist, however; although this hypothesis is appealing, it may not explain, for example, observed metabolic responses to physiologic challenges such as famine. In utero epigenetic modifications may produce the observed correlation between intrauterine and postnatal conditions. The "drifty gene" hypothesis has been postulated as well, i.e., that permissive drift is a viable alternative explanation for the existence of genetic variants conferring increased risk for metabolic efficiency and obesity. Clearly, the role of genetics in modifying obesity risk in an era of nutrient excess and deficient activity is complex and far from being completely understood. Estimates of the relative contribution of genetic and individual or shared environmental factors are presented here.

Heritability

The heritability of any condition refers to the proportion of phenotypic variability accounted for by genetics; for a detailed consideration of techniques for heritability estimates, the reader is referred to any number of reviews on the topic e.g., [10]. For a condition that is becoming increasingly prevalent, and apparently occurs more often in relatives, disentangling the contributions of genes and shared adverse environmental influences becomes challenging. Two frequently employed strategies for generating heritability estimates are twin studies and adoption studies.

Twin Studies

The value of twin studies lies in the high degree of genetic similarity between monozygotic (100 %) as compared to nonidentical dizygotic (50 %) twins; both sets of individuals also shared both intrauterine and, to some extent, extrauterine environments. The degree of similarity, or concordance, between monozygotic as compared to dizygotic twins would be expected to be greater in proportion to the

relative importance of their shared genetic information. Indeed, as expected for a heritable trait, estimates of concordance for fat mass between pairs of monozygotic twins are between 70 and 90 %, while for dizygotic twins, they are closer to 35–45 % [11–13]. Fatness is thus considered highly heritable, similar to adult height. The variability in these estimates illustrates a recurrent theme in these studies, that is, the developmental specificity of heritability. In general, estimates of heritability for body mass index (BMI) tend to increase with age, and suggest that interacting gene–environment effects may be cumulative. In another important male twin-pair study, heritability increased from 77 to 84 % over the course of 25 years of longitudinal follow-up [14]. In another study, aggregate data from 23 twin-cohorts demonstrated that heritability for BMI was lowest at birth and increased to over half or more of the variance by as early as 5 months of age [15]. Finally, a study carried out during the obesity epidemic concluded that heritability estimates remained constant, and emphasized the relative importance of individual, non-shared environments [16].

Beyond the biological insights they offer about physiologic regulation of appetite and energy balance, these types of estimates have public health and policy implications, with respect to which individuals to target and by what means to achieve the biggest reduction in overweight and obesity. Adoption studies provide additional information.

Adoption and Family Studies

Adoption studies have provided additional evidence of the genetic contribution to obesity. In one study, adoptees demonstrated more similarity to their biological than their adoptive parents with respect to BMI [17]. In this seminal work, BMI of the biological mother was found to be most closely related to BMI, although a positive association existed for the biological father as well. The authors noted that this association is present across the range of BMI categories (i.e., very lean through obese), pointing to the heritability of low as well as high measures of body fatness. No relationship between adoptees' BMI and the BMI of adoptive parents was identified. In a separate investigation, identical twins raised apart, but not nonidentical twins raised apart, retain some concordance in BMI [18], again indicating the importance of shared genetics in determining common risk for adiposity. Therefore, age-specific methodological effects may be important in such studies.

Racial/Ethnic Differences

Racial and ethnic differences exist in the prevalence of obesity, which also suggests that there may be an effect of shared ancestry, with some populations having an excess risk for obesity, perhaps exacerbated in the context of particular environmental influences. For example, the risk of obesity is 50 % or more in Pima Indians,

conferring an excess risk for diabetes mellitus in this group [19]. By WHO estimates for 2010, the rate of obesity (BMI > 30 kg/m^2) among men ages 30–100 was substantially less than 1 % in Eritrea but over 80 % in Nauru, a Micronesian island in the South Pacific; similar results were seen in women. (Median percentage of obese individuals over 30 was approximately 12 % for both men and women.)

In the USA, there is variation in the prevalence of obesity by racial group [20], including, for example, ongoing increases in non-Hispanic black and Hispanic women, and a recent Endocrine Society scientific and policy statement emphasized the importance of elucidating the complex interacting social, cultural, biological, and genetic factors that may underlie these differences [21].

Some evidence implicates differences in coupling of oxidative phosphorylation to ATP production (as opposed to thermogenesis, through controlled leak of protons across the mitochondrial matrix) through variation of uncoupling proteins [22, 23]. Although the contribution of mitochondrial variation (either nuclear- or mitochondrial-encoded) to obesity is not clear [24], it may be that variation needs to be studied in context of important population and environmental influences including migration patterns, ambient temperature, and altitude [25].

Overall, findings from twin and adoption studies, and differences between racial and ethnic groups do suggest that genetic variation underlies much of the pathogenesis of obesity, but many of the complex mechanism(s) by which this occurs remain elusive.

Previous Genetic Studies in Obesity and the Need for GWAS Approaches

Families with monogenic forms of early onset, apparently isolated childhood obesity have been studied in the context of known candidate genes (see below) and have also yielded loci that remain incompletely characterized. Regarding syndromic obesity (where obesity occurs along with congenital and developmental anomalies), Prader–Willi syndrome [26], Bardet–Biedl syndrome [27–29], and Alström's syndrome [30] are better understood examples, both with respect to underlying molecular mechanisms, and the potential relevance of these for common obesity, as reviewed in the Table 1.1. Overall, candidate gene approaches have yielded some insights prior to the era of GWAS, as summarized by the Table 1.1, but many studies were underpowered to detect more modest effects.

Many candidate genes were chosen for studies on the basis of the known neurophysiology of appetite. The neural circuitry underlying hypothalamic control of appetite and energy balance has been the focus of intensive ongoing research, and seminal work in model systems identified targets for further studies in humans. Indeed, we will describe several important examples of mutations identified in these genes in forms of familial, early-onset morbid obesity where there are either no or only subtle other congenital anomalies or developmental manifestations. These discoveries required comprehensive phenotyping of large numbers of families

with apparently isolated severe obesity [31]. We focus in more detail on the genes directly implicated in hypothalamic regulation of appetite, and also include in the Table studies on enteroendocrine regulation of appetite (ghrelin and its receptor, peptide YY) as well as glucose- and lipid-sensing and regulation, including the peroxisome proliferator-activated receptor family (PPARs) and beta-adrenergic receptors.

Hypothalamic Leptin–Melanocortin Pathway

In mice, the *ob* gene is expressed primarily in white fat and encodes a secreted protein called leptin; although this gene was initially cloned and characterized in mice, a human homolog has also identified [32]. Homozygous mutations in the rodent *ob* gene lead to severe obesity, and adult mice outweigh their lean littermates by more than three times [33]. This phenotype is characterized by severe leptin deficiency, and can be rescued by administration of recombinant leptin [34–36]. In contrast, a different strain of mouse with mutations in the primarily hypothalamic leptin receptor (the so-called *db*/*db* mouse, a model of type 2 diabetes mellitus [37]), exhibit no response to recombinant leptin [38]. Taken together, these results indicate that circulating leptin serves as a homeostatic indicator of the degree of adiposity, and that, physiologically low levels promote food-seeking behavior while high levels inhibit this same behavior. In further support of this proposed mechanism, in humans serum concentrations of leptin do exhibit positive association with obesity [39].

Two children in the same highly consanguineous family with nearly undetectable levels of leptin despite extreme obesity were the first identified human cases of congenital leptin deficiency; they had homozygous frameshift mutations in the leptin gene detected via sequencing of this candidate gene [40]. Later, using a similar strategy, humans with homozygous mutations in the leptin receptor mutation were found [41]. In the latter cases, affected individuals exhibited other endocrinopathies, including hypogonadotropic hypogonadism, and decreased secretion of growth hormone and thyrotropin. When 300 individuals with severe, early-onset obesity all underwent sequencing of the leptin receptor, 3 % had pathogenic mutations, all of which were homozygotes or compound heterozygotes due to a high proportion of individuals from consanguineous families (90 out of 300) [42]. These individuals also displayed hypogonadotropic hypogonadism, as well as defects in immune function.

Although early follow-up studies could not identify evidence for the association between variation within the leptin gene itself and common obesity [43], variation in its 5′ region of the gene has been reproducibly associated with propensity for weight loss, as well as common obesity [44]. These pre-GWAS studies suggested some potential sex-dependence of these effects [45]. With respect to the leptin receptor, one small series of Greek individuals identified a modest contribution of variation in the leptin receptor to BMI [46]; like leptin, variation in the leptin receptor may contribute more readily to other physiologic traits like blood pressure [47] or insulin secretion [48]. The difficulty of concluding whether variation in these loci

primarily contribute to common obesity highlights the value of subsequent GWAS studies with much greater power in both discovery and validation cohorts.

As the role of the hypothalamus in regulating appetite and energy balance was investigated further, other components of the so-called leptin–melanocortin pathway were elucidated and characterized, both in model organisms and in humans. The melanocortin family of receptors (MCR) is a G-protein coupled receptor class that, like the leptin receptor, is highly expressed in the hypothalamus and modulates food-seeking behavior. Mice with disruptions in the melanocortin 4 receptor (MC4R) activity are hyperphagic. Unlike the leptin or leptin receptor mutants, obesity develops later in life, and is accompanied by increased, rather than decreased, linear growth; in addition, hyperinsulinemia is also present [49]. Sequencing the gene encoding MC4R in obese humans has led to the discovery of mutations responsible for a co-dominantly inherited form of familial obesity [50–53]. In one seminal series of 500 children with severe, early-onset obesity, 5.8 % were found to have *MC4R* mutations [54], supporting the conclusion that variation at this locus is the most common genetic cause of obesity identified before the era of GWAS. Similar to the affected rodents, individuals were tall with increased lean mass and hyperinsulinemia. Gene dosage and degree of receptor function modified the phenotype. Later, decreased energy expenditure was also implicated in the etiology of obesity in these individuals [55].

Leptin signaling and MC4R activity are connected via multiple signaling pathways, most notably, pro-opiomelanocortin. Pro-opiomelanocortin is a precursor protein encoded by a gene of the same name. It is cleaved, in part by a prohormone convertase (PC1/3) encoded by the *PCSK1* gene, into melanocortin peptides, including adreoncorticotrophin (ACTH), beta-endorphin, and the melanocyte-stimulating hormones, including anorexigenic α-MSH [56]. This latter is a ligand for MC4R. Multiple ligands for this receptor exist, including agouti-related peptide, an orexigenic peptide.

Expression of POMC is regulated by leptin via activation of its receptor on POMC neurons [57]. Two unrelated individuals of German descent were found to have mutations in *POMC*; their phenotypes included adrenal insufficiency (related to insufficiency in ACTH) and red hair (related to decreased melanin) in addition to early onset obesity [58]. When examined with respect to common obesity, variations in the *POMC* gene have also been related to obesity in individuals of European [59] and Hispanic American [60] descent.

The prohormone convertase 1/3 is encoded by the *PCSK1* gene, and affects processing of other hormones besides POMC, most notably proinsulin, the precursor of insulin C-peptide. A woman who was a compound heterozygote for mutations in PC1 was identified [61] whose phenotype recapitulated that of the so-called *fat*/*fat* mouse that harbors mutations in the carboxypeptidase E gene, encoding another prohormone convertase. This included early-onset obesity with hyperphagia, pituitary hypofunction (hypogonadotropic hypogonadism and hypocortisolism), as well as disordered glucose homeostasis. Other individuals with mutations in this gene were also described [62]. The consistent role for variation in *PCKS1* in common obesity was not demonstrated until the GWAS era [63].

The initial report of the *MC4R* mutant mouse noted phenotypic similarities to a mouse that overexpresses agouti ("yellow") protein [64], see Table 1.1; disruption of pigment proteins related to POMC leads to this coloring. Variation at the agouti-related protein locus may be related to age-dependent onset of common obesity [65], and there is another possible association of a different variant with reduced fat mass identified in a candidate gene study [66]. The syndecan family of cell surface heparan sulfate proteoglycans can mechanically potentiate the ability of agouti-related protein to inhibit α-MSH; transgenetic alteration of the endogenous hypothalamic syndecans in mice can lead to hyperphagia and maturity-onset obesity suggesting reduced α-MSH signaling [67].

Rodent studies of downstream targets of MC4R have identified brain-derived neurotrophic factor (BDNF), a nerve growth factor expressed in the ventromedial hypothalamus. It has been shown to modulate appetite and energy balance in response to MC4R signaling [68]. Animals missing BDNF, hyperinsulinemic, grow rapidly, and have increased locomotor activity, and the phenotype is rescued with central infusion of BDNF [69]. In humans, loss of only one copy of the *BDNF* gene leads to obesity, hyperphagia, intellectual impairment and hyperactivity [70]. Indeed, it may be haploinsufficiency of *BDNF* that leads to the childhood onset obesity in some individuals with WAGR syndrome (Wilms' tumor, aniridia, genito-urinary abnormalities, and mental retardation) if the extent of the responsible deletion includes that gene [71]. In additional support for the role of this effector, a de novo mutation in a tyrosine kinase receptor downstream of BDNF also produces a similar phenotype [72]. The role for variation in *BDNF* in common obesity per se was not clearly demonstrated, although it was found to be associated with neuropsychiatric conditions also characterized by disordered eating (e.g., [73]).

Sim1 (Single-minded, drosophila, homolog) is another gene whose disruption and/or haploinsufficiency [74] have been reported to cause hypothalamic obesity with hyperphagia. *Sim1* (+/–) heterozygous mice demonstrate high levels of leptin, in keeping with their elevated fat mass, as well as hyperinsulinemia and increased linear growth. Human studies demonstrate similar results. A de novo balanced translocation disrupting *Sim1* was observed in a girl with severe early-onset obesity and normal resting energy expenditure [75]. Mechanisms invoked have included decreased cellularity of the paraventricular nucleus (PVN) whose development is regulated by *Sim1*; PVN cells also express MC4R that may be regulated by α-MSH. A candidate gene study suggested a possible association of common variants in *Sim1* with BMI and weight gain in individuals of European descent [76]. An association was also identified in the Pima Indians where the risk allele is the major allele [77].

Another focus of investigation has been the *CART* gene (Cocaine- and Amphetamine-Regulated Transcript), which encodes an anorectic peptide activated by leptin [78]. One family has been identified [79] where a mutation in this gene co-segregated with an extreme obesity phenotype. An initial study in Europeans did not find an association with obesity, but did find a relationship with metabolic traits that mediated its effect via fat distribution (waist-to-hip ratio) [80]. Sequencing of the putative promoter region also identified variants that were associated with BMI and, in particular, extreme obesity [81].

Enteroendocrine Hormones

Ghrelin was discovered initially as the endogenous ligand for the growth hormone secretagogue receptor; studies in rodents demonstrated that, like leptin, ghrelin responds to a peripheral signal regarding nutrient availability and appears to convey this information to the hypothalamus. Ghrelin is produced in the stomach, and its level rises with fasting and promotes increased food seeking, as well as decreased fat utilization, and is hypothesized to reflect a signal to increase metabolic efficiency in times of low nutrient availability [82]. Circulating levels of ghrelin have been shown to play a role in long-term weight loss, likely by helping to homeostatically defend a metabolic "set-point" [83]. In one study, 6.3 % of severely obese adult women of European descent were found to be heterozygotes for an amino acid change in the last residue in mature ghrelin; this change was not found in controls, but the functional significance of this change was not defined [84]. Initial work suggesting variation contributed to common obesity was not confirmed [85]; later work, however, demonstrates an association of variation in the gene encoding ghrelin's receptor (*GHSR*) and BMI [86].

Peptide YY (PYY) is an enteroendocrine hormone present in highest concentrations in the ileum and colon that rises in response to food ingestion, in particular, of fat-containing foods [87]. In addition to inhibiting gastric and pancreatic secretion, it is also expressed in some its target tissues suggesting a paracrine feedback mechanism in addition to its known endocrine function [88]. Rare variants in the *PYY* gene with altered function have been reported to segregate with severe obesity in one family [89]. In addition, a *PYY* haplotype has been related to circulating PYY levels as well as metabolic traits [90]. Later studies have demonstrated a possible association with obesity, particularly in young African-American adults [91].

Fat and Glucose Utilization

The peroxisome proliferator activated receptor family (PPARs) is a class of nuclear hormone-sensing transcription factors that convey signals about nutrient availability and coordinate the appropriate cellular metabolic responses [92]. Of particular relevance for obesity, subsets of PPARs act as lipid sensors and may control adipocyte expansion in relation to BMI [93], and are also the target of insulin-sensitizing thiazolidinediones [92]. In recognition of their importance in lipid homeostasis, variation in the genes encoding PPARs has been examined with respect to adiposity. An early finding of an association of the Pro12Ala variant in *PPARγ* with obesity in Caucasians [94] was later also demonstrated in the general population [95]. Initial observations about the relevance of variation in one of the PPAR coactivator genes (*PPARGC1B*) have also been published [96].

Another family of nuclear receptors, the nuclear receptor subfamily 0, group B, member 2 (NR0B2), has also been the subject of investigation. The gene encodes an orphan nuclear receptor [97] that is known to interact with HNF4-α, one of the genes mutated in a monogenic form of diabetes. As a result, variation in this gene

was initially studied with respect to risk for diabetes mellitus, but was found instead to confer risk based on its association with obesity. Specifically, in initial studies, variation was associated with obesity in a Japanese population [98], but less often in Danish individuals [99]. Its relationship with insulin resistance [100] was also the motivation to study ectonucleotide pyrophosphatase/phosphodiesterase type 1 (ENPP1), a plasma membrane glycoprotein that may be associated with obesity. Mutations in the gene encoding this glycoprotein have been identified in patients with generalized arterial calcification of infancy and rickets [101, 102]. Its relationship to common obesity remains to be fully characterized.

Another receptor that influences fuel use in response to circulating signals is the beta-adrenergic receptor [103], which coordinates response to catecholamines as part of the physiologic stress response. Polymorphisms in this receptor are frequent, with functional consequences [104]; while other investigations have focused on asthma phenotypes, in particular, response to sympathomimetic bronchodilator therapy, catecholamine-induced lipolysis is relevant in determining obesity risk. In the longitudinal Bogalusa Heart Study, a cohort of individuals of European and African-American descent, males with the Arg16Gly polymorphism demonstrated an increased association of BMI with age over time [105]. This result also demonstrates the developmental specificity of testing for association of genetic variants with BMI. The functional consequence of differential expression, and presumably, receptor action as well, includes resistance to catecholamine-induced lipolysis [106].

Reviews written prior to the era of GWAS describe the success of approaches used to identify monogenic disorders (linkage and candidate gene studies), but acknowledge the limitations of these strategies in addressing the complex pathogenesis of multigenic traits like common obesity, e.g., [7]. Subsequent investigative strategies have varying capacities to identify the "missing heritability," or the genetic variation that remains to be explained after accounting for what is known. With respect to variation in nuclear DNA, the hypothesized risk allele frequency and effect size are important determinants of experimental power of association studies [107]. Other factors, including more complex structural variation like copy-number variants, or epigenetic modification, may require unique approaches.

Interestingly, some of the first GWAS fulfilled the promise of these innovative technologies, but also highlighted new challenges for discovery of novel biologic pathways and explanation of additional variation. One of the first GWAS of BMI identified variation near *INSIG2*, a gene that encodes a protein that interacts with sterol regulator element binding proteins (SREBPs), transcription factors that control the reverse transport of cholesterol and therefore may have functional significance as well [108]. Inconsistencies and difficulties with replication efforts have been instructive, and remain the subject of ongoing investigation [109]. The first consistently reproducible signal from GWAS for obesity came instead from investigations into type 2 diabetes mellitus; variation near the *FTO* (fat-mass and obesity associated) locus affected diabetes risk by modulating BMI [110–112]. No matter the strategy to identify additional sources of genetic variation, the functional significance of novel findings, interpreted in the context of what is already understood about the complex regulation of energy homeostasis, will continue to be of primary importance.

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Chapter 2
Genetic Obesity Syndromes

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Abstract A number of genetic obesity syndromes have been identified by sequencing candidate genes in patients with severe obesity. Many of the initial findings emerged from studying families who displayed a classical Mendelian pattern of inheritance; however, with more comprehensive genome wide approaches, increasingly more complex models of inheritance are likely to emerge. The functional and physiological characterization of the human obesity syndromes has provided information that has diagnostic value (Fig. 2.1), has led to specific treatments in some patients and continues to provide insights into the mechanisms involved in the regulation of body weight in humans.

Introduction

Traditionally, patients affected by genetic forms of obesity were identified as a result of their association with developmental delay, dysmorphic features and/or other developmental abnormalities, i.e. a pattern of clinical features which represented a recognizable syndrome. However, the identification of genetic disorders that disrupt the hypothalamic leptin–melanocortin signalling pathway has led to the recognition that obesity is the predominant presenting feature in a significant subset of individuals. Based on case series of patients with genetic obesity syndromes, childhood onset of obesity is a consistent feature. For the purposes of clinical assessment, it remains useful to categorize the genetic obesity syndromes as those with dysmorphism and/or developmental delay and those without these features; however, in some cases the spectrum of clinical features can be quite variable (Fig. 2.1).

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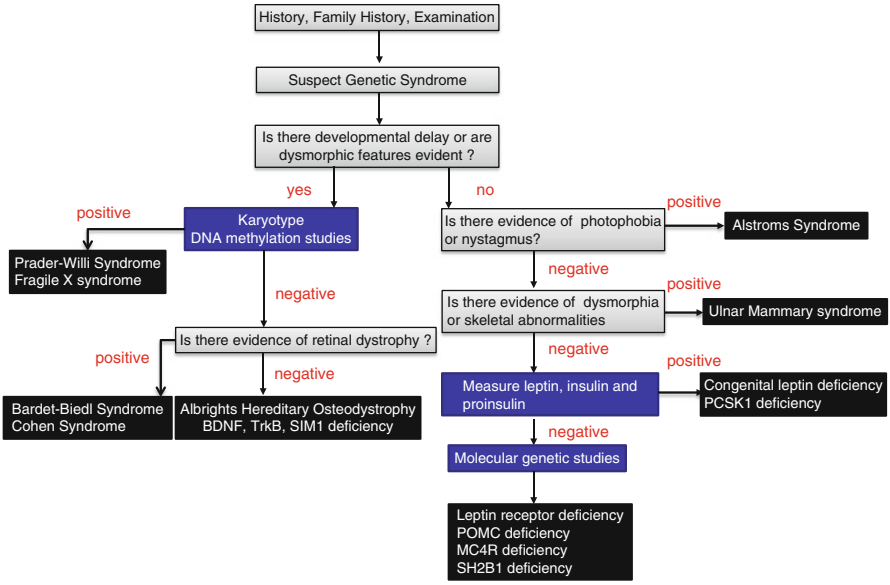

Fig. 2.1 A diagnostic approach to obesity syndromes

Obesity with Developmental Delay

To date, there are at least 30 disorders where obesity is a consistent clinical feature but often associated with mental retardation, dysmorphic features and organ-specific developmental abnormalities. High-throughput next-generation sequencing technologies, and in particular copy number variant detection, are likely to result in the identification and recognition of multiple new syndromes where obesity and developmental delay are closely associated.

Prader–Willi Syndrome

The Prader–Willi syndrome is the most common obesity syndrome (estimated prevalence of about 1 in 25,000). Key clinical features include hypotonia and failure to thrive in infancy, mental retardation, short stature, hyperphagic obesity and hypogonadotropic hypogonadism [1]. Children with Prader–Willi syndrome (PWS) have reduced lean body mass and increased fat mass, abnormalities which resemble those seen in growth hormone (GH) deficiency; GH treatment decreases body fat and increases linear growth, muscle mass, fat oxidation and energy expenditure [2]. Children and adults with PWS have fasting plasma ghrelin levels that are several-fold higher than equally obese controls and patients with other genetic obesity

syndromes [3]. The significance of this finding and its possible role in the pathogenesis of hyperphagia in these patients is unknown.

PWS is caused by deletion of a critical segment on the paternally inherited copy of chromosome 15q11.2-q12, or loss of the entire paternal chromosome 15 with presence of two maternal copies (uniparental maternal disomy). Most chromosomal abnormalities in PWS occur sporadically. Deletions account for 70–80 % of cases; the majority are interstitial deletions, many of which can be visualized by karyotype analysis. There are distinct differences in DNA methylation of the parental alleles, and DNA methylation can be used as a reliable postnatal diagnostic tool in PWS. Small deletions encompassing only the HBII-85 family of snoRNAs have been reported in association with the cardinal features of PWS including obesity [4, 5], suggesting that these noncoding sequences and the genes they regulate may be important.

Albright Hereditary Osteodystrophy

Mutations in *GNAS1* that decrease expression or function of G alpha s protein result in Albright hereditary osteodystrophy (AHO), which is an autosomal dominant disorder. Maternal transmission of *GNAS1* mutations leads to classical AHO (characterized by short stature, obesity, skeletal defects and impaired olfaction) plus resistance to several hormones (e.g. parathyroid hormone) that activate Gs in their target tissues (pseudohypoparathyroidism type IA), while paternal transmission leads only to AHO (pseudopseudohypoparathyroidism). Studies in both mice and humans demonstrate that GNAS1 is imprinted in a tissue-specific manner, being expressed primarily from the maternal allele in some tissues and biallelically in other tissues; thus multi-hormone resistance occurs only when Gs (alpha) mutations are inherited maternally [6].

Bardet–Biedl Syndrome

Bardet–Biedl syndrome (BBS) is a rare (prevalence <1/100,000), autosomal recessive disease characterized by obesity, mental retardation, dysmorphic extremities (syndactyly, brachydactyly or polydactyly), retinal dystrophy or pigmentary retinopathy, hypogonadism and structural abnormalities of the kidney or functional renal impairment. BBS is a genetically heterogeneous disorder that is now known to map to at least 16 loci, with mutations in more than one locus sometimes required for complete expression of the phenotype. Many BBS genes appear to affect proteins localized to the basal body, a key element of the monocilium thought to be important for intercellular sensing in mammalian cells including neurons [7]. Other disorders of ciliary function (e.g. Alström syndrome and Carpenter syndrome) are also associated with obesity. Recent studies in mice have suggested a connection between ciliary function and leptin signalling [8].

BDNF and TRKB Deficiency

Brain-derived neurotrophic factor (BDNF) is one of several nerve growth factors which activate signalling by the tyrosine kinase receptor tropomycin-related kinase B (TrkB) which may lie distal to melanocortin 4 receptor (MC4R) signalling. We reported a child with severe obesity, impaired short-term memory and developmental delay who had a de novo missense mutation impairing the function of TrkB [9]. We also identified a patient with a de novo chromosomal inversion, which encompasses the *BDNF* locus and disrupts *BDNF* expression [10]. Yanovski and colleagues showed that in patients with WAGR syndrome, a subset of deletions on chromosome 11p.12 which encompass the *BDNF* locus, were associated with early-onset obesity [11].

SIM1 Deficiency

Single-minded 1 (SIM1) is a basic helix–loop–helix transcription factor involved in the development and function of the paraventricular nucleus of the hypothalamus. Obesity has been reported in a patient with a balanced translocation disrupting SIM1 [12] and multiple heterozygous missense mutations have been identified in severely obese patients. *SIM1* variants with reduced activity co-segregate with obesity in extended family studies with variable penetrance. The phenotypic similarities between patients with SIM1 deficiency and MC4R deficiency suggests that some of the effects of SIM1 deficiency are mediated by altered melanocortin signalling. In some cases, *SIM1* variant carriers have been reported to exhibit a spectrum of neurobehavioural features including autistic type behaviours. These features are not recognized features of MC4R deficiency but show some overlap with the behavioural phenotypes seen in Prader–Willi Syndrome. As the hyperphagia of *sim1* haplo-insufficient mice is partly ameliorated by the central administration of oxytocin [13], a neurotransmitter involved in the modulation of emotion and social interaction, impaired oxytocinergic signalling is one possible mechanism implicated in the obesity and behavioural phenotype seen in *SIM1* variant carriers.

Obesity Without Developmental Delay

Severe obesity can result from a multiplicity of defects involving the leptin–melanocortin pathway. Leptin is an adipocyte-derived hormone whose circulating levels correlate closely with fat mass. The physiological effects of leptin are mediated through the long isoform of the leptin receptor which is widely expressed in the hypothalamus and other brain regions involved in energy homeostasis. Leptin stimulates the expression of pro-opiomelanocortin (POMC) in primary neurons located

in the arcuate nucleus of the hypothalamus. POMC is extensively post-translationally modified to generate the melanocortin peptides, which activate the melanocortin receptors to modulate diverse functions in the central nervous system, the adrenal gland and the skin. The melanocortins are agonists at melanocortin receptors and suppress food intake. In addition, leptin inhibits orexigenic pathways, mediated by neurons expressing the melanocortin antagonist Agouti-related protein and neuropeptide Y (NPY); NPY can suppress the expression of POMC. These two sets of primary leptin-responsive neurons project to second-order neurons expressing MC4R. Targeted genetic disruption of MC4R in mice leads to increased food intake and increased lean mass and linear growth [14].

Leptin and Leptin Receptor Deficiency

Amongst patients with hyperphagic obesity of early onset from consanguineous families, the prevalence of leptin mutations is approximately 1 % and of leptin receptor mutations, 2–3 %. Leptin receptor mutations have been found in some non-consanguineous families, where both parents are unrelated but happen to carry rare alleles in heterozygous form. Serum leptin is a useful test in patients with severe early onset obesity as an undetectable serum leptin is highly suggestive of a diagnosis of congenital leptin deficiency due to homozygous loss of function mutations in the gene encoding leptin. Serum leptin concentrations are appropriate for the degree of obesity in leptin receptor deficient patients and as such an elevated serum leptin concentration is not necessarily a predictor of leptin receptor deficiency [15].

The clinical phenotypes associated with congenital leptin and leptin receptor deficiencies are similar. Leptin and leptin receptor deficient subjects are born of normal birth weight but exhibit rapid weight gain in the first few months of life resulting in severe obesity [16]. Affected subjects are characterized by intense hyperphagia with food seeking behaviour and aggressive behaviour when food is denied, and energy intake at an ad libitum meal is markedly elevated. While measurable changes in resting metabolic rate or total energy expenditure have not been demonstrated, abnormalities of sympathetic nerve function in leptin deficient adults suggest that autonomic dysfunction may contribute to the obesity phenotype observed. Leptin and leptin receptor deficiency are associated with hypothalamic hypothyroidism; normal pubertal development does not occur in adults with leptin or leptin receptor deficiency, with biochemical evidence of hypogonadotropic hypogonadism. However, there is some evidence for the delayed but spontaneous onset of menses in some leptin and leptin receptor deficient adults. Leptin and leptin receptor deficient children have normal linear growth in childhood and normal IGF1 levels. However, because of the absence of a pubertal growth spurt the final height of adult subjects is reduced. Children with leptin deficiency have impaired T cell number and function, consistent with high rates of childhood infection and a high reported rate of childhood mortality from infection.

Although leptin deficiency appears to be rare, it is entirely treatable with daily subcutaneous injections of recombinant human leptin with beneficial effects on the degree of hyperphagia, reversal of the immune defects and infection risk and permissive effects on the development of puberty [16]. Such treatment is currently available to patients on a named patient basis. The major effect of leptin administration is on food intake, with normalization of hyperphagia and enhanced satiety [16, 17]. Leptin is also involved in mediating food reward [18, 19]. Leptin administration does not result in a change in energy expenditure; however, as weight loss by other means is associated with a decrease in basal metabolic rate, the absence of an effect is notable.

Disorders Affecting Pro-opiomelanocortin (POMC) and POMC Processing

Children who are homozygous or compound heterozygous for mutations in the POMC gene present in neonatal life with adrenal crisis due to ACTH deficiency, as POMC is a precursor of ACTH in the pituitary, and they require long-term corticosteroid replacement [20]. Such children have pale skin and white Caucasians have red hair due to the lack of MSH function at melanocortin 1 receptors in the skin. Although red hair may be an important diagnostic clue in patients of Caucasian origin, its absence in patients originating from other ethnic groups should not result in this diagnostic consideration being excluded as children from different ethnic backgrounds may have a less obvious phenotype such as dark hair with red roots. POMC deficiency results in hyperphagia and early-onset obesity due to loss of melanocortin signalling at the MC4R. The clinical features are comparable to those reported in patients with mutations in the receptor for POMC derived ligands, MC4R (see below).

Heterozygous point mutations in POMC have been described which significantly increase obesity risk but are not invariably associated with obesity. R236G disrupts a di-basic cleavage site between β-MSH and β-endorphin, resulting in a β-MSH/β--endorphin fusion protein that binds to MC4R but has reduced ability to activate the receptor [21]. A rare missense mutation in the region encoding β-MSH, Tyr221Cys has impaired the ability to bind to and activate signalling from the MC4R, and obese children carrying the Tyr221Cys variant are hyperphagic and showed increased linear growth, features of MC4R deficiency [22]. These observations support a role for β-MSH in the control of human energy homeostasis. Selective MC4R agonists of melanocortin analogues may be feasible therapies for such patients in the future.

PCSK1 Deficiency

Proprotein convertases (PCs) are a family of serine endoproteases that cleave inactive pro-peptides into biologically active peptides [23]. Two family members, Proprotein Convertase Subtilisin/Kexin type 1 and 2 (PCSK1 and PCSK2) are selectively

expressed in neuroendocrine tissues where they cleave prohormones including pro-opiomelanocortin (POMC), prothyrotrophin releasing hormone (TRH), proinsulin, proglucagon and progonadotrophin releasing hormone (GnRH) to release biologically active peptides. Compound heterozygous or homozygous mutations in the *PCSK1* gene, which encodes PC1/3, cause small bowel enteropathy and complex neuroendocrine effects (including diabetes insipidus) due to a failure to process a number of prohormones as well as severe, early onset obesity [24, 25].

MC4R Deficiency

Heterozygous *MC4R* mutations have been reported in obese people from various ethnic groups. The prevalence of pathogenic *MC4R* mutations has varied from 0.5 to 2.5 % of people with a BMI > 30 kg/m^2 in UK and European populations to 5 % in patients with severe childhood obesity [26, 27]. As *MC4R* deficiency is the most common genetic form of obesity, assessment of the sequence of the *MC4R* is increasingly seen as a necessary part of the clinical evaluation of the severely obese child.

Given the large number of potential influences on body weight, it is perhaps not surprising that both genetic and environmental modifiers will have important effects on the severity of obesity associated with *MC4R* mutations in some pedigrees. Co-dominance, with modulation of expressivity and penetrance of the phenotype, is the most appropriate descriptor for the mode of inheritance.

The clinical features of *MC4R* deficiency include hyperphagia in early childhood. Alongside the increase in fat mass, *MC4R*-deficient subjects also have an increase in lean mass and a marked increase in bone mineral density, thus they often appear “big-boned” [27]. They exhibit accelerated linear growth, which may be a consequence of disproportionate early hyperinsulinemia and effects on pulsatile growth hormone (GH) secretion, which is retained in MC4R-deficient adults in contrast to common forms of obesity [28]. Despite this early hyperinsulinemia, obese adult subjects who are heterozygous for mutations in the *MC4R* gene are not at increased risk of developing glucose intolerance and type 2 diabetes compared to controls of similar age and adiposity [27]. The proportion of visceral to subcutaneous fat is not altered in MC4R deficiency. Reduced sympathetic nervous system activity in MC4R-deficient patients is likely to explain the lower prevalence of hypertension and lower systolic and diastolic blood pressures [29]. Thus, central melanocortin signalling appears to play an important role in the regulation of blood pressure and its coupling to changes in weight.

At present, there is no specific therapy for *MC4R* deficiency, but patients with heterozygous MC4R mutations do respond to Roux-en-Y-bypass surgery [30], which can be considered in adults. As most patients are heterozygotes with one functional allele intact, it is possible that small molecule MC4R agonists or pharmacological chaperones which improve receptor trafficking to the cell surface might be appropriate treatments for this disorder.

SH2B1 Deficiency

Severe obesity without developmental delay is associated with a significantly increased burden of rare, typically singleton copy number variants (CNVs) [31]. Deletion of a 220-kb segment of 16p11.2 is associated with highly penetrant familial severe early-onset obesity and severe insulin resistance [32]. This deletion includes a small number of genes, one of which is *SH2B1*, known to be involved in leptin and insulin signalling. These patients gain weight in the first years of life, with hyperphagia and fasting plasma insulin levels that are disproportionately elevated compared to age- and obesity-matched controls. Several mutations in the *SH2B1* gene have also been reported in association with early onset obesity, severe insulin resistance and behavioural abnormalities in some patients [33].

Clinical History, Examination and Investigation

The assessment of severely obese children and adults should be directed at screening for potentially treatable endocrine and neurological conditions and identifying genetic conditions so that appropriate genetic counselling and in some cases treatment can be instituted. Much of the information needed can be obtained from a careful medical history and physical examination, which should also address the potential complications of severe obesity such as sleep apnoea [34]. In addition to a general medical history, a specific weight history should be taken carefully establishing the age of onset and the presence of hyperphagia. A careful family history to identify potential consanguineous relationships, the presence of other family members with severe early onset obesity and the ethnic and geographical origin of family members should be taken. The history and examination can then guide the appropriate use of diagnostic tests.

Conclusions

Given the rapid application of next-generation sequencing technologies such as whole exome sequencing, it is very likely that new genes and mechanisms will emerge to explain a variety of previously unrecognized obesity syndromes. As more is learned about these genes and more syndromes are described, it is likely that the need to perform a comprehensive evaluation of severely obese patients will be recognized. Knowledge of the specific molecular mechanisms affected by these genetic disorders may lead to better mechanism-directed, stratified pharmacotherapy in the future.

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Chapter 3
Genome-Wide Association Studies of Obesity

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Abstract Genome-wide association studies (GWAS) have accelerated the discovery of genetic variants associated with susceptibility to common complex diseases, such as obesity. Following the first robust GWAS of BMI and risk of obesity identified in 2007, GWAS have delivered 70 additional common loci associated with a wide range of obesity-related traits. These loci highlight a variety of molecular and physiological mechanisms involved in shaping these traits. However, even in combination, these loci explain only a small proportion of overall phenotypic heritability indicating that much of the genetic variation in obesity traits remains unexplained. Here, we discuss how the GWAS approach has been applied to the study of anthropometric phenotypes related to overall obesity and fat distribution and describe some of the clues to trait biology that are emerging. We also highlight some of the limitations of this work and future directions for research in this field.

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Abbreviations

BMI	Body mass index
CNV	Copy number variation
GIANT	Genetic Investigation of ANthropometric Traits
GWAS	Genome-wide association studies
LD	Linkage disequilibrium
MAF	Minor allele frequency
SNP	Single nucleotide polymorphism
T2D	Type 2 Diabetes
WC	Waist circumference
WHR	Waist–hip ratio

The rise in the prevalence of obesity in recent decades has been spectacular: recent estimates indicate that over 500 million adults worldwide are now classed as obese [1]. While the increased prevalence of obesity is almost certainly a reflection of secular changes in environmental and lifestyle factors, including an increased intake of nutrient-dense foods coupled with reduced physical activity [2], the familial aggregation of obesity is consistent with some degree of genetic influence on body mass index (BMI) and individual predisposition to obesity. More conclusive evidence for a genetic component comes from studies that have examined the correlation of BMI between identical twins raised apart and the relationship between the BMI of adoptees and both their biological and adoptive parents [3–5]. These consistently highlight the importance of genetic factors in modulating individual susceptibility to obesity in contemporary environments. Furthermore, in controlled experiments of excessive calorie intake, consequent changes in weight and body composition were highly correlated in monozygotic twins, once again consistent with a powerful role of genetic variation in the regulation of weight [6]. Estimates for the heritability of BMI vary widely between studies, but typical figures range between 0.47 and 0.90 in twin studies and between 0.24 and 0.81 in family-based studies [7].

Other obesity related traits, including measures of fat distribution are also heritable (even after adjusting for BMI). Estimates for the heritability of waist–hip ratio (WHR), a proxy of fat distribution, range between $h^2 \sim 0.31$–0.70; and ~0.22–0.61 after accounting for BMI [8–11]. The heritability of WHR is higher in women and estimates of genetic correlation of WHR between men and women indicate a sex specific genetic influence on the trait [11].

The Genetics of BMI and Obesity Pre-GWAS

Genetic studies aim to find DNA sequence variants that are causally associated with the trait of interest, in the expectation that such discoveries will help to reveal fundamental mechanisms responsible for human disease. The earliest studies in this

field focused on the application of family-based linkage studies to individuals and families with rare monogenic forms of obesity. The rare variants of large effects revealed by these efforts, such as those in *LEP* (encoding the hormone leptin, a crucial component of energy balance mechanisms) [12–14], *LEPR* (encoding the leptin receptor) [15], and *POMC* (encoding the proopiomelanocortin protein which is cleaved to form a number of key neuroendocrine messengers) [16], helped to define components of hypothalamic circuitry involved in body weight regulation in man. However, the application of linkage approaches to population-level variation in BMI and risk of common forms of obesity met with little success in terms of robust, replicated signals even in relatively well-powered meta-analysis [17]. This indicates that the genetic contribution to these traits is not dominated by the kinds of highly penetrant variants which linkage methods are best suited to detect [18].

The shift from linkage to association approaches was initially focused on the analysis of candidate genes [19], a strategy reliant on the quality of the prior biological hypotheses used to select them. One of relatively few successes from this approach was the demonstration that low frequency variants in the gene encoding the melanocortin 4 receptor (*MC4R*) were associated with severe, early-onset obesity [20]. These variants remain the commonest known genetic cause of morbid obesity contributing to a few percent of these cases [21]. These findings provided confirmation of the role of signalling through the hypothalamic leptin–melanocortin pathway for the maintenance of body mass in man [22]. However, the major impetus to the discovery of BMI- and obesity-associated variants has been provided by the ability to perform genome-wide scans for association.

Genome-Wide Association Studies

Genome-wide association studies (GWAS) (reviewed in [23, 24]) use dense genotyping arrays to determine how variation in genomic sequence (predominantly that due to single nucleotide polymorphisms, SNPs) associates with phenotypic traits of interest. Those traits may be categorical (e.g., obese cases and non-obese controls) or continuous (e.g., BMI or WHR). Array content and the correlation structure of variation across the genome (i.e., linkage disequilibrium) mean that GWAS to date have favored the interrogation of common variants (minor allele frequency [MAF] > 5 %). Since GWAS assay such variants across the genome, suitably powered studies enable the discovery of associated loci in an agnostic fashion, without the need for prespecified hypotheses concerning the genomic location of the association and the transcripts through which they may operate.

In the remainder of this chapter, we focus on the loci which have been shown by GWAS to be associated with overall obesity or fat distribution. We distinguish between studies of traits of overall obesity (including BMI, fat percentage, and dichotomized indices of extreme obesity) and those of fat distribution (including WHR, waist circumference (WC), and measures of visceral and subcutaneous fat). In total 70 genome-wide significant loci have been associated with these traits and most of these (50 in number) are common variant loci influencing continuous

obesity-related traits found in European samples. Others derive from equivalent studies in non-European samples (4 loci), and some have emerged exclusively from case–control studies in individuals selected from the extremes of the BMI distribution (9 loci) or by clinical classifications of overweight and obesity (7 loci).

Overall Obesity

Genome-Wide Association Studies of BMI

The first report from a GWA study claiming to have identified variants associated with common forms of overall obesity came in 2006 [25]. The researchers used a two-stage family-based design to identify a signal mapping close to the *INSIG2* encoding insulin induced gene 2 [25]. However, this association has not been proven robust to replication in the much larger samples that have been examined in subsequent studies (see below). In fact, the association p-value observed in this study fell short of the now-widely accepted threshold ($p < 5 \times 10^{-8}$: based on $p < 0.05$ corrected for a million independent tests [26]), highlighting the value of such stringent criteria as a means of avoiding inflation of the type 1 error, and the attribution of biological significance to loci which, like *INSIG2*, are likely to have been false positives.

The first report of a robust genome-wide significant locus influencing BMI and risk of obesity locus came from Frayling et al. [27] in 2007, and concerned a cluster of common variants close to the *FTO* ("Fat mass and obesity-associated") gene. These variants account for ~0.35 % of the phenotypic variance in BMI in Europeans [28] such that the two groups of homozygotes differ in weight by around 2.5 kg. The BMI association has now been widely replicated [28–33] and it is also clear that the same *FTO* variants are associated with risk of obesity at all grades of severity [27, 34].

Given that the only locus emerging from this first round of GWA studies [25, 27] had a relatively modest effect size, it was clear that larger sample sizes would be needed to extend these discoveries, both to common alleles of lesser effect, and to less frequent risk alleles. This provided the motivation for ever-larger meta-analyses efforts, which have dominated discovery efforts over the past few years. The largest of the studies published to date assembled data from almost 250,000 individuals [28]. The current count of BMI-associated loci detected in Europeans by these studies, most of them conducted under the aegis of the Genetic Investigation of ANthropometric Traits (GIANT) consortium [28–30], is 32 (Fig. 3.1).

The first such meta-analyses uncovered common regulatory variants influencing BMI near *MC4R* (encoding melanocortin receptor 4) [29]: low-frequency coding variants in this same gene had previously been implicated in severe obesity [20]. Subsequently, the parallel publications from GIANT [30] and the deCODE group [31] added nine BMI loci (mapping near *GNPDA2*, *KCTD15*, *MTCH2*, *NEGR1*, *SH2B1*, *TMEM18*, *BDNF*, *ETV5*, and *SEC16B*) to the list. It is of note that *BDNF*, encoding a brain derived neurotrophic factor involved in regulation of development

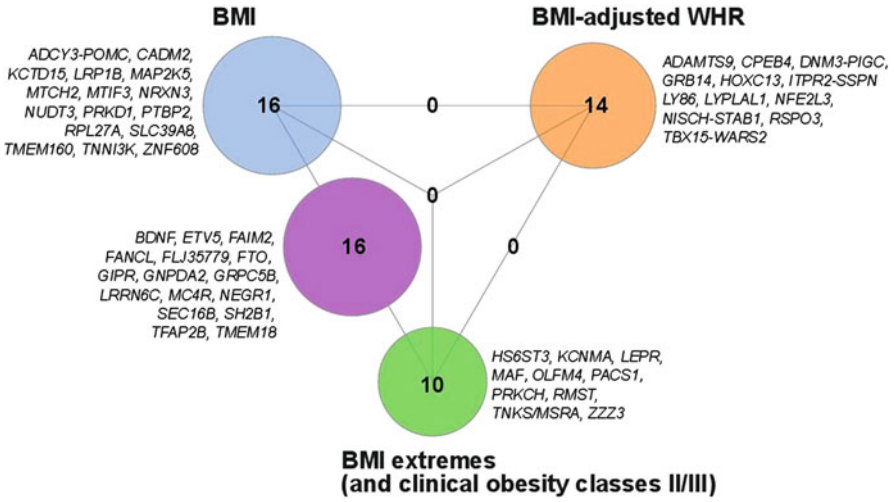

Fig. 3.1 Overlap of genome-wide significant loci of overall obesity (BMI), fat distribution (BMI-adjusted WHR) and BMI extremes (or clinical obesity classes) in European populations. Diagram depicts the overlap of reported GWAS loci ($p < 5 \times 10^{-8}$) of BMI [27–31], BMI-adjusted WHR [98], and BMI extremes or clinical obesity classes II–III [34, 38, 51–53, 55, 120]

of neuronal circuits [35], is also involved in monogenic forms of obesity [36]. The largest meta-analysis of BMI associations added 18 further loci to the tally [28] including regions near known obesity genes such as *POMC* (proopiomelanocortin) [16], known to be involved in neuroendocrine regulation of weight, as well as associations in or near novel genes such as *GPRC5B* (G protein-coupled receptor, family C, group 5, member B), implicated in regulation of adipose inflammatory processes and progression to insulin resistance in obesity in mice [37].

The studies above mostly focused on the analysis of SNPs but there is some evidence that copy number variations (CNVs) may be causal at some loci. For example, in the study by Willer et al. the BMI-associated SNP at the *NEGR1* (neuronal growth regulator 1) locus detected by GWAS was shown to tag a 45 kb deletion that might have stronger functional grounds for being causal [30]. More detailed studies published recently locate the causal allele at this locus to a second 8 kb deletion near *NEGR1* [38]. Rare CNVs have also been implicated in syndromic forms of obesity. For example, a rare deletion in the 16p11.2 region is associated with the combination of severe obesity and mental retardation [39, 40], and duplication of the same region is associated with underweight [41].

To date, most GWAS studies have been performed in populations of European origin but studies in other ethnic groups can help to identify novel loci, to characterize the extent of aetiological overlap, and to fine-map causal variants (such as in the *FTO* locus [42, 43]). Two large GWAS meta-analyses of BMI in East Asian populations were recently published [32, 33]. Between them, seven of the known loci in Europeans could be replicated to genome-wide levels of significance.

Furthermore, evaluating the associations in East Asians of previously reported BMI loci, an additional 11 loci, besides those genome-wide significant, were associated with BMI at lesser levels of significance (Okada et al. [32], $p < 0.02$; Wen et al. [33], $p < 0.05$), indicating considerable overlap in signals between East Asian and European populations. As in populations of European origin, the association at *FTO* locus explained the largest proportion of phenotypic variance (~0.2 %).

In addition, these studies identified four novel loci, mapping near *CDKAL1*, *KLF9*, *PCSK1* and *GP2* [32, 33]. Mutations in *PCSK1* cause monogenic obesity [44] and, while a candidate study previously associated nonsynonymous variants in *PCSK1* with common obesity risk in a European population [45], the *PCSK1* signal in East Asians (also nominally associated with BMI in Europeans in GIANT [28]) likely represents an independent signal. Genetic variants in *CDKAL1* (a CDK5 regulatory associated protein 1-like 1 with methythiotransferase function [46]), in strong LD ($r^2 \sim 0.8$) with the BMI GWAS SNPs in East Asians, have previously been associated with increased risk of Type 2 Diabetes (T2D) [47, 48]. The T2D risk allele(s) is associated with decreased glucose-stimulated insulin secretion [47, 49, 50]. Furthermore, the BMI-lowering allele of rs2206734 (also nominally associated with BMI in Europeans) was associated with increased risk of T2D in the same study population [32], indicating that variation near *CDKAL1* may play a complex role with respect to variation in both BMI and T2D-risk.

Case–Control Studies of Dichotomized BMI

In addition to studies of the variance in BMI in population-based studies, a complementary approach treats obesity in terms of a dichotomous "case–control" analysis. A variety of different schemes for this dichotomization are possible (Table 3.1). In the largest study of this type [51], featuring case–control analyses restricted to the "tails" of the BMI distribution using data from studies previously included in GIANT meta-analyses [28], Berndt et al. found considerable overlap in the pattern of association signals seen as compared to those observed in population- or cohort-wide analyses. However, where such studies focus on cases of more extreme definitions of obesity and/or leanness (that is, individuals several standard deviations away from the population mean), there may be the opportunity to detect additional, novel, signals that may have limited impact on overall population-level variance and which are therefore difficult or impossible to detect using GWAS approaches. The rare, penetrant variants causal for monogenic and syndromic forms of obesity provide the most obvious example of this phenomenon.

Indeed, whilst several of the loci reaching genome-wide significance in dichotomous analyses focused on extreme obesity in adults overlap with those previously reported (e.g., *BDNF*, *FTO*), there are several signals that appear unique to dichotomous analyses including *KCNMA1*, *NPC1*, *PTER*, and *HS6ST3* (Table 3.1, Fig. 3.1) [51–53]. However, most of these have appeared in a single study and have not, as yet, been replicated, even in other extreme case–control analyses. In equivalent

Table 3.1 Novel GWAS loci identified in case–control analyses of dichotomized BMI

Study type	Selection criteria for cases	Sample size in stage 1, cases/controls	Loci not described in BMI GWAS	Reference
Extreme obesity in children and adults	Early onset obesity (≤6 years) and extreme adult obese (BMI ≥ 40)	1,380/1,416	*MAF* (rs1424233), *NPC1*[a] (rs1805081), *PTER*[a] (rs10508503)	[52]
Extreme obesity in children and adolescents	BMI >97 % percentile	1,138/1,120	*TNKS/MSRA* (rs17150703), *SDCCAG8*[a] (rs12145833)	[55]
Extreme obesity in adults	BMI ≥ 40	164/163	*KCNMA1* (rs2116830)	[53]
Distributional tails in children	BMI ≥95 % percentiles	5,530/8,313	*BC041448* (rs4864201), *HOXB5* (rs9299), *OLFM4* (rs9568856)	[54]
Extreme obesity in children	BMI standard deviation score (SDS) ≥3, and onset at 10 years	1,509/5,380	*LEPR* (rs11208659), *PACS1* (rs564343), *PRKCH* (rs1957894) *RMST* (rs11109072)	[38]
Clinical class: obesity II	BMI ≥ 35	9,889/62,657	*HS6ST3* (rs7989336), *ZZZ3* (rs17381664)	[51]
Clinical class: obesity I	BMI ≥ 30	32,858/65,839	*GNAT2* (rs17024258), *HNF4G* (rs4735692), *MRPS33P4* (rs13041126), *ADCY9* (rs2531995)	[51]
Clinical class: overweight	BMI ≥ 25	93,015/65,840	*HNF4G* (rs4735692), *RPTOR* (rs7503807)	[51]

[a]Not genome-wide significant ($p < 5 \times 10^{-8}$)

case–control analyses in children, the more relaxed criteria adopted by Bradfield et al [54] detected many of the known adult BMI association signals but also highlighted novel signals near *OLFM4* and *HOXB5*. In contrast, studies of children selected from the extremes of the distribution have detected signals at (or approaching) genome-wide significance near *LEPR*, *PACS1*, *PRKCH*, *RMST*, *SDCCAG8*, and *TNKS/MSRA* (Table 3.1, Fig. 3.1) [38, 55], the latter locus also detected in some studies of fat distribution [56].

Genome-Wide Association Studies of Fat Percentage

BMI, although a widely used proxy of overall obesity, represents an aggregate measure of the lean and the fat mass of the individual. In an effort to better define the genetic determinants of obesity, Kilpeläinen et al. focused on body fat

percentage, as a more direct measure of adiposity, generating a GWAS meta-analysis of 36,626 individuals [57]. As well as detecting *FTO*, these analyses recovered two loci (*IRS1* and *SPRY2*) not previously associated with BMI. The body fat-increasing alleles at the *IRS1* (insulin receptor substrate 1 signalling protein) signal are, intriguingly, associated with a healthy metabolic profile (including reduced risk of T2D [58] and unhealthy lipid profile [59]). The *IRS1* locus is associated with measures of subcutaneous, but not visceral fat, indicating that the effect on fat mass at the *IRS1* locus is through regulation of subcutaneous fat deposition [57, 60]. The *SPRY2* locus has also been implicated in T2D risk [61, 62], though the body fat-associated SNP is not coincident with this previously reported T2D SNP. Contrary to the observations at *IRS1*, the body-fat increasing allele at the *SPRY2* locus is associated with an adverse metabolic profile [57].

Genetic Architecture of Overall Obesity (BMI)

Despite the success in identifying a growing numbers of loci to genome-wide significance, in European populations these signals, in combination, explain no more than 1.5 % of phenotypic variance in BMI. Of the established loci, the *FTO* locus has the largest effect accounting for ~0.35 % of population variance [28]. These numbers fall well short of estimates of the heritability of this trait (see above). The basis for this "missing" genetic variance remains unclear, though there is no lack of possible explanations [63, 64]. At least part of the "missing" genetic variance can be attributed to the effects of additional common variants that lie below the genome-wide significance threshold. Using full GWAS data sets (not just the "proven" hits), Yang and colleagues could recover approximately 17 % of the phenotypic variance in BMI that was tagged by common variants [65]. Part of the remaining shortfall likely reflects incomplete linkage disequilibrium between the variants genotyped on GWAS arrays and those which are causally responsible for the BMI associations [66], but other mechanisms are almost certainly involved [63, 67].

There is also the possibility that the estimates of heritability against which these measures of explained variance are evaluated, are themselves inaccurate. For example, intrauterine events that lead to epigenetic modifications with long-term phenotypic impacts can lead to increased sibling resemblance, inflating heritability estimates under some designs. Similarly, estimates derived from the comparison of the phenotypic correlations observed between monozygotic and dizygotic twin pairs are based on the assumption that both types of twin are exposed to a similar degree of shared environment [68], an assumption that may not be appropriate for intrauterine exposures.

Notwithstanding the above, it seems likely that an appreciable component of the genetic variance remains unexplained, and that at least part of this will be attributable to low frequency and rare variants not well captured by GWAS studies to date. The current wave of sequencing studies should shed some light on the extent to which these variants are contributing to inherited risk.

From GWAS Associations to Potential Functional Roles in Overall Obesity

As we have seen, GWAS have powered the identification of many genetic regions associated with BMI and obesity. However, this information is of limited value unless it can be translated into improved understanding of the pathophysiology of disease, and thereby into novel clinical approaches. However, in BMI, as with most other complex traits, the regions revealed by GWAS do not lend themselves to easy biological inference. The effect sizes are modest, and most signals map to non-coding sequence, frustrating efforts to identify the "causal" transcript (that is, the specific gene that is mediating the association signal). At the same time, the extensive local correlations between common variants (that is, linkage disequilibrium) can make fine-mapping of the causal variants challenging.

The *FTO* locus provides an excellent example of the difficulties inherent in moving from an association signal—in this case, a comparatively strong one—to a clear mechanism of action. We have now known for more than 6 years that a cluster of highly correlated common variants in the first intron of the *FTO* gene is associated with BMI and obesity [27]. Epigenetic analyses have suggested that the BMI-associated haplotype may influence local methylation status [69] but fine-mapping efforts have yet to provide compelling localization of the causal variant. When it comes to defining downstream effects, we still have no convincing evidence from man that the *FTO* transcript itself is in any way involved. There is for example, no instance of the co-occurrence of loss of function alleles in *FTO* and severe obesity in humans [70, 71]. On the other hand, the adjacent gene *RPGRIP1L* (or *FTM*), which is known to be coordinately regulated with *FTO* via a common promoter [72], and to display a similar pattern of hypothalamic expression, has an intriguing connection to obesity through its known causal role with respect to monogenic ciliopathies [73] some of which result in marked early obesity.

In fact, the most compelling evidence implicating *FTO* comes from mouse models: transgenic knockdown of the murine homologue *Fto* results in reduced weight, and overexpression to weight gain compared to control mice [74, 75]. One possible explanation consistent with these data is that the common intronic variants within *FTO* identified by GWAS, exert their effects on energy balance in man through coordinate dysregulation of both *FTO* and *RPGRIP1L*.

The identification of the signal at *FTO* naturally prompted interest in the normal function of this transcript. In humans, *FTO* encodes a 2-oxoglutarate-dependent nucleic acid demethylase [76] thought to be involved in nucleic acid repair. In vitro studies have suggested a role for *FTO* demethylation in cellular sensing of amino acids [77], which could be relevant to regulation of appetite control in the hypothalamus. Nonetheless, it is clear that we remain some way from a complete description of how these variants influence BMI and obesity risk.

At certain other BMI GWAS loci, the situation is better understood. At four GWAS loci (near *BDNF*, *PCSK1*, *POMC* and *MC4R*) the common variant associations overlap genes in which coding mutations have been shown to be causal for

monogenic or syndromic forms of obesity [20, 36, 78, 79]. In the case of three of these—*PCSK1* (proprotein convertase 1), *POMC* (proopiomelanocortin), and *MC4R* (melanocortin receptor 4)—there are strong mechanistic ties to the hypothalamic leptin–melanocortin signalling pathways that regulate energy balance [80]. *BDNF* encodes a brain derived neurotrophic factor involved in neurogenesis and thought to be involved in food intake [81]. These GWAS signals therefore demonstrate that the neuroendocrine mechanisms documented in monogenic forms of obesity extend to population level variance in BMI and to more common forms of obesity.

At other BMI-associated GWAS, efforts to define the causal transcript are supported by additional sources of genomic data (regulatory annotations [82] or mRNA expression [83]). For example, it can be very useful through integration with mRNA and/or miRNA transcriptomic data [83–88] to demonstrate that the set of BMI-associated variants at a given locus also drives *cis*-expression of one of the regional transcripts. In the most recent GIANT meta-analysis [28], this approach led to positional candidates being identified at almost half the 32 BMI-associated loci.

These candidacy assignments can often be bolstered by other sources of data. Consider for example the association signal mapping close to the *SH2B1* gene, encoding SH2B adapter protein 1. *Cis*-expression data point to *SH2B1* [28], as does the high expression of this transcript in the hypothalamus [30]. The neuronal isoform of *SH2B1* is involved in regulation of energy balance via effects on leptin and insulin signalling, and systemic deletion of the gene in mice results in severe leptin resistance [89].

For some loci, the data seem to point towards peripheral rather than central mechanisms of action. The BMI association on chromosome 19 lies close to the *GIPR* gene, encoding the gastric inhibitory polypeptide receptor, and the lead SNP is in strong LD with a missense SNP in that transcript (though the functional consequences of that mutation are not yet established). GIPR plays an important role in mediating the incretin response, which augments insulin release in response to the ingestion of food. The same locus has also been shown to associate with glucose response and insulin secretion in response to a glucose challenge [90]. Another example, mentioned earlier, is the mechanistic relationship between insulin signalling and obesity implicated by the association between *IRS1* variants and fat percentage [57]. Though both central and peripheral mechanisms may be involved at *IRS1*, the fact that the fat percentage-associated allele is associated with improved insulin sensitivity and a healthy metabolic profile [58, 59] is consistent with enhanced insulin-mediated adipogenesis as the driver of the adiposity.

For several other BMI-associated loci such as *TMEM160-ZC3H4* [28], there are few clues on the biological relevance in obesity, and any one of several transcripts could be responsible. One way of leveraging the combination of genetic and prior biological data to make provisional mechanistic inference in such situations is to perform pathway-based analyses (reviewed in Wang et al. [91]), which test for enrichment of GWAS loci for transcripts that have been mapped to defined biological processes or pathways. Applied to BMI GWAS data, these analyses have tended to support the evidence for broad neuroendocrine involvement, whilst also highlighting processes that are more difficult to assimilate within the current knowledge base (e.g., platelet-derived growth factor signalling) [28].

Fat Distribution

The clinical consequences of adipose tissue excess depend not only on its quantity but also its distribution, with the accumulation of visceral (abdominal) fat leading to particularly adverse metabolic and cardiovascular effects [92, 93]. After accounting for overall obesity (as measured by BMI), fat distribution (commonly measured by WHR) shows substantial residual heritability ($h^2 \sim 0.22–0.61$) consistent with mechanisms of genetic control distinct from those influencing overall energy balance and BMI [9, 10]. The distinct genetic regulation of patterns of fat distribution is also supported by rare monogenic syndromes of selective adipose tissue loss (collectively, the lipodystrophies) [94]. Given the checkered history of efforts to target neuronal pathways related to overall obesity in the search for effective, safe treatments for obesity, there is considerable interest in defining the mechanisms responsible for individual variation in patterns of fat distribution, and in particular, in identifying peripheral (rather than central) targets for therapeutic intervention.

Genome-Wide Association Studies of WHR and WC

Initial efforts to map variants influencing fat distribution focused on the standard clinical traits, WHR and WC. In the first GWAS for WHR, Lindgren et al. discovered an association to a genetic variant on chromosome 1 (close to the *LYPLAL1* gene encoding lysophospholipase-like 1) associated with WHR in women exclusively: this effect was independent of BMI [56]. Studies of WC generated their strongest signals at previously reported BMI loci such as *FTO* and *MC4R*, reflecting the strong correlation between these traits [27, 56, 95, 96]. With the possible exception of the association near *TFAP2B*, at which adjustment with BMI seems to increase the magnitude of the effect on central obesity [97], other WC-associated loci identified by GWAS (*MSRA*, *NRXN3*) are likely to reflect a primary association with BMI [28].

Given these strong trait correlations, more recent fat distribution GWAS efforts have adopted the approach of adjusting WHR (or WC) for BMI before performing the association analyses, thereby seeking to emphasize those signals that influence patterns of relative fat deposition independent of the overall obesity component. In the largest analysis to date, involving data from around 190,000 subjects, Heid et al. [98] used this approach to identify 13 novel loci for BMI-adjusted WHR as well as to replicate the signal near *LYPLAL1*. As might have been expected given the adjustment for BMI, the loci identified by this endeavor were completely distinct from those previously reported to influence overall obesity (Figs. 3.1 and 3.2). In line with the metabolic consequences of visceral fat accumulation, these fat distribution associated variants are also enriched for association with related metabolic traits including fasting insulin, lipids and indices of insulin resistance [98]. The obvious gender dimorphism of WHR prompted efforts to evaluate these signals in terms of their potential for different effects in males and females.

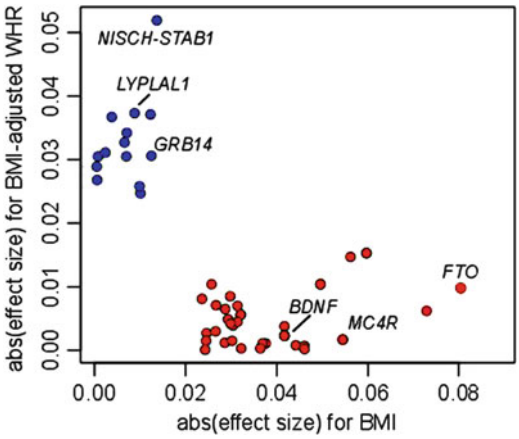

Fig. 3.2 Effect sizes for BMI in GIANT meta-analyses vs. BMI-adjusted WHR in GIANT meta-analyses for genome-wide significant BMI and BMI-adjusted WHR loci. In the *scatterplot*, data for BMI in GIANT meta-analyses [28] are shown on the *X*-axis and data for BMI-adjusted WHR in GIANT meta-analyses [98] on the *Y*-axis. The points are colored according to if they represent loci associated with BMI (*red*) or BMI-adjusted WHR (*blue*)

Half of the 14 loci showed evidence of gender-specific effects: in each case, the effect was stronger in women [98].

In a complementary approach to studies of WHR in population-wide analysis, Berndt et al. restricted analysis to the "tails" of the WHR distribution (upper and lower 5th percentiles) and analyzed WHR in terms of dichotomous "case–control" analyses [51]. This analysis demonstrated a similar pattern of association signals as that of previous population-wide analysis [98], indicating that WHR at the "tails" of the distribution has a similar genetic architecture as that of the full distribution.

Genome-Wide Association Studies of Abdominal Fat Distribution

The use of imprecise, but widely available, clinical measures such as WHR facilitates large meta-analysis, but there is much to be gained by complementary analyses in smaller numbers of more carefully phenotyped subjects. In a recent study, more direct measurements of the extent of abdominal subcutaneous and visceral adiposity were obtained by computed tomography (CT) [60]. This analysis was able to demonstrate that the fat distribution association signal near *LYPLAL1* [51, 56, 98] could also be detected using CT (as the ratio between subcutaneous and visceral fat area). It also highlighted a signal near *THNSL2* that was associated with visceral adiposity in women: this survived adjustment for BMI, and has not previously been associated to obesity traits [60].

Genetic Architecture of Fat Distribution

Combined, the 14 loci for BMI-adjusted WHR uncovered by GWAS account for approximately 1 % of variance in this trait (1.34 % in women; 0.46 % in men) [98]. Using methods analogous to those for BMI described above [65], Vattikuti et al. showed that ~13 % of the overall variance in WHR could be explained by common GWAS SNPs, and thus estimated that 46 % of heritability in WHR may be captured by common variants [99]. A similar range of explanation for the missing genetic variance is possible as for overall obesity [63, 64, 67], and ongoing sequence-based efforts will help to define the extent to which this deficit can be plugged by the contribution of low frequency and rare variants.

From GWAS Associations to Potential Functional Roles in Fat Distribution

As with BMI, progress towards characterization of the mechanisms operating at each of these loci has been patchy. Expression-QTL mapping in adipose tissue, blood, and other tissues has identified promising candidate transcripts at six of the loci (*AA553656*, *GRB14*, *PIGC*, *STAB1*, *TBX15*, and *ZNRF3*) [98].

For several of these transcripts, the genetic data integrates well with the corpus of existing biological data. For example, *GRB14*, encoding a growth factor receptor-binding protein, is known to acts as a negative regulator of insulin receptor signalling [100, 101]. The WHR-associated variant shows directionally consistent associations with triglyceride and insulin levels [98] and other (statistically independent) variants at the same locus influence BMI-adjusted insulin and HDL-cholesterol levels [102, 103]. *TBX15* encodes a mesodermal developmental transcription factor and has been indicated in adipocyte differentiation and triglyceride accumulation [104]. This transcript is also differentially expressed between visceral and subcutaneous adipose tissue, and there is evidence that visceral adipose tissue expression is negatively correlated with BMI [105].

The most consistent signal for fat distribution maps to the *LYPLAL1* locus [51, 56, 60, 98]. As might be expected variants at this locus are associated with a range of related metabolic and anthropometric traits including adiponectin [106], fasting insulin adjusted for BMI [103] and height [107]. So far, there is limited evidence to demonstrate that the signal is mediated through the *LYPLAL1* transcript and the region contains several other potential candidates. However, expression of this gene is induced in subcutaneous fat following obesity [108] and its presumed function as a lysophospholipase is consistent with a causal role.

As with the GWAS loci associated with BMI, pathway enrichment approaches have been applied across the 14 WHR-associated loci. Though the enrichment signals were relatively weak, they highlighted developmental processes and mRNA transcript regulation [98]. The known functions of some of the stronger positional

candidates—such as angiogenesis (*VEGFA*), adipocyte differentiation (*GRB14*) and developmental function (*TBX15*, *HOXC13*)—seem to point towards peripheral mechanisms.

These enrichment patterns, when compared with those seen for BMI, seem consistent with the hypothesis that overall obesity is primarily defined by variation at genes involved in central neuroendocrine regulation, whereas fat distribution is largely influenced by variation at genes, which control peripheral aspects of adipose function and development.

Challenges for the Present and for the Future

Whilst there is no doubt that GWAS studies have accelerated our understanding of the genetics and biology of obesity, there remains much to do. At most of the loci discovered, we have yet to identify the causal variant (or variants) or to define with certainty which regional transcript is responsible for mediating the association effect. The accumulation of transethnic association data [109–111] combined with the growing use of next-generation sequencing to generate reference sets for imputation [112] and to interrogate phenotypically selected individuals (e.g., the morbidly obese) should help to address the former. The latter depends in part on the generation of improved annotations (particularly those from relevant tissues) that connect non-coding variation to transcript regulation, and on the development of appropriate functional assays. As always, the ability to refine the phenotypic consequences of allelic differences at variants of interest in human subjects (for example through imaging of fat tissues) will play a crucial role in defining a mechanistic understanding of these traits.

Missing Genetic Variance

As we have seen the loci identified by GWAS loci explain a surprisingly small proportion of phenotypic variance, far less than appears to be the case for other "similar" quantitative traits, such as height and lipids. Approaches that combine effects across the entire GWAS dataset, rather than considering only those signals reaching genome-wide significance, do a better job of recovering variation (indicating a long "polygenic" tail of common variant susceptibility) but still leave a substantial component of estimated heritability unexplained [65]. To discover further genome-wide significant associations to common genetic variants with increasingly smaller effects would require even larger studies than to date. The latest wave of grand meta-analyses of BMI and BMI-adjusted WHR (involving over 320,000 and 210,000 European samples respectively), currently underway, promise to reveal some of these common variant signals, as do the studies emerging from analyses in a variety of non-European samples.

It has been suggested that rare (MAF < 1 %) or low frequency (MAF 1–5 %) variants beyond the range of the historical GWAS approach, may contribute to this missing genetic variance [24, 64, 113, 114]. The rapidly decreasing cost and increasing accuracy of next-generation sequencing are bringing variants in this class under the microscope for the first time [115].

It is clear that individual risk of obesity reflects the integration of genetic and non-genetic factors including variation in food availability and extent of physical exercise [116]. Indeed, these may directly interact such that variant effects are modulated by these lifestyle factors: under some circumstances these interactions may contribute to the missing "genetic" variance [67]. The detection of such interactions at the genome scale requires massive sample sizes, unless the interaction terms are substantial. Nevertheless, there are several examples now emerging of interaction effects at obesity loci: these include an interaction between *FTO* and exercise [117] as well as sex-specific effects reported for WHR [56, 98] and visceral adiposity [60].

Risk Prediction, Intervention and Medication

One might hope that improved knowledge about the genetics of obesity would help to generate predictive models. These might be used to identify individuals at highest future risk of obesity who could be targeted for early intervention, and/or define genetic markers related to treatment outcome that can be used to guide therapeutic choices. However, the common variants so far identified by GWAS have too weak an effect, even in combination, to have value in this respect. Indeed, genetic risk factors are currently outperformed by traditional risk factors [118] including present BMI (a good predictor of future obesity risk [119]).

Instead, the most valuable translational benefits are likely to accrue from the biological knowledge, which grows from the genetics. Currently, there are few effective pharmaceutical treatments for obesity, and the most successful clinical intervention requires radical (bariatric) surgery. The clinical burden of obesity urgently requires the identification of novel validated therapeutic targets based around a better understanding of underlying mechanisms. The wider behavioral effects of drugs acting on central processes such as appetite may continue to prove problematic in this respect and efforts to target peripheral mechanisms of fat distribution, and thereby ameliorate the adverse metabolic consequences of obesity may prove more productive.

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Chapter 4
Copy Number Variants and Their Contribution to the Risk of Obesity

Julia Sarah El-Sayed Moustafa and Philippe Froguel

Abstract Obesity is becoming an increasingly serious health concern, given its associated health risks and the growing number of people affected. Understanding the genetic factors underlying body weight regulation and obesity susceptibility has thus become an issue of paramount importance. Obesity has a high estimated heritability, yet much of this remains unexplained. Copy number variants (CNVs) represent a relatively understudied class of genetic variants which may account for some of this unexplained heritability. This chapter explores how copy number variation contributes to body weight regulation and obesity susceptibility.

Common CNVs associated with body mass index (BMI) and obesity have recently been identified, including variants on chromosomes 1p31.3, 8p21.2, 10q11.22, 11q11, and 16p12.3 at the *NEGR1*, *DOCK5*, *PPYR1*, *OR4P4*, *OR4S2*, and *OR4C6*, and *GPRC5B* loci. A number of rare CNVs have also recently been associated with extreme forms of obesity, including two on chromosome 16p11.2, consisting of a 593 kb deletion whose reciprocal duplication has been associated with increased risk of underweight, as well as a 220 kb deletion encompassing the *SH2B1* gene, which has been associated with overweight and obesity. Several studies have also reported enrichment in the global burden of large, rare CNVs among obese subjects, as well as the presence of several rare CNVs uniquely among obese cases.

In the case of large CNVs encompassing multiple genes, functional studies will be required to establish which gene or genes within each CNV are causative for the

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observed phenotype. Improved methodologies, both laboratory and statistical, are also required to enable systematic investigation of complex structural variants such as VNTRs and multi-allelic loci. While developments in this field are promising, analysis of CNVs, both common and rare, has proven challenging, and reported associations require extensive follow-up in large replication samples in order to confirm their role in obesity susceptibility. It is hoped that such in-depth investigations will provide increased understanding of the role of CNVs in body weight regulation and risk of obesity.

Introduction

The study of the genetic factors underlying obesity susceptibility is a subject which has captured the attention of many within the scientific community, particularly due to the serious health risks faced by affected individuals, and the increased risk of obesity in their relatives. This chapter explores the contribution of copy number variants (CNVs) to body weight regulation and risk of obesity.

The Missing Heritability of Obesity

The current obesogenic environment, characterized by an increased consumption of widely available calorie-dense foods among many other factors, has no doubt driven the recent rise in obesity rates [1]. A question of extreme interest in the study of obesity, however, is why individual risk of obesity differs even between subjects exposed to the same environmental risk factors [1]. The answer to this question lies in the fact that obesity is a complex disease arising from a complex interplay of environmental risk factors, affecting all individuals within any given population, and individual genetic predisposition, which renders certain individuals more susceptible to obesity in the face of these environmental risk factors [1].

Despite this complex interaction, numerous studies have shown obesity to be a highly heritable trait. Several twin, adoption, and family studies examining the heritability of adiposity have reported heritability estimates for obesity ranging from approximately 40–70 %, with increased concordance levels between monozygotic twins, even those reared apart, compared to dizygotic twins [2–7].

Conversely, genetic variants associated with adiposity and obesity identified to date explain only approximately 2–4 % of the heritability of these traits [8, 9], with the vast majority of studies having focussed on the analysis of common single nucleotide polymorphisms (SNPs). This discrepancy between the estimated heritability of corpulence and the proportion of which has been explained to date has raised the important question of whether the heritability of obesity has been overestimated, or whether this "missing heritability" [10] could in fact be accounted for by forms of genetic variation not captured by genome-wide association studies (GWAS) of common SNP variants. One such class of variants that have received increased attention in recent years are CNVs.

Introduction to Copy Number Variation

A CNV is defined as a segment of DNA differing in the number of diploid copies carried by individuals within the population [11–14]. CNVs include simple bi-allelic deletions and duplications, as well as more complex, multi-allelic variants showing highly polymorphic patterns of copy number distribution at the population level (Fig. 4.1).

CNV discovery studies to date all concur that CNVs are widespread throughout the human genome, and are also observed in phenotypically healthy individuals [11–16]. While precise estimates of CNV frequencies and their average size have differed between studies, in the highest resolution genome-wide CNV discovery study carried out to date [14], a total of 8,599 CNVs above 443 bp, covering approximately 3.7 % of the genome, were independently validated, with a median CNV size of 2.7 kb and a median of 1,117 and 1,488 CNVs in European (CEU) and Yoruban African (YRI) subjects, respectively [14]. Of the approximately 5,000 validated CNVs which were subject to further investigation, 77 % were deletions, 16 % were duplications and 7 % were multi-allelic variants, although it is essential to consider that these frequencies may also be influenced in part by the respective ease of detection of these three forms of structural variation [14].

As shown in Fig. 4.2, CNVs were found to overlap 13.4 % of RefSeq genes, with a smaller proportion of deletions than duplications and multi-allelic variants overlapping genes [14]. CNVs were detected genome-wide, with CNVs shown to result in loss of function mutations at over 260 genes [14]. Any two subjects were found to differ in copy number at an average of approximately 0.78 % of the genome, affecting structure of approximately 2.7 % of gene transcripts [14]. Multiple studies have concurred that common bi-allelic CNVs are well-tagged by surrounding SNPs [13, 14, 17], while significantly less linkage disequilibrium has been detected between duplications and multi-allelic variants and their surrounding SNPs [14, 17]. In addition to tandem duplications, numerous dispersed duplications have also been detected, indicating that this may be an overlooked class of CNV [14] (Fig. 4.3).

Population genetic analyses of genomic structural variation thus suggest that CNVs are widely distributed in the human genome, with the majority of CNVs being of small size, with significant overlap between CNVs detected in different subjects [11–16]. Furthermore, CNV hotspots prone to recurrent recombination

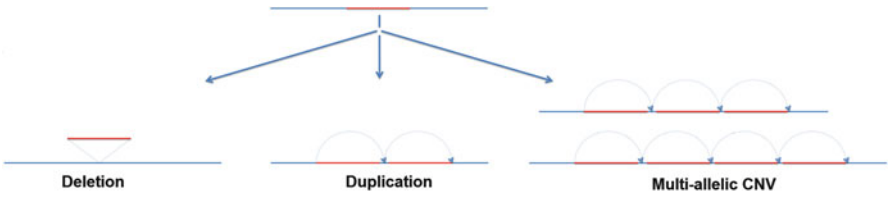

Fig. 4.1 Copy number variant (CNV) classes. CNVs may consist of simple deletions or duplications, or more complex rearrangements such as multi-allelic CNVs, where several allelic configurations exist for the same locus, varying in the number of copies of the duplicated region

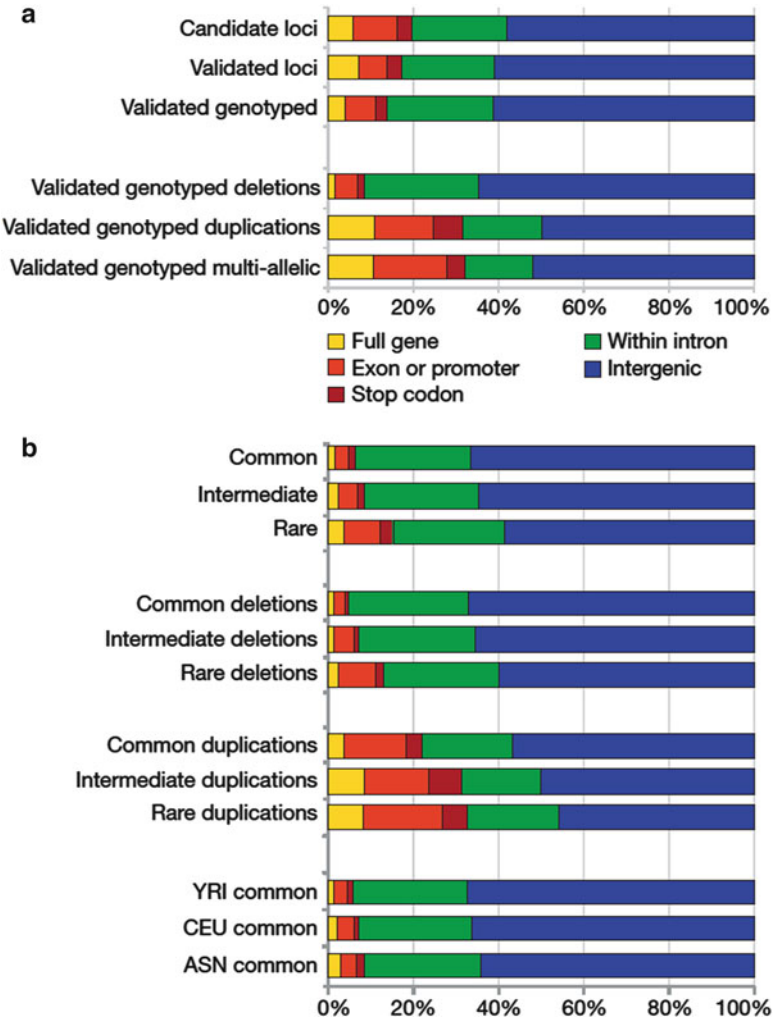

Fig. 4.2 Functional impact of CNVs in the genome. (**a**) Overall functional consequences of CNVs, stratified by level of validation and CNV type. (**b**) Functional impact of CNVs, stratified by CNV type, frequency, and sample geographic origin. YRI: Yoruba in Ibadan, Nigeria; CEU: CEPH (Utah residents with ancestry from northern and western Europe); ASN: Japanese in Tokyo, Japan + Han Chinese in Beijing, China. Figure reproduced with permission from Conrad et al. (2010) [14]

exist in the genome, particularly in the vicinity of segmental duplications [18] and sequence motifs such as *Alu* repeats [14, 19, 20]. In addition to common CNVs with identical breakpoints shared by multiple individuals, a multitude of rare and recurrent CNVs exist, a higher proportion of which overlap genes than do common structural variants, and might thus contribute significantly to interindividual phenotypic differences [14]. Similarly, a higher degree of overlap exists between genes and

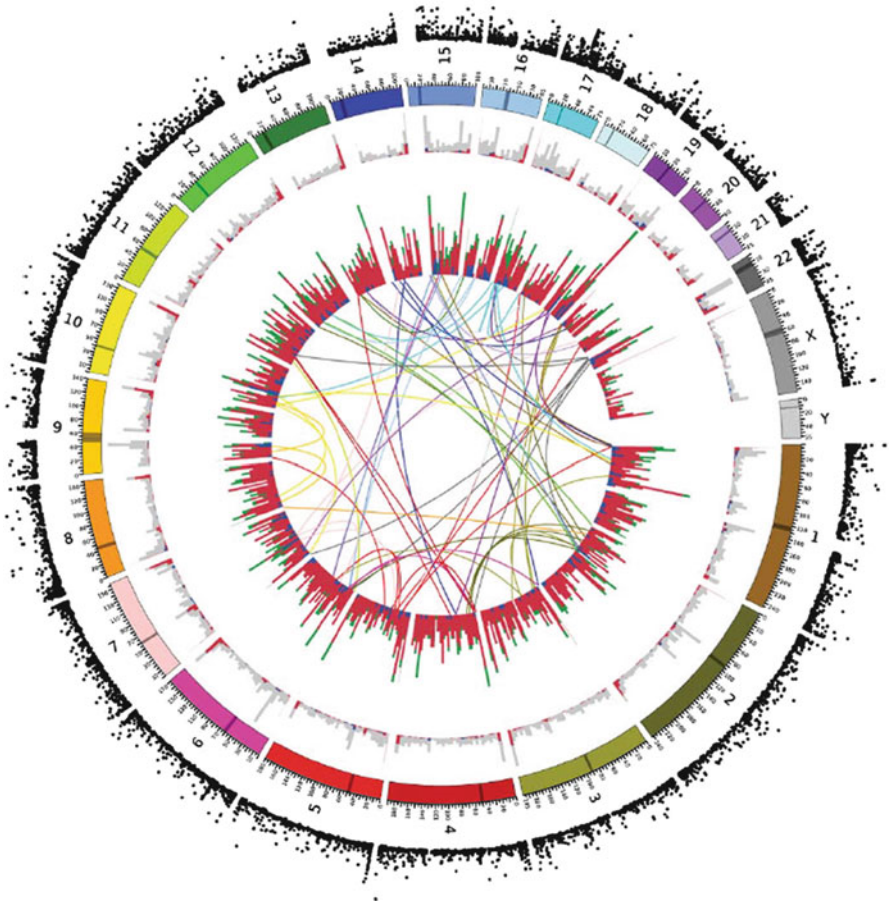

Fig. 4.3 Circular plot of genome-wide CNV distribution reported by Conrad and colleagues [14]. The concentric circles depict, from inside to outside, stacked histograms of the numbers of deletions, duplication, and multi-allelic CNVs in *red*, *green*, and *blue*, respectively, the number of CNVs by mechanism of formation (NAHR, VNTR, and other shown in *blue*, *red*, and *grey*, respectively), and the degree of population differentiation between the Yoruban and European study samples of detected CNVs in the outermost circle, with the innermost circle depicting the origin and new location of dispersed duplications in the genome. Figure reproduced with permission from Conrad et al. (2010) [14]

complex structural variants such as multi-allelic CNVs and VNTRs, implicating these complex and understudied variants in phenotypic variability and disease susceptibility [14].

CNVs may influence gene expression levels either directly or indirectly through a number of different mechanisms, including deletion or duplication of entire genes, gene-disrupting CNVs, or through long-range effects mediated through disruption or insertion of regulatory elements such as enhancers or repressors [21, 22]. In the case of multi-allelic CNVs encompassing dosage sensitive genes, expression levels

Fig. 4.4 Dosage-sensitive genes. Dosage-sensitive genes are those at which changes in gene copy number result in changes in the quantity of mRNA produced

may be directly correlated with gene copy number [22] (Fig. 4.4). The phenotypic effects of CNVs and their potential contribution to disease susceptibility have thus become a topic of considerable interest.

Copy Number Variation in Adiposity and Obesity Susceptibility

The Contribution of Common Copy Number Variants to Body Weight Regulation

Given the previously noted potential functional influences of CNVs, a natural progression from CNV discovery studies was the investigation of their potential contribution to human disease susceptibility and the so-called "missing heritability" [10] of common diseases.

A large number of SNP association analyses have been conducted to date in both case–control samples and population cohorts for numerous common diseases [23], and the development of CNV prediction algorithms has enabled CNV prediction using these genome-wide SNP array data [24–27]. This has permitted the reuse of these data for CNV association studies. Similar to SNP GWAS, genome-wide CNV association studies have often focussed on common CNVs, usually defined as those having a population frequency above 5 %, with several associations between common CNVs and complex diseases, including obesity, having been reported in recent years.

Marginal association of a common CNV on chr10q11.22 encompassing the pancreatic polypeptide receptor 1 (*PPYR1*) gene with BMI has been reported in a Chinese population sample, with low copy number associated with increased BMI [28]. *PPYR1* ligands have previously been linked to the regulation of food intake in both human and animal studies [29–31], lending support to a potential role for CNVs encompassing this gene in body weight regulation. Furthermore, a common CNV at 11q11 encompassing the olfactory receptor genes *OR4P4*, *OR4S2*, and *OR4C6* has also been reported to show association with early-onset extreme obesity [32].

As well as CNVs consisting of di-allelic variants, more complex structurally variable regions may also contribute to increased risk of disorders such as obesity [33]. We have recently shown a complex copy number variable region on chromosome

8p21.2 to be significantly associated with susceptibility to severe obesity [33]. The region encompasses two variable number tandem repeats (VNTRs) flanking a 3,975 bp common deletion. Two of these three variants are located within the dedicator of cytokinesis gene (*DOCK5*), and all three structural variants were shown to be significantly associated with *DOCK5* gene expression levels [33]. The *DOCK5* gene is a member of the DOCK family of guanine nucleotide exchange factors (GEFs) [34], which are thought to be involved in a variety of cellular functions such as growth, differentiation, regulation of the actin cytoskeleton, vesicle transport, cell signalling, cell movement, phagocytosis, and apoptosis [35] through their role in the activation of members of the Rho/Rac-family GTPases [34]. Further investigation is required in order to establish the precise mechanism by which CNVs within the *DOCK5* region contribute to obesity susceptibility.

In addition to studies directly measuring copy number, some studies have also identified common CNVs potentially contributing to disease susceptibility through linkage disequilibrium with nearby SNPs. Using this approach, two common CNVs, one upstream of *NEGR1* and another near *GPRC5B*, have been linked to body weight through association of tagging SNPs with BMI in two large meta-analyses [8, 36]. Although the effect sizes observed at each of these loci were small, given the LD between these structural variants and their tagging SNPs, it has been suggested that these variants could potentially be causal variants [8, 36].

In spite of these reports, the role of common CNVs in disease susceptibility remains an issue of contention, with little replication of reported associations. A large study conducted by the Wellcome Trust Case Control Consortium (WTCCC) reported association of common CNVs at *IRGM* and *TSPAN8* with Crohn's disease and type 2 diabetes, respectively, as well as association of copy number at the HLA locus with each of Crohn's disease, rheumatoid arthritis, and type 1 diabetes [37]. However, apart from these reported hits, the authors found little evidence of association between common CNVs included in their analyses and any of the eight complex diseases in their study. The authors did however highlight the complexity of CNV prediction and association studies, reporting the confounding effects of several sources of systematic bias such as DNA source and quality, as well as batch effects, on CNV analyses [37]. Moreover, the authors also acknowledged that due to the extensive challenges in assaying more complex structural variants such as multiallelic CNVs and VNTRs, their study was largely limited to common bi-allelic CNVs [37]. These observations highlight the need for additional investigation of the role of CNVs in complex disease susceptibility, focussing in particular on complex structural variants.

The Role of Rare Genomic Structural Variants in Adiposity and Risk of Obesity

Given the large proportion of the estimated heritability of obesity which remains unexplained, it has been suggested that some of this "missing heritability" [10] may

Fig. 4.5 UCSC genome browser view of the proximal 16p11.2 CNV region. The presence of two segmental duplications with high sequence similarity (depicted in *red*) results in the recurrent occurrence of deletions and duplications of the intervening 593 kb segment of unique DNA sequence in this region. Plot generated using the UCSC genome browser [56]

be accounted for by the collective effect of a large number of individually rare variants, each of large effect size [38]. Consistent with what has been observed in the case of SNPs, an increasing body of evidence is supporting the potential contribution of rare CNVs to susceptibility to complex diseases such as obesity, which will be the focus of this section.

Rare CNVs are generally defined as those with frequencies below 1 % in the general population [39, 40]. The rarity of these CNVs generally means that they are not well-tagged by surrounding common SNPs genotyped on GWAS panels. Given the inherent difficulties in accurately genotyping CNVs, analysis of rare CNVs has also principally focussed on variants of large size, often above 200–500 kb [39, 40]. Several large, rare CNVs have thus been reported to show association with body weight and risk of obesity.

Structural Variants Within the 16p11.2 Region

Several CNVs have been identified within the 16p11.2 region, with CNVs at two loci in this region showing association with either underweight, or increased risk of overweight or obesity [38, 39, 41, 42].

Copy Number Variation at the Proximal 16p11.2 Locus

In 2010, we reported association of a heterozygous deletion on chromosome 16p11.2 (chr16: 29,514,353–30,107,356) with highly increased risk of obesity [38]. The deletion encompasses 593 kb of unique sequence and contains 29 genes (Fig. 4.5), including multiple candidates for the obesity phenotype. The presence of two segmental duplications with high sequence similarity renders this locus prone to de novo structural rearrangements (Fig. 4.5), resulting in the occurrence of both deletions and duplications of the intervening DNA sequence [38].

This deletion was initially identified in our study at a frequency of approximately 2.9 % in a study sample of patients suffering from obesity-plus syndromes, whereby patients presented with obesity coupled with additional clinical features such as developmental delay and/or congenital abnormalities [38]. Further investigation revealed an additional 22 deletion carriers among subjects referred to clinical services for cognitive impairment, and 19 subjects among obesity case–control and population GWAS samples [38]. This variant was also reported concurrently in a study by Bochukova et al. [39].

Deletions at this locus resulted in a 30-fold increase in risk of obesity and 43-fold increased risk of morbid obesity, and were identified in 0.7 % of morbidly obese subjects included in our analysis [38]. The obesity phenotype observed among deletion carriers was frequently coupled with hyperphagia, suggesting it to be of potentially neurological origin. While no gender bias was detected in our analysis, an age-dependent effect for this CNV was observed, where penetrance of the obesity phenotype in deletion carriers was positively correlated with subject age [38]. A 0.4–0.7-fold reduction in gene expression levels was also observed for transcripts of genes located within the deleted segment, suggesting that haploinsufficiency for one or more of these genes may be causative for the obesity phenotype observed in deletion carriers [38]. In addition to increased risk of obesity, the deletion was also associated with increased head circumference [38].

A recent study also confirmed the association of this deletion with macrocephaly, but also reported significantly reduced cognitive functioning and an increased frequency of gross motor delay among deletion carriers [43]. Psychiatric comorbidities were reported in greater than 80 % of deletion carriers, while penetrance of obesity was over 70 % of carriers of the deletion in this study sample [43].

Apart from its association with obesity, copy number variation at this 16p11.2 locus has previously been associated with neurodevelopmental and psychiatric conditions, implicating this locus in a number of phenotypes. Both microdeletions and microduplications of the same locus at 29.5 Mb in the 16p11.2 region were shown to be associated with increased risk of autism spectrum disorders (ASD), accounting for approximately 1 % of ASD cases in one study [44]. On the other hand, duplications, but not deletions, at this locus were also linked to increased susceptibility to schizophrenia, with duplication carriers showing a 14.5-fold increased risk of schizophrenia [45]. These findings have since been replicated in a number of studies [46–48], confirming the contribution of these loci to increased risk of these disorders, and raising the interesting question of the interrelationship between the obesity and neurodevelopmental and psychiatric phenotypes associated with copy number variation at this locus.

A retrospective analysis of 16p11.2 deletions in a clinical sample of approximately 7,000 subjects—the majority of whom had presented with phenotypes such as developmental delay, autism spectrum disorder (ASD) or dysmorphism—identified 28 deletion carriers among this sample [49]. The age-dependence and juvenile onset of the obesity phenotype was confirmed, with obesity generally developing within the first decade of life. Furthermore, a gender-dependence for the 16p11.2 was reported, with male deletion carriers exhibiting a more severe phenotype than female carriers [49]. The incidence of obesity among deletion carriers diagnosed

with ASD was also noted to be higher than among autistic subjects not carrying the deletion, providing further support for the independent association between deletions at this locus and increased risk of obesity [49].

In a second study in 2011, we investigated the impact of the reciprocal 16p11.2 duplication on body mass and head circumference [41]. In a fascinating example of a mirror effect of gene dosage at this locus on phenotype, the reciprocal 16p11.2 duplication was associated with strongly increased risk of being underweight, with carriers of this duplication showed significantly reduced postnatal weight and BMI compared to non-duplication carriers [41]. For the purpose of this study, underweight was defined as a BMI $\leq$ 18.5 kg/m^2 in adults and BMI z-score $\leq$ 2 standard deviations from the mean for age and sex in children [41]. Underweight can have serious health repercussions, and is frequently associated with failure to thrive during childhood, eating and feeding disorders, as well as anorexia nervosa. Despite the potentially serious nature of this condition, little is known of the factors underlying its genetic susceptibility [50]. In this analysis, 50 % of the male duplication carriers under the age of 5 were diagnosed with a failure to thrive, while adult carriers of this duplication showed an 8.3-fold increased risk of being clinically underweight [41]. A gender effect was also observed, with males showing a trend towards increased severity. In addition to its observed effect on weight, the duplication was also associated with an increased frequency of restrictive and selective eating behaviors, mirroring the hyperphagic phenotype observed in carriers of the reciprocal deletion [41]. Similarly, duplication carriers were noted to show significant reduction in head circumference, which mirrored the macrocephaly associated with the reciprocal deletion [38].

The 16p11.2 duplication was also observed at a higher frequency among medically ascertained patients, recruited on the basis of developmental and cognitive delay or psychiatric phenotypes, than in non-medically ascertained population cohorts in this study, supporting the previously reported association of this duplication with cognitive, neurodevelopmental, and psychiatric phenotypes [44, 45].

A 220 kb Deletion on Chromosome 16p11.2 Encompassing the SH2B1 *Gene*

In addition to the previously described proximal 16p11.2 deletion and duplication shown to be associated with body weight regulation, additional CNVs within the 16p11.2 region have also been associated with obesity susceptibility.

A 220 kb deletion in this region encompassing nine genes, including the SH2B adaptor protein 1 (*SH2B1*) gene, has been reported to be associated with severe, hyperphagic, early-onset obesity [39]. Although carriers of this deletion have been reported to exhibit elevated fasting plasma insulin levels, conflicting observations have also been reported [39, 51].

SH2B1 is known to enhance leptin and insulin signalling, and animal studies have shown mice harboring homozygous null mutations in the *SH2B1* gene to

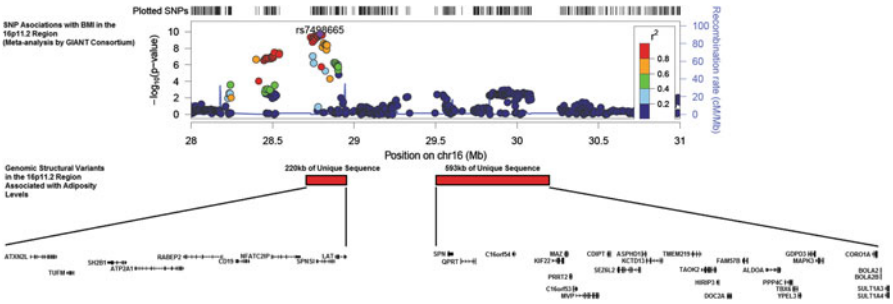

Fig. 4.6 The chromosome 16p11.2 region. (**a**) Association results for SNPs in the 16p11.2 region with BMI in a recent meta-analysis carried out by the GIANT consortium [8] Chromosome 16 genomic coordinates are plotted on the *x* axis, with minus $\log_{10}$(*P*-value) plotted on the *y* axis. An association peak can be seen at approximately 28.8 Mb. Plot generated using LocusZoom [57]. (**b**) The positions of two genomic structural variants associated with adiposity levels are depicted. A 220 kb deletion at chr16: 28.73–28.95 Mb and a 593 kb deletion at chr16: 29.51–30.11 Mb have been associated with obesity [38, 39] while a duplication of the latter 593 kb of unique sequence has also been associated with risk of being underweight [41]. The genes falling within each of the two CNVs are also shown. *CNV* copy number variant, *GIANT* Genetic Investigation of ANthropometric Traits, *SNP* single nucleotide polymorphism. First published in *Nature Reviews Endocrinology*, 2013, doi: 10.1038/nrendo.2013.57 by Nature Publishing Group

exhibit signs of metabolic syndrome, with a phenotype including obesity, hyperphagia and insulin resistance [52]. SNPs within *SH2B1* have also shown association with BMI in several meta-analyses [8, 36, 53], making it a strong candidate for the obesity phenotype observed in carriers of this 220 kb deletion. Figure 4.6 depicts association results for SNPs within the 16p11.2 region from a recent BMI meta-analysis, as well as the positions and gene content of both CNVs within the 16p11.2 region described in this chapter.

In addition to its association with severe obesity, this deletion encompassing *SH2B1* has also been linked to developmental delay. In an analysis of a clinical sample of approximately 23,000 patients referred for array comparative genome hybridization (aCGH) for phenotypic abnormalities including developmental delay and cognitive deficits, this medically ascertained sample was found to be enriched for this deletion, and assessment of additional anthropometric data available for a subset of the deletion carriers supported its association with early-onset obesity [42].

Global Burden of Rare Copy Number Variants in Obesity

In addition to the analysis of individual CNVs and their contribution to obesity susceptibility, another area of particular interest is whether the global burden of large, rare CNVs may be higher among subjects suffering from obesity. This is assessed by comparing the total number of rare CNVs above a defined size threshold observed in obese cases versus normal-weight control subjects.

Large, rare deletions have been reported to be enriched among obese cases compared to normal-weight controls in case–control analyses of global CNV burden [39, 40]. In these analyses, large CNVs were found to be overrepresented among obese cases, with this enrichment driven largely by deletions [39, 40]. Furthermore, a larger effect was observed when the analysis was limited to those CNVs which disrupt genes [40], highlighting the potential significance of genes located within these variants to obesity susceptibility.

Rare CNVs Present Exclusively in Cases

Another method of identifying CNVs which might be relevant to obesity susceptibility is to identify CNVs observed exclusively in obese cases and not in normal-weight controls. One study identified 17 CNVs present exclusively in three or more Caucasian obese subjects, eight of which were also observed only among African American obese subjects and no normal-weight controls [54]. Their presence solely in obese cases might suggest a potential role for these variants in obesity susceptibility, and replication of these observations in study samples of different ethnicities provides further support for their relevance to the pathogenesis of obesity [54].

While several studies have provided intriguing evidence for the involvement of rare CNVs on obesity susceptibility, it is essential to note that in the analysis of rare variants, wider replication in larger study samples will be necessary to firmly establish their contribution to disorders such as obesity.

From Genetic Variants to Their Physiological Impact: The Importance of Follow-up Studies in CNV Analyses

The identification of structural variants, both common and rare, associated with obesity susceptibility is providing insight into its pathogenetic origins and helping explain some of the missing heritability of this disorder. However, similar to what is observed in the case of common SNPs, there is often difficulty in translating these genetic findings into clear understanding of the underlying biological pathways and mechanisms responsible for this disease. In the case of CNVs, this problem is compounded by the fact that CNVs are often large and may encompass several genes, making it difficult to decipher which gene or genes are responsible for the phenotypic effects observed. Furthermore, CNVs may also have long-range effects, with variants shown to influence expression levels of genes up to several megabases away.

For this reason, it is important to follow up genetic associations with functional studies which attempt to understand how specific variants affect phenotype. As previously discussed, deletion and duplication of a 593 kb region on chromosome 16p11.2 has been associated with a mirror effect on various phenotypes, one of which is head circumference. Duplication of this region has been associated with microcephaly [41], while the reciprocal deletion has been associated with increased

head circumference. Through systematic over-expression and knockdown of each of the orthologous genes within the CNV region in zebrafish, the gene responsible for the variation in head circumference associated with copy number in this region was shown to be the potassium channel tetramerization domain containing 13 (*KCTD13*) gene [55]. Further studies of this type should be undertaken to identify the causal gene or genes for the obesity phenotype associated with the proximal 16p11.2 CNV. Similarly, functional exploration of other CNVs reported to be associated with obesity susceptibility would help in better delineating their physiological effects and in identifying the causative genes located within them.

Future Directions in the Study of CNVs in Obesity

The contribution of CNVs—both common and rare—to obesity susceptibility is becoming increasingly recognized, with progressively more reports of CNVs associated with adiposity levels. However, in spite of this mounting body of evidence, our understanding of the contribution of structural variants to complex diseases such as obesity remains rudimentary, particularly in the case of rare CNVs.

Extensive replication studies including larger numbers of subjects are now required in order to study reported structural variants more comprehensively, verify their reported associations with obesity susceptibility and provide better estimates of their effect sizes. As previously discussed, functional studies will also be necessary to uncover the underlying mechanisms by which such variants may contribute to body weight regulation.

Furthermore, novel methodologies, both technical and statistical, will be required to enable more systematic investigation of complex CNVs such as multi-allelic CNVs. It is hoped that such further in-depth analyses of structural variation may improve our understanding of the genetics factors underlying susceptibility to complex diseases such as obesity.

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Chapter 5
Genetics of Childhood Obesity

Struan F.A. Grant

Abstract Obesity is increasingly becoming a major health issue for both the USA and the rest of the world, and presents health care systems with a huge economic problem. The rate at which children are becoming obese is dramatically increasingly, particularly since the turn of the twenty-first century. Although environmental factors are known to play a key role, childhood obesity is also known to have an underlying genetic component contributing to its complex etiology. Elucidating the genetic architecture of childhood obesity will not only help prevention and treatment of pediatric cases but also will have fundamental implications for diseases that present later on in life. Furthermore, the execution of genome-wide surveys of childhood obesity have uncovered novel loci that turned out not to be within the detection range in an adult setting as a consequence of environmental factor clouding, supporting the notion that the pediatric setting may be optimal for uncovering obesity genes. This new era of genome-wide association studies (GWAS) is delivering compelling signals associated with obesity, particularly with peer research groups sharing a very strong consensus on what the key loci are that contribute to the pathogenesis of this trait. Although we suggest that the pediatric setting can be harnessed for obesity gene discovery, the fact is that most BMI-associated loci identified to date were found in the adult setting, so there is a requirement to elucidate which of these variants contribute early on in life and therefore predisposing an individual to related diseases in later life. In this chapter, we outline what advances have been made in determining which genetic factors are conferring their effects on childhood obesity and which ones go on to have an impact in adulthood.

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Epidemiology of Childhood Obesity

Obesity is considered a major health concern for all industrialized societies, with an ever increasing incidence occurring specifically in children [1]. This disorder, along with the associated insulin resistance [2, 3], is known to be the underpinning of a number of major causes of death in the USA largely due to acting as a key risk factor for type 2 diabetes, cardiovascular disease, and other chronic diseases.

Understanding the early indictors for risk of obesity could play a crucial role in combating this mortality rate. Although an obese adult may not have been necessarily obese as a child, the converse is much more notable, where roughly three quarters of obese adolescents go on to present with obesity in adulthood [4–6]. Apart from the obvious chronic comorbidities and increased overall mortality for obese children in later life [7], where overweight children followed over decades [8, 9] are more likely to have cardiovascular and digestive diseases, they also have to contend with the main direct adverse effects of presenting with this trait, which include orthopedic complications, sleep apnea, and psychosocial disorders [10, 11].

Obesity, as presented as excess in adipose tissue mass, presents when there is imbalance between energy intake and energy expenditure. However, what is now considered a disadvantage and recognized as a disease, could well have been an advantage in previous times, under the "thrifty phenotype" hypothesis, when food availability was much more scarce and physical activity was a more natural part of everyday life [12].

Overweight and obesity is defined by the World Health Organization (WHO) as "abnormal or excessive fat accumulation that may impair health" [13]. In order to ascertain a metric of excess body fat, the most readily available method is to simply leverage an individual's height and weight to determine their body mass index (BMI), defined as kilograms divided by meters squared (kg/m^2). Indeed this metric has been shown to correlate well with fat content in most people, where adults with a BMI equal to 25–30 are defined as being overweight while a BMI greater than or equal to 30 is the definition of adult obesity. Although an excepted standard in the adult setting, BMI does not serve the same purpose in children well, due to large fluctuations tracking with pubertal status, age, and gender; instead, a BMI-for-age percentile is used to ascertain a sense of pediatric adiposity, where a BMI from 85th to 95th percentile is considered by the Center for Disease Control and Prevention as overweight, while a BMI equal to or greater than the 95th percentile is considered obese [14, 15]. Once a child reaches late adolescence, these percentiles do start to serve as relatively good proxies for adult definitions, where the 95th BMI-for-age percentile gets closer to representing a BMI of 30 kg/m^2 [11].

Getting a good handle on the prevalence of childhood obesity has proven challenging due to no internationally accepted definition currently existing. That said, the widely held view is that childhood obesity has reached epidemic levels in the developed world. For instance, in the USA approximately a quarter of all children in the USA are considered overweight and approximately one in ten are obese. More empirically, in the decade separately the two National Health and Nutrition

Examination Surveys (NHANES) II (1976–1980) and NHANES III (1988–1991), the prevalence of overweight children in the USA increased by 40 % [1]. Indeed, when one looks at many countries, the distribution of BMI is clearly becoming increasingly skewed toward overweight [16], with the lower part of the distribution having changed relatively little, while the upper part has widened substantially. As such, it is increasingly obvious that children are becoming more susceptible to obesity, either as a consequence of genetics or exposures in the environment.

A Genetic Component to Childhood Obesity

Despite societal changes plus strong behavioral and environmental factors, there is very strong evidence that there is a genetic component to obesity pathogenesis [17, 18]. As such, elucidating the genetic architecture of childhood obesity could have fundamental implications for both treatment and prevention of many diseases occurring much later on in life.

Twin studies have revealed much about the genetic component to many complex traits, where monozygotic twins are completely genetically identical while nonidentical dizygotic twins only share 50 % of their genetic material. When looking in the context of fat mass, the concordance among monozygotic twins has been shown to be approximately 80 % while only approximately 40 % in dizygotic twins [19–21].

Adoption and family studies have yielded even further evidence. For instance, adopted children have a strong correlation with the BMI of their biological parents but not their adoptive parents [22]. Furthermore, identical twins are significantly concordant for BMI while their nonidentical counterparts are not [23].

Looking at prevalence difference in racial/ethnic groups presents further clues of a genetic component to obesity, such as 35% or less observed in Caucasian and Asian populations while a prevalence 50% or more is seen in Pima Indians and South Sea Island populations [24].

All this genetic epidemiological evidence points to a substantial inherited component to obesity; however due to the obvious interactions with environmental factors, it has proved challenging to tease apart and characterize the genetic component to this trait.

Pre-GWAS Approaches

Linkage scans in families allow for a non-hypothesis approach to assess regions of the genome shared within and across families presenting with a given trait. In the case of the common form of childhood obesity, a number of loci have been reported, but the underlying causative event has still to be elucidated. On the other hand, syndromic forms of childhood obesity have been readily solved using this approach, with chromosomal loci for Prader–Willi syndrome [25], Alström's syndrome [26], and Bardet–Biedl syndrome [27–29] having been mapped.

Single gene disorders that present with obesity features have given us the first insight in to the genetic etiology of this trait, with early studies in rodents shedding much needed light on the issue.

The *ob*/*ob* mutant mouse [30, 31], exhibiting excess adipose tissue, revealed a mutation in the leptin gene [32, 33], with another strain of severely obese mice, *db*/*db*, revealing a mutation in its receptor [34]. Variants within these genes have subsequently been reported for human obesity-related traits [35–39], in particular a skipped exon 16 in the human leptin receptor gene leading to impaired growth hormone secretion, early-onset morbid obesity, and failure of pubertal development [40].

A notable developmental trajectory for children with disturbances of the hypothalamic leptin–melanocortin pathway as a whole has been reported. Mutations in the pro-opiomelanocortin (*POMC*) gene have been shown to impact metrics of early onset obesity in children [41–44]. In addition, individuals with genetic mutations in the *PCSK1* gene, which encodes neuroendocrine-specific prohormone convertase 1/3 (PC1/3), present with childhood obesity, hyperphagia, diarrhea, pituitary hypofunction, and disordered glucose homeostasis [45–47].

MC4R is widely considered to be the first established gene to confer morbid human obesity when mutated. Its encoded protein also plays a vital role in the hypothalamic leptin–melanocortin pathway. Multiple nonsense and missense mutations have now been reported in *MC4R*, many of which are strongly correlated with obesity related traits [48–50].

Brain-derived neurotrophic factor (BDNF) is a downstream target of MC4R activity and has also been implicated in the pathogenesis of childhood obesity, most notably a chromosomal inversion leading to the loss of one functional copy of *BDNF* in an 8-year-old girl, resulting in increased food intake, severe early-onset obesity, hyperactivity, and cognitive impairment [51].

Other loci implicated include a Y722C missense variant in *NTRK2* causing severe obesity and impaired memory in an 8-year-old boy [52] and haploinsufficiency of *SIM1* leading to severe early-onset human obesity due to a balanced translocation between chromosomes 1p22.1 and 6q16.2 [53].

More pronounced syndromes of obesity were our only means to isolate genetic factors before genome-wide association studies (GWAS) emerged after such technology became available around 2005. These classical approaches provided key insights in to the underlying mechanisms involved in energy homoeostasis and are now being complemented by the findings arising from GWAS.

Genome-Wide Association Studies

As outlined above, it has become relatively clear that family-based linkage analyses have had limited success in isolating genes contributing to obesity, particularly the common form of the disease, largely due to the fact that this approach is not well suited to detect common variants in the population conferring relatively modest risk [54, 55]. Candidate gene association studies have also struggled to bear fruit as such

approaches are limited to known biology of the given trait mechanisms; indeed from GWAS approaches described below, many of the key loci identified were never on anybody's candidate gene list.

Conversely, the GWAS approach has empowered investigators to execute a more comprehensive and unbiased strategy to identify causal genes related to complex traits, including obesity, through non-hypothesis based methodologies.

GWAS was made possible by the International HapMap project, which arose out of the human genome sequencing project. This large-scale effort went about systematically characterizing human sequence variation, a vital precursor to comprehensively investigate the genetic basis of complex disease [54–56]. Genome-wide genotyping of in excess of 500,000 single nucleotide polymorphisms (SNPs) can now be readily achieved in an efficient, cost effective and highly accurate manner [57–60]. These SNPs represented on the arrays coming out of these efforts are not selected based being putatively causal, rather they are statistically selected to simply act as "tag-SNPs" for capture of common haplotypic variation information stored in a given region of the human genome. This approach has much higher resolution than the previous linkage approaches for complex traits, where a given signal signifies that the underlying causative variant is typically within just a few hundred kilobases of the tag-SNP.

Unlike the linkage and candidate gene eras that tackled complex traits, GWAS has proven to be a very successful approach yielding robust associations that fellow researchers can replicate and agree on (see the continually updated NIH Catalog of Published Genome-Wide Association Studies at http://www.genome.gov/gwastudies).

Findings from First GWAS Analyses of Obesity

In the past 6 years, tens of genetic loci have been implicated and established for BMI from the outcomes of GWAS, but primarily in adults. These findings will be briefly outlined below in order to give context to the pediatric findings made subsequently.

The first GWAS-implicated locus for obesity was close to the insulin-induced gene 2 (*INSIG2*) gene employing only employing 100,000 SNPs [61]. The tag-SNP, rs7566605, captured the association, which represented a common genetic event with modest relative risk (relative risk = ~1.2). The locus was reported to be associated with both adult and childhood obesity and in individuals of both European and African American and ancestry. However, this study has been largely not replicated by other investigative groups [62–66] and disagreement on this observation remains in the obesity research community.

On the other hand, the second obesity locus to be reported, within the fat mass- and obesity-associated gene (*FTO*) gene [67], has been extensively replicated [68–71], including children [72]. *FTO* is now widely regarded as the most strongly associated obesity locus reported to date [69]. Of note, this locus was actually

implicated in type 2 diabetes initially, from one of the first GWAS of that disease [73, 74] but it became very obvious early on in the analyses that the primary trait was obesity susceptibility which was in turn impairing glycemic control [67]. It was subsequently shown that the minor allele of the *FTO* tag-SNP, rs9939609, is correlated almost exclusively to greater fat mass and that it influences fat distribution [75].

Like almost every other GWAS-implicated locus, the causative variant at the *FTO* locus has still to be determined. In addition, the mechanism by which *FTO* confers its effect on the pathogenesis of obesity is still far from clear. It is known that the gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase [76], is expressed in areas of the brain that influence appetite [77] and as such may explain its association with increased energy intake [78]. Compelling studies of both *FTO* knockout and *FTO* over-expressing mice strongly support the role of this gene in the regulation of energy intake and metabolism, showing that a lack of *FTO* expression leads to leanness and the converse promotes obesity [79, 80].

A sequencing effort in Caucasians, consisting of primarily adults reported a set of exonic mutations in *FTO*; however, these variants largely did not confer risk for obesity [81]; in addition, a comparable sequencing endeavor in African American children drew the same conclusion [82]. These data show that exonic events are unlikely to be the process by which this gene confers its effect, rather it is more likely to harbor a causative event somewhere in the regulatory machinery of *FTO*.

Meta-analyses

With the sample sizes required and the cost to run the arrays, GWAS represents a sizeable investment. In order to get the maximum from their datasets, investigators subsequently combine their datasets with other groups in order to discover additional loci as a consequence of the extra statistical power gained. In addition, imputation can enable an increase in the number of SNPs available for analysis in these existing datasets [83] through computationally inferring them based on neighboring variant frequencies.

Although these "meta-analyses" represent a substantial statistical power gain, the additional loci detected do have substantially smaller effects than *FTO*, but do provide additional insights in to the biology of the BMI/obesity phenotype.

The first GWAS meta-analysis of BMI, again primarily in adults, revealed a signal that coincided with the well-known *MC4R* gene [84]. The GIANT consortium then revealed six more genes [transmembrane protein 18 (*TMEM18*), potassium channel tetramerization domain containing 15 (*KCTD15*), glucosamine-6-phosphate deaminase 2 (*GNPDA2*), SH2B adaptor protein 1 (*SH2B1*), mitochondrial carrier 2 (*MTCH2*), and neuronal growth regulator 1 (*NEGR1*)] [85], five of which were confirmed in an Icelandic GWAS (but not *GNPDA2* due to an unavailable proxy SNP), who also uncovered and reported loci on 1q25, 3q27, and 12q13 [86] and verified association with the brain-derived neurotrophic factor (*BDNF*) gene [87].

The largest meta-analysis reported to data, by the GIANT consortium, revealed multiple additional BMI loci through the leveraging of data available on 249,796

individuals [88]. Thirty-two loci reached genome-wide significance, of which ten were known from the BMI studies described above, four were known loci from previous studies of weight and/or waist-hip ratio, namely, *SEC16B*, *TFAP2B*, *FAIM2*, *NRXN3*, and eighteen were entirely novel BMI loci, namely, *RBJ-ADCY3-POMC*, *GPRC5B-IQCK*, *MAP2K5-LBXCOR1*, *QPCTL-GIPR*, *TNNI3K*, *SLC39A8*, *FLJ35779-HMGCR*, *LRRN6C*, *TMEM160-ZC3H4*, *FANCL*, *CADM2*, *PRKD1*, *LRP1B*, *PTBP2*, *MTIF3-GTF3A*, *ZNF608*, *RPL27A-TUB*, and *NUDT3-HMGA1*. Interestingly, apart from the *GPRC5B* association to SNPs, a 21 kb associated deletion was identified 50 kb upstream of this gene. This study also made use of a pediatric cohort to provide further support for their findings.

The same study group subsequently went on to look at extremes of the distribution in 263,407 individuals of European ancestry and identified 7 additional loci (*HNF4G*, *RPTOR*, *GNAT2*, *MRPS33P4*, *ADCY9*, *HS6ST3* and *ZZZ3*) that contributed to clinical classes of obesity [89].

Testing Adult-Discovered Loci in Children

There is increasing evidence that that many of the common complex diseases observed in adults have their developmental origins in childhood, in particular obesity, and the path to these disorders are laid out at a young age, or even *in utero* [90, 91]. As described above, a number of genetic loci have now been established to be robustly associated with adult BMI so it would be interesting to know how these loci operate in childhood to see if they confer risk for the pediatric form of obesity.

Leveraging an existing GWAS dataset of pediatric BMI variation from 6,000 children, investigators were able to ask if these SNPs influenced this trait [92]. Nine of the loci in fact did reveal evidence of association with pediatric BMI, of which the *FTO* locus was the strongest. *TMEM18* followed by *GNPDA2* were the next most strongly associated adult-implicated loci, showing a similar magnitude to that of *FTO* in this pediatric setting. The remaining weaker loci were *INSIG2*, *MC4R*, *NEGR1*, 1q25, *BDNF* and *KCTD15* (Table 5.1). These findings were much in line with the findings made in the initial adult report, where they checked in a smaller pediatric setting [85].

Going on to check the full 32 loci reported in the more recent GIANT meta-analysis through the leveraging of 1,097 childhood obesity cases (BMI ≥95th percentile CDC definition), together with 2,760 lean controls (defined as BMI <50th percentile), aged between 2 and 18 years old [93], the same investigative group reported evidence of association for nine of the loci, namely, at *FTO*, *TMEM18*, *NRXN3*, *MC4R*, *SEC16B*, *GNPDA2*, *TNNI3K*, *QPCTL*, and *BDNF*. Overall, 28 of the 32 loci revealed directionally consistent effects to that of the adult BMI meta-analysis. As such, it is abundantly clear that the majority of obesity-conferring variants initially uncovered in adults are indeed operating early on in life; however these adult-discovered loci only explain less than 2% of the total variation for BMI in children, which is less striking than reported by the GIANT consortium in their adult cohorts [85] so many more genes need to be characterized.

Table 5.1 Childhood obesity loci that have been identified to date and the context by which they were implicated

Category	Loci	Citations
Adult BMI GWAS loci also associated with childhood BMI/obesity in independent studies	*FTO*, *TMEM18*, *GNPDA2*, *INSIG2*, *MC4R*, *NEGR1*, *1q25*, *BDNF*, *KCTD15*, *POMC*, *FAIM2*, *TNNI3K*, *SEC16B*, *GNPDA2*, *BDNF*, *NRXN3*, *QPCTL*	[92, 93, 109]
Adult 2 type diabetes GWAS loci also associated with childhood BMI/obesity	*HHEX-IDE*	[94]
GWAS of extreme childhood obesity—novel loci	*SDCCAG8*, *TNKS-MSRA*	[108]
GWAS of common childhood obesity (BMI ≥ 95th percentile)—novel loci	*OLFM4*, *HOXB5*	[109]
CNV analyses of childhood obesity—novel loci	*SH2B1*, *EDIL3*, *S1PR5*, *FOXP2*, *TBCA*, *ABCB5*, *ZPLD1*, *KIF2B*, *ARL15*, *EPHA6-UNQ6114*, *OR4P4-OR4S2-OR4C6*	[126, 127, 129, 131]

Similarly, a number of loci have been implicated by GWAS for type 2 diabetes and their mechanism of action in this regard is equally far from clear. Using a similar tactic, the same investigative group analyzed the role of these loci with respect to childhood BMI [94]. Interestingly, only a single locus showed association, where the same variant in *IDE-HHEX* that increases type 2 diabetes risk also showed evidence of association with increased pediatric BMI. This finding provides some guidance on how this particular locus is conferring its diabetic effect.

Subsequently, a comparable study was carried out with loci implicated by GWAS for influencing adult bone mineral density and/or osteoporosis risk [95–99] and found that the same variation near *Osterix* that increases BMD is also associated with higher BMI in girls [100], further implicating body size and skeletal loading in its potential mechanism of action.

FTO in Childhood

As *FTO* represents the first robustly established locus associated with obesity, it has received much more attention to date than other similarly found loci due to the fact that it has been known longest, including in the pediatric setting.

It has become clear from cross-sectional studies that there is an age-dependent effect of *FTO* genetic variation on BMI. Although there is consistent evidence that *FTO* has no obvious impact on fetal growth or birth weight, there is marked effect by the age 7 years old [67]; however, one study has suggested that there is in fact a negative association between *FTO* and BMI before the age of 2 years old, but a

positive correlation after that age, peaking at approximately 20 years old followed by a decline [101].

Longitudinal cohort studies have added context to some of these initial reports. Specific statistical modeling has already been developed that can characterize important milestones in pediatric BMI trajectories. For children with normal growth patterns, BMI rises steeply after birth until reaching "infancy peak" [102], which has been suggested to be at a median of 7 months old [103]. Following this maximum, BMI declines gradually, and then reaches a low point called the "adiposity rebound" [104], which occurs just before the onset of puberty, and then continues to rise until adulthood. Adiposity rebound can occur anywhere in the range of 2–7 years old, but typically in the 4–5 year old age band [103]. Children carrying the obesity risk variants harbored at the *FTO* locus do not just present with a higher BMI throughout their lives, but also present with a specific developmental trajectory that is known to lead to higher incidences of future obesity [105] and comorbidities [106]. In a meta-analysis of eight Caucasian pediatric cohorts, adult obesity associated *FTO* alleles were associated with lower BMI at adiposity peak, higher BMI at adiposity rebound, and most interestingly, with an earlier age of adiposity rebound [107], thus supporting the initial study suggesting a negative association in the less than 2 years old age band. This in turn reflects a possible steeper downward inflection of BMI trajectory after infancy peak leading towards an earlier adiposity rebound in those genetically predisposed individuals [107].

Loci Specifically Identified in Childhood Obesity GWAS Analyses

A widely recognized notion is that the distillation of the genetic component for a number of complex traits, including obesity, should be easier in children, where the period of environmental exposure is substantially. As such, if a child is presenting with a given trait that is also seen in adults, it is more likely that has presented due to a genetic predisposition rather than a prolonged environmental stressor.

Although multiple studies have revealed many loci in the context of relatively simple syndromic forms of the disease, there has been relatively little progress in identifying genes that directly impact the less extreme, common form of childhood obesity; after all this is the primary trait that is on the massive increase observed over recent years.

The first attempt at a full GWAS of childhood obesity was carried out in 2010, where French and German study groups carried out a joint analysis of genome-wide genotyped data generated on their early-onset obesity cohort [108], defined as BMI in the range of the 97th to the 99th percentile; as a consequence, two novel loci were identified, namely, *SDCCAG8* and *TNKS*/*MSRA* (Table 5.1). In the initial step of the analyses, association was tested for both genotyped and imputed SNPs in a combined French and German sample consisting of 1,138 extremely obese children plus 1,120 normal or underweight controls. For the follow-up replication

attempts, all SNPs that yielded a degree of strong evidence for association were genotyped and tested in an independent cohort of 1,181 obese children and 1,960 normal- or underweight controls plus 715 nuclear families with at least one extremely obese offspring. Although this observation for these two loci was relatively compelling, their effect within the most recent adult GIANT meta-analysis [88] was marginally associated at best, suggesting that the effect was limited to a relatively extreme pediatric setting.

Subsequently, a large-scale meta-analysis was carried out with a definition of the disease with a less extreme cutoff, i.e., BMI ≥95th percentile, in order to address the more common form of childhood obesity [109]. This study consisted of 14 existing GWAS datasets, made up of 5,530 cases plus 8,318 controls defined at the <50th percentile of BMI. Apart from robustly detecting seven known loci, namely, *FTO*, *TMEM18*, *POMC*, *MC4R*, *FAIM2*, *TNNI3K*, and *SEC16B*, this study also detected two novel loci when taking any signal that reached a $P < 5 \times 10^{-5}$ in the discovery stage in to the replication stage, namely, rs9568856 near *OLFM4* and rs9299 within *HOXB5*. It became clear that these loci were also associated with adult BMI when querying the large GIANT meta-analysis dataset, but were not detected by those investigators in that setting as the signals were below detection at the genome-wide level. As such, this pediatric model did detect additional variants that were below the bandwidth in the environmentally more complex setting of adulthood; in addition, the study "rediscovered" loci like *TNNI3K* at the fraction of the cohort size that was required in the adult setting. These two loci are also functionally interesting where studies in mice have shown that OLFM4 plays a role in the host gastric mucosa immune response against *H. pylori* infection [110], while HOXB5 is a member of the homeobox transcription factor family and has been implicated in gut development and fat loss [111, 112]. As such, these findings point to a possible role of the gut in determining BMI in early childhood and beyond, and presents investigators with possibly novel therapeutic entry points in to both preventing and treating the disease.

A subsequent study performed SNP association analyses in 1,509 children at the very extreme end of the obesity tail, i.e., greater than 3 standard deviations from the mean of the BMI distribution plus 5,380 controls [113]. Following up 29 SNPs that reached $P < 1 \times 10^{-5}$ in the discovery stage in an additional 971 severely obese children and 1,990 controls revealed four new loci, namely, *LEPR*, *PRKCH*, *PACS1*, and *RMST*. This is clearly different from what was observed in the more common form of the disease and could be possibly used in the future to partition the disease in to different forms of obesity, i.e., sub-categorization.

Other Ethnicities

Genomes from people of different ancestries can provide geneticists with a lot of information. In particular if a locus is found to confer risk of a disease in multiple populations, then that genomic region will be considered to have more global

relevance to the disease and thus may be more attractive targets and pathways from a potential diagnostic and therapeutic point-of-view. In addition, cohorts of African ancestry have been leveraged on multiple occasions to fine map a locus down to the closest point to the underlying causative variant by taking advantage of the fact that these populations are more ancient, have had more time for genetic recombination and therefore have, on average, shorter stretches of linkage disequilibrium, e.g., the association of T2D with *TCF7L2* [114] has been refined utilizing a West African patient cohort [115].

With respect to outcomes form GWAS analyses of obesity, the bulk of those studies have been carried out in Caucasians to date. This is partly due to the relatively low haplotypic complexity of genomes from European ancestral populations and thus less SNPs are needed on the array to capture the bulk of the common variation, but is also partly to best deal with issues related to admixture. When considering the well-established association between *FTO* and obesity, and the fact that it shows the strongest association with BMI among children European ancestry [92], the picture has been less clear investigating the correlation in populations of African ancestry [70, 116]. However, there is growing consensus from recent large cohort studies in both adults [117] and children [72], that SNP rs3751812 captures the *FTO* association with the trait in both ethnicities.

GWAS meta-analyses for BMI and obesity are now emerging for other ethnicities, and with respect to the first African American effort [118], where a pediatric cohort of the same ethnicity was used to support the findings. In addition to supporting 32 of the 36 loci previously reported in Caucasians, the large group of investigators found robust evidence for association at loci harboring *GALNT10* and *MIR148A-NFE2L3* plus suggestive support for association at *KLHL32*.

Missing Heritability

A major restriction built in to any GWAS approach in that it is based on the concept of the common disease, common variant hypothesis, where it is presumed that the genetic component to the complex trait under study falls in to that category. It has turned out to be partly correct, where a proportion of such a genetic component is due to a moderate number of common variants, but which individually only explain a small proportion of predicted genetic susceptibility to the trait in a population. Like most complex and common diseases, tens of loci have now been identified for obesity, but only a small proportion of the calculated heritability has been explained, representing approximately 10 % of the estimated heritability in most disease settings. In fact, the statistically robust obesity associations with *FTO* and *MC4R* only account for less than 2 % of the variance in adult BMI, with the combined results of all obesity GWAS loci found to date still only accounting for a very small proportion of the heritability of BMI [85, 119]. This has led to a great deal of debate on what the missing heritability could look like [120], with the main consensus settling around the notion that it will be made up of much rarer variants, copy

number variants and epigenetic changes which are not within the detection range of GWAS [121], rather they will need to be characterized through the use of whole genome sequencing related approaches.

Interestingly, there is a counter argument to this prevailing view, where it could be said that the current estimates of missing heritability are incorrect. In particular, Zuk et al. [122] suggested that the estimated missing heritability not detected by GWAS may in fact be hugely overinflated, due to the fact that the community has assumed incorrectly that genetic variants contributing to a given trait do not interact with each other. Even if this interaction occurred at a relatively modest level, then missing heritability estimates would need to be substantially reassessed.

Irrespective of some of these estimate, it is clear that additional variants need to be found that cannot be readily picked up by GWAS and some alternative approaches are outlined below.

Rare SNPs

Commercially available genotyping arrays have typically restricted content to simply feature products comprehensive coverage of common variants with a minor allele frequency greater than 5 % based on information gained from the HapMap. However, this changing to a degree as more information comes out from the successor to the HapMap, the 1000 Genomes project, where newer arrays are ensuring comprehensive coverage down to a 1 % frequency plus exome coverage. However this information is still far from exhaustive as not every variant known in the genome can as yet be represented on this platform and so signals may be missed. For instance, a rare SNP within intron 4 of *NAMPT* has been implicated in patients with severe obesity [123] but would not be detected with standard high throughput genotyping methods. As such, genotyping array products will need to be much more comprehensive in genomic coverage going forward or extremely large sample sizes will be required to overcome multiple testing statistical power issues in whole genome sequencing setting in order to elucidate the remaining genetic component of obesity, the latter of which remains cost prohibitive.

Copy Number Variation

Genomic rearrangements resulting in deletions, duplications, inversions, and translocations are collectively referred to as copy number variants (CNVs). As described above, much of the genetic diversity in the human genome is due to single base pair variations, but there is also variation in copy number throughout the genome too [124]. With the array technology available for GWAS-related approaches, the underlying single-base extension (SBE) biochemistry and hybridization/detection to synthetic oligonucleotides allows for accurate genotyping and quantitation of

allelic copy number [58, 125]. As such SNP arrays allow for characterization of CNVs genome-wide for a given individual [57, 58, 125].

CNVs have been particularly implicated in subjects with the combination of extreme obesity and coexisting developmental delay (Table 5.1). Analyses in this regard has revealed what is now considered the most established CNV in the obesity field to date, namely, a large, rare chromosomal deletion of at least 593 kb at 16p11.2. Two groups in the UK plus their collaborators independently detected rare deletions at this chromosomal location, observing that they were present in excess among extreme obese cases when compared with normal and regular obese subjects [126, 127]. Heterozygotes for this deletion at this location are significantly enriched in Caucasian patients with severe early-onset obesity and developmental delay [127, 128]. These deletions, albeit very rare, have been shown not to be present in healthy non-obese controls but in 0.7 % of morbidly obese cases (BMI ≥40 kg/m^2), resulting in a striking odds ratio of 43.0. This deleted region, estimated to be in the size range of 220 kb to 1.7 Mb, and thus coincides with a number of genes making it more challenging to determine which is the causative gene. However, Bochukova et al. [126] observed that *SH2B1* was the only consistently impacted genes when deleted regions were overlaid among the five cases studied. In addition, it is well known that *SH2B1* plays a role in leptin and insulin signaling and energy homeostasis [126], plus common SNPs near *SH2B1* locus have been strongly associated with BMI in GWAS analyses [85, 126].

Examining CNVs in common childhood obesity is a good complement to what was described above in the more extreme setting. Investigators examined children in the upper 5th percentile of BMI but excluded any subject greater than 3 standard deviations in order to rigorously adhere to the pursuit of genetic factors underpinning the common form the disease rather than rare syndromes [129] (Table 5.1). Through the use of a European American pediatric cohort at the discovery stage to detect any associated events, a very conservative bar was set for the replication stage, where the observed CNVs also had to be exclusive to cases in a second ethnicity, i.e., African Americans. 34 putative CNVR loci (15 deletions and 19 duplications) that were exclusive to EA cases were detected in a cohort of European American (EA) childhood obesity cases (n = ~1,000) and lean controls (n = 2,500). However, following a physical validation step to ensure the events were real, three of the putative deletions turned out to be false positives during the quantitative PCR (qPCR) checks. Ultimately 17 of these CNV impacted loci were unique to the cohort, not appearing in any public databases, so those findings were further evaluated in the independent African American (AA) cohort (n = ~1,500) of childhood obesity cases and lean controls (n = ~1,500). Interestingly, almost half of these variants were also exclusive to AA cases (6 deletions and 2 duplications). The established loci from this study that were impacted by a deletion were *EDIL3*, *S1PR5*, *FOXP2*, *TBCA*, *ABCB5*, and *ZPLD1*, while *KIF2B* and *ARL15* were impacted by a duplication event. When the AA cohort was used as the discovery set, evidence was also gained for a deletion at the *EPHA6-UNQ6114* locus.

As with findings from GWAS, most of the loci represented novel biology for obesity, with no reports of these genes playing a role in the disease previously.

Although it was comforting to see, from a positive control point of view, that one locus came up that had been implicated in a related trait previously, i.e., *ARL15* in a GWAS of adiponectin levels, a known risk factor for CVD and T2D [130].

In the same study, the presence of large rare deletions in childhood obesity was assessed, where they had to present in <1 % of individuals and >500 kb in size. The result was that no excess of large rare deletions was observed genome-wide. This is consistent with the 16p11.2 story, where significance in this regard was only achieved once developmental delay subjects were included but not severe early-onset obesity on its own [126, 127].

A novel common CNV for early onset extreme obesity was subsequently reported on chromosome 11q11, harboring a cluster of related genes *OR4P4*, *OR4S2*, and *OR4C6* genes [131] (Table 5.1). Furthermore, a very recent study performed CNV association analyses in 1,509 children at the extreme tail obesity (>3 standard deviations from the mean) plus 5,380 controls [113]. A previously reported 43 kb deletion at the *NEGR1* locus was significantly associated with the trait but it appeared that this signal was entirely driven by a flanking 8 kb deletion; absence of this deletion increased the risk for obesity dramatically. They also reported significant burden of rare, single CNVs in severely obese cases ($P < 0.0001$), while integrative gene network pathway analysis of rare deletions indicated enrichment of genes affecting G protein-coupled receptors (GPCRs) involved in the neuronal regulation of energy homeostasis. This pathway is of course great relevance to the pathogenesis of obesity.

As greater and greater resolution genome-wide scans are executed, one should envisage further reports of such findings. The hunt for CNVs in the context of childhood obesity has proven fruitful up to now and it has become quite clear they contribute to the missing heritability for the trait.

Epigenetics

Looking beyond the underlying DNA sequence to explain the reminder of the missing heritability of childhood obesity and other complex traits, one should also consider heritable changes in gene expression or cellular phenotype, in particular when they are caused by DNA methylation and histone modification.

Prader–Willi is a syndrome that presents with developmental defects, cognitive disabilities, excessive eating and pronounced life-threatening obesity. This imprinted disorder is due to genetic and epigenetic errors in the region of chromosome 15q11-q13. The phenotype occurs when the paternal copy is deleted, while the maternal copy is inactivated by methylation [132, 133].

Although the underlying genes have yet to be identified, linkage scans of European and African ancestral populations have revealed that several obesity-related genetic loci have different parental effects or maternal effects [134]. In addition, variation in DNA methylation patterns on the human leptin promoter has been

observed between alleles and cells, suggesting that imprinting occurs at this locus and that its actions are cell type specific.

Observations like these strongly point to epigenetic mechanisms contributing to obesity risk, but of course this still needs to be fully elucidated. New techniques, such as ChIP-Seq [135] and whole genome sequencing, will provide us with much needed insight in to global methylation patterns and histone modifications in the context of childhood obesity.

Conclusions

Only until recently, genetic studies of childhood obesity were largely reliant on family studies, animal models and candidate gene efforts, with monogenic obesity providing crucial clues. However, GWAS has now taken on much of the heavy lifting with respect to gene discovery in the context of the more polygenic form of the disease, with its unbiased, whole genome scan approach.

Although such studies have revealed several new biomolecular pathways not previously associated with obesity, it is still a concern to many complex trait geneticists that the well-established and robust associations only explain very little of the genetic risk for a given complex, in particular childhood obesity, suggesting that more loci are out there to find but may be conferring their effects more subtly or through rare variants.

The current situation has given the genetics community pause for thought on what the best strategy is to adopt in order to fill in the gaps and elucidate the full repertoire of the genetic component of a given trait so that the current "missing heritability" [120] can ultimately be all accounted for. It is becoming increasingly clear that larger and larger cohorts combined in to mega meta-analyses will be required, but new discoveries can only be made when leveraging the ultimate resolution of whole genome sequencing technologies rather than the variant snapshots we get with GWAS. Although not a reality yet, these approaches will become feasible as the price of sequencing continues to drop.

In addition, as GWAS is designed with representative tag-SNPs present on the array, the signals that we are seeing and robustly associated with given traits are in no way to be considered the underlying causal susceptibility variant at a given loci. The move from association to causality remains a big challenge for common complex diseases like obesity so new approaches to characterize the true causative genes will need to be developed and subsequent functional studies will be required to fully understand how they feed in to the risk profile for childhood obesity. This in turn will help us produce more efficacious therapies and will guide us on the path to personalized medicine.

Clearly using the pediatric model can aid in elucidating the role of many variants first shown to be associated with an adult trait. As shown above, many of the loci initially found for BMI determination in adults turn out to operate early on in life and influence childhood obesity risk. And as we suggest, leveraging the pediatric

model may be a more efficient way to distill out genetic variants underpinning many complex traits, being more cost effective in sample collection and genotyping, due to the fact that the environmental confounder is substantially less in the childhood setting. As such, recognizing this angle can increase the efficiency of efforts to detect, treat, and ultimately, prevent obesity and its comorbidities.

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Chapter 6
Genetic Pleiotropies of Obesity

Bratati Kahali and Elizabeth K. Speliotes

Abstract Advances in the knowledge of variation in the human genome and reproducible results from the Genome-wide Association Studies (GWAS) have led to the identification of susceptible loci contributing to obesity and related disorders. Obesity is associated with and may contribute to the development of many metabolic diseases including, but not limited to, diabetes, hypertension, dyslipidemia, liver disease, and cardiovascular disease, often leading to morbidity and mortality; however the mechanisms linking the genetic polymorphisms associated with obesity to these metabolic complications are extremely complex and remains to be fully elucidated. In this chapter, we review a number of genetic perturbations that predispose to obesity as well as obesity-associated metabolic complications. Further understanding on how these variants act may help toward personalized treatment for obesity-related comorbidities based on individual needs.

Obesity Is a Worldwide Epidemic with Much Comorbidity

Obesity has become an epidemic that threatens the health of billions of people worldwide. Obesity is associated with and may contribute to the development of many metabolic diseases including, but not limited to, diabetes, hypertension, dyslipidemia, liver disease, cardiovascular disease, and cancer (Fig. 6.1). Worldwide, more than 700 million adults are obese and global projections estimate there will be 2.16 billion overweight and 1.12 billion obese individuals by 2030 [47]. According to

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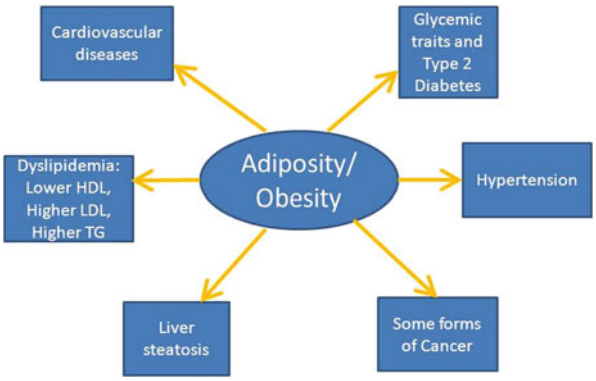

Fig. 6.1 Obesity-related metabolic diseases

data released by the National Bureau of Economic Research in 2010, the annual health costs related to obesity in the USA reach $168 billion, and nearly 17 % of the medical costs in the USA result from obesity. An obese person generates health costs of $3,271 annually compared with $512 for a nonobese person [11]. A better understanding of the causes of obesity and its related comorbidities is essential to mitigate the immense medical and economic impact of obesity worldwide.

Interestingly, obesity and related metabolic diseases are in part genetically influenced. Recent work into the genetic basis of these conditions is beginning to reveal a possible common genetic basis to obesity and related traits, sometimes in directions not predicted by epidemiological relationships. Here we discuss the genetic basis of some obesity related traits as they relate to and possibly contribute to associated metabolic diseases.

Types of Adiposity and Their Measures

Adiposity can increase throughout the whole body or in particular depots. Specialized methods to measure body-fat percentage, like dual energy X-ray absorptiometry (DXA—formerly DEXA), and air displacement plethysmography (ADP) are specific but expensive and cumbersome to implement for large groups. Body mass index (BMI) is commonly used as an inexpensive, noninvasive measurement of adiposity, but it is less specific since it also includes lean body mass. Internationally, BMI is commonly used to classify adult obesity. The World Health Organization, considers individuals of European ancestry with a BMI $\geq$ 30 kg/m^2 to be obese and those with a BMI $\geq$ 40 kg/m^2 to be extremely obese. Interestingly, comorbidities can develop at lower BMIs in some people of non-European ancestry; individuals of Indian, Chinese, Korean, and Japanese ancestry have a higher prevalence than those of Caucasian ancestry, of diabetes and other metabolic conditions with similar BMI and waist circumference measurements [41]. One possible explanation for this disparity is fat distribution. Visceral and hepatic fat deposition is more strongly

associated with the presence of metabolic disease than generalized adiposity [41]. Individuals of Asian ancestry then may have a greater proportion of visceral fat deposition than individuals of European ancestry [41]. One pathophysiological explanation for how increased visceral fat accumulation may lead to an increased risk for type 2 diabetes is the production by visceral fat of cytokines that promote insulin resistance [8]. Noninvasive, inexpensive measures of visceral adiposity include waist circumference (WC) and waist-to-hip ratio (WHR) but these are not as specific for measuring this and other specific depots as using imaging modalities such as magnetic resonance imaging, computed tomography, or ultrasound.

Obesity Is Associated with and May Contribute to the Development of Other Diseases

Obesity is excess body fat accumulation caused by a chronic positive energy balance (energy intake exceeding energy expenditure) due to deregulation in the complex process of energy homeostasis [73]. Unfortunately, the increased prevalence of obesity worldwide has led to an increase in the prevalence of diabetes, hypertension, dyslipidemia, coronary artery disease, nonalcoholic fatty liver disease, and some forms of cancer [53]. In individuals of Caucasian ancestry with a BMI above 29 versus below this cutoff, there is a greater risk of developing these diseases, independent of gender [63, 112, 113]. Increased waist circumference is a measure of abdominal obesity. Men and women that have a waist circumference greater than or equal to 102 cm and 88 cm respectively are at substantially increased risk for metabolic complications like coronary heart disease, hypertension, and dyslipidemia [32] compared to those with a waist circumference less than these values. Increased adiposity is associated with elevated fasting plasma insulin and an exaggerated insulin response to an oral glucose load, and may contribute to the development of type 2 diabetes [52]. In the Nurses Cohort Study of 116,000 women, the risk of diabetes increased fivefold for women with a BMI of 25, 28-fold for women with a BMI of 30, and 93-fold for women with a BMI of 35 or higher, compared with women with a BMI of less than 21, even after adjustment for age [16]. In the Health Professionals Study of 51,529 men, the risk of diabetes, adjusted for age, is increased 2.2-fold for men with a BMI between 25 and 26.9, 6.7-fold for men with a BMI between 29 and 30, and 42-fold for men with a BMI of 35 or higher, compared with men with a BMI of less than 21 [13]. Obesity is also a primary risk factor for nonalcoholic fatty liver disease (NAFLD), but not all obese individuals are affected [21]. NAFLD includes a spectrum of disease in individuals without a history of excessive alcohol ingestion: from fatty infiltration of the liver (steatosis), to histological evidence of inflammation (nonalcoholic steatohepatitis, NASH), to fibrosis or cirrhosis [15]. NAFLD can lead to liver failure and is accompanied by substantial morbidity and mortality, with few known effective treatments [33]. Dyslipoproteinemia (abnormal blood lipid concentrations) with decreased HDL cholesterol, increased LDL triglyceride, and increased LDL concentrations is associated with increased adiposity (reviewed by Kopelman [53]).

Genetic Basis of Obesity

Although decreased physical activity and excess calorie intake may partially explain the increased prevalence of obesity in modern times, some individuals are more susceptible to these lifestyle changes than are others, suggesting that they may have a genetic predisposition to obesity [75]. Indeed, results from twin studies suggest that genetic factors explain 50–90 % of the variance in BMI [62]. Individual variation in waist-to-hip ratio is heritable, even after accounting for BMI, with heritability estimates ranging from 22–61 % [36]. Hepatic adiposity is also 26–27 % heritable [98]. In the last decade much has been learned about the genetic basis of adiposity. Specific genetic variants have been associated with measures of human obesity. These include rare chromosomal aberrations and gene defects that have a large effect on afflicted individuals, as well as common variants that have smaller effects but influence the overall distribution of obesity in the population [96]. Some obesity conditions are caused by large chromosomal aberrations; whereas in many obesity conditions the causal variant has been narrowed down to particular intervals in the genome. This work suggests that there is an allelic spectrum of genetic variants that contribute to human obesity.

Obesity Syndromes with Developmental Delay or Mental Retardation

Over 30 pleiotropic syndromes have been reported that include obesity and developmental delay or mental retardation as components (Refer to other chapter in book). The genetic basis for some of these has been identified. A large chromosomal aberration involving multiple genes is sometimes the cause. One example is Prader Willi syndrome which causes obesity together with hyperphagia, hypotonia, mental retardation, learning disabilities, short stature, and hypogonadotropic hypogonadism. Complications co-occurring with the syndrome include abnormal glucose tolerance, type 2 diabetes, right-sided heart failure, and bone problems. Prader–Willi syndrome is a contiguous gene syndrome resulting from deletion of paternal copies of the imprinted *SNRPN* gene, the *NDN* (necdin) gene, and possibly other missing genes on part of chromosome 15 (15q11-13) (OMIM); which phenotypes are due to disturbance of which genes remains to be determined.

For other syndromes, such as Bardet–Biedl syndrome (where there are 15 BBS genes and 3 modifier genes identified), we have a better understanding of how disruption in the functioning of one of these genes in the set results in pleiotropic phenotypes. BBS is characterized by retinal degeneration, obesity, polydactyly, and hypogonadism, as well as developmental delay, speech disorder, anosmia, ataxia/imbalance, behavioral problems, polycystic kidneys, hearing loss, congenital heart defects, situs inversus, and Hirschsprung disease ([105]; a clinical synopsis available in OMIM id 209900). Afflicted individuals are at increased risk for diabetes mellitus,

hypertension, and congenital heart disease [70, 71]. BBS genes are involved in basal body and centrosome function, relating to ciliary development and function in many tissues. Defects in ciliary processes in many tissues are thought to underlie the pleiotropic phenotypes observed in this syndrome [69, 72]. For example, dysfunction of BBS genes in preadipocytes and the hypothalamus may contribute to the obesity seen in BBS [64]. Human preadipocytes form a transient primary cilium during differentiation. *BBS10* and *BBS12* localize to the basal body of this primary cilium and knockdown of *BBS10* and *BBS12* expression reduces the number of ciliated cells and promotes adipogenesis. Furthermore, differentiation of *BBS10* and *BBS12* patients' fibroblasts into fat-accumulating cells contain increased triglycerides compared with control cells [64], supporting the assertion that primary dysfunction of adipogenesis may contribute to the obesity seen in BBS. A second mechanism by which BBS genes contribute to obesity may involve altered leptin receptor (*LEPR*) signaling in the hypothalamus leading to deregulation of energy balance. *Bbs2 –/–*, *Bbs4 –/–*, and *Bbs6 –/–* mice are resistant to the action of leptin to reduce body weight and food intake regardless of serum leptin levels and obesity [90]. Activation of hypothalamic *Stat3* by leptin, but not downstream *MC4R* signaling, was significantly decreased in *Bbs2 –/–*, *Bbs4 –/–*, and *Bbs6 –/–* mice indicating that LEPR signaling was specifically impaired in these animals. The human *BBS1* protein physically interacts with *LEPR*, and loss of BBS proteins perturbs LEPR trafficking and decreases proopiomelanocortin (*POMC*) expression in human cells, suggesting a mechanism by which some BBS proteins may mediate their effects. Some BBS proteins are also required for proper trafficking of G protein-coupled receptors (GPCRs) in and out of neuronal cilia, suggesting disrupted ciliary GPCR trafficking is the basis for the neurological defects in BBS [7]. Better understanding of ciliopathies like Bardet–Biedl syndrome allow the identification of signaling pathways potentially involved in common diseases that share phenotypic features like obesity.

Obesity Syndromes Without Developmental Delay or Mental Retardation

Obesity that segregates within families but that is not associated with severe developmental delay or mental retardation has been reported and characterized. Genes that are part of a hypothalamic axis for appetite regulation include leptin, leptin receptor, *POMC*, and *MC4R*. Defects in leptin in humans lead to severe early-onset obesity and intense hyperphagia, as well as hypogonadotropic hypogonadism [77]. *POMC* mutations are characterized by hyperphagia, and impaired melanocortin signaling in the hypothalamus with early-onset obesity [54]. Heterozygous mutations in *MC4R* have been reported to cause a dominantly inherited obesity in various ethnic groups. Mutations in *MC4R* are by far the most common forms of monogenic obesity in humans. Defects in *MC4R* have been reported in multiple families, and associated phenotypes include obesity, an increase in lean body mass and bone mineral density, an increase in linear growth throughout childhood, hyperphagia, and

severe hyperinsulinemia; homozygotes are more severely affected than heterozygotes [22]. MC4R is expressed in human and rodent brain and targeted disruption of *Mc4r* in rodents leads to increased food intake, obesity, severe early hyperinsulinemia, and increased linear growth; heterozygotes have an intermediate phenotype between homozygous mutant and wild-type mice [43]. The results of *MC4R* disruption are similar in humans, suggesting that aspects of the *MC4R* pathway may be conserved between rodents and humans. These genes may have a common effect on appetite because they act on the central leptin–melanocortin axis in the hypothalamus, thus playing a critical role in the regulation of feeding behavior. However, individuals with defects in leptin, leptin receptor, *POMC*, and *MC4R* do not display the same set of related pleiotropisms. For example, individuals with defects in *MC4R* show increased lean body mass, hyperinsulinemia, and increased linear growth, which are not seen in individuals with the defects in the other genes [74]. Individuals with defects in leptin/leptin receptor have hypogonadotropic hypogonadism and central hypothyroidism, which are not seen with defects in the other genes [74]. Adrenal crisis and pale skin are seen in individuals with *POMC* defects [54] but not with defects in the other genes [74]. The basis for gene unique phenotypes may be due to particular genes having effects in other neurons/cells outside of the feeding pathways or to them having effects via downstream mediators that are not part of the core feeding circuit. For example, *MC4R* is expressed in many neurons in the hypothalamus in addition to those that control feeding [92]. It is therefore probable that its effects on non-obesity phenotypes are mediated through neurons in non-feeding pathways. Alternatively, *POMC* undergoes extensive and tissue-specific posttranslational processing by prohormone convertases (PCs) to yield a range of biologically active peptides [17]. Specifically, *POMC* is cleaved by PC1 and PC2 to make α-, β-, and γ-MSH (the melanocortins) and ACTH respectively. The expression of PC2 within the hypothalamus leads to the production of α-, β-, and γ-MSH (the melanocortins), which mediate the effect of POMC on feeding. In contrast, adrenocorticotropic hormone (ACTH) is the predominant peptide produced from POMC in pituitary corticotrophs that express PC1 and mediate its effect on the adrenal axis. Therefore, expression of the genes themselves or of the downstream mediators of their effects in particular cell types may help to explain the diversity of pleiotropisms observed when these genes are disrupted.

Common Variants Affect Adiposity and Related Metabolic Traits in Human Populations

BMI, WC, WHR, and Obesity-Associated Variants

Genome-wide association studies (GWAS) have identified genetic variants that associate with measures of overall obesity (e.g., BMI) that also have effects on related metabolic traits. Interestingly, some of these common variants fall in or near genes

GWAS trait	Variant identified	Nearby Gene	HDL-cholesterol	LDL-cholesterol	Triglycerides	Fasting Glucose	2-hour Glucose	Fasting Insulin	HOMA-IR	Type 2 Diabetes	NASH	Neuropsychiatric traits***
BMI	rs2229616, rs12970134 rs17782313**	MC4R	↓						↑			
BMI	rs1558902	FTO						↑	↑	↑		
BMI	rs7359397	SH2B1				↑		↑				@ ↑
BMI	rs10767664*	BDNF										↓
WC	rs10146997	NRXN3										↑
BMI	rs2287019	GIPR				↑	↓			↓		
BMI	rs6232	PCSK1				↑	↓		↓			
WHR	rs10195252	GRB14		↑	↑			↑	↑			
WHR	rs2605100	LYPLAL1			↑				# ↑			
Body Fat Percentage	rs2943650	IRS1	↑		↓					↓		
VAT (Women only)	rs1659258	THNSL2	↓			↑						
Liver steatosis	rs780094	GCKR		↑	↑	↓			↓		↑	
Liver steatosis	rs738409	PNPLA3									↑	
Liver steatosis	rs12137855	LYPLAL1									↑	
Liver steatosis	rs4240624^	PPP1R3B	↑	↑		↓						
Liver steatosis	rs2228603	NCAN		↓	↓						↑	

Fig. 6.2 The associations with other metabolic traits for the trait-increasing allele for various measures of obesity

^Variant associates with CT hepatic steatosis but not histologic fibrosis (Speliotes et al., PLoS Genetics, 2011)

*Variant is in r2 = 0.76 with rs6265 which encodes a Val66Met missense change in BDNF. A strong association of the BDNF 196G/A (Val66Met) polymorphism has been demonstrated with the restricting type of anorexia nervosa and binge-eating/purging type of bulimia nervosa (Ribases et al., Mol Psychiatry, 2003; Koizumi et al., Am J Med Genet, 2004).

**rs17782313 identified as in a large-scale GWAS as susceptible to fat mass, overweight and risk of obesity in European population, these authors suggest that rs17782313 influence BMI independently from rs2229616 V103I polymorphism (Loos et al., Nature Genetics, 2008); rs2229616 encodes the V103I missense change in MC4R (Heid et al., Obesity, 2008). The association of rs17782313 with insulin resistance was assessed in a recent study population based study (Povel et al., Cardiovasc Diabetol, 2012). rs12970134 (r2 = 0.81 with rs17782313) associated significantly with waist circumference and insulin resistance in Indian Asians and Europeans (Chambers et al., Nature Genetics, 2008).

***Implies Anorexia nervosa and Bulimia nervosa for the variant near BDNF and Impulsivity for the variant near NRXN3.

@Aggressive behavior for SH2B1 loss-of-function mutations.

#rs4846567 G allele carriers showed a 5.2% lower HOMA-IR in women, this SNP is in moderate LD (r2 = 0.64) with the WHR SNP.

that have been previously identified to play a role in obesity because when they are deregulated in humans or model organisms, they result in obesity. In particular, common variants have been identified in or near *MC4R* and *POMC* that affect population-based BMI. As noted above, severe perturbation of these genes results in monogenic obesity syndromes. Indeed, for lipid and height traits when monogenic disease genes that affect these traits are present near a GWAS variant that affect these same traits, the monogenic disease gene is the nearest gene two-thirds of the time [55, 102]. This suggests that examining genes near GWAS signals may give us insights into genes that may affect the biology of the trait of interest. Interestingly, common variants identified by large-scale GWAS near *MC4R* [60] and *POMC* [96,

97] that associate with higher BMI also associate with higher and lower height, respectively, consistent with observations in individuals with monogenic mutations affecting these genes. Thus, GWAS-associated variants can provide direct insights into the genetic basis of obesity pleiotropisms. For example, variants near *MC4R* have been found to associate with increased risk of metabolic syndrome and increased insulin resistance in human populations of diverse ancestry [12, 37, 78] (Fig. 6.2).

The first common variants identified by GWAS to associate with overall fat measures were located in the first intron of *FTO* (fat mass and obesity-associated gene). Variants in high linkage disequilibrium with each other were associated with BMI, severe childhood and adult obesity, as well as fat mass: rs9939609 [111]; rs9930506 [89]; rs1421085 [66]; rs8050136 [49]. The BMI-increasing allele (rs9939609) at this locus is strongly associated with increased risk for type 2 diabetes [26]. Further, the BMI-increasing alleles at the FTO locus are also significantly associated with increased fasting insulin, increased homeostatic measure for insulin resistance (HOMA-IR) [96, 97], elevated diastolic blood pressure [104], and increased risk for metabolic syndrome [93] (Fig. 6.2). *FTO* affects adiposity; ubiquitous overexpression of *FTO* leads to a dose-dependent increase in body and fat mass in mice fed either a standard or high-fat diet [14]. FTO has a potential role in nucleic acid demethylation and is highly expressed in parts of the brain that govern energy balance and feeding behavior [27, 28], which make it a likely candidate through which these variants exert their effects on BMI.

The common variant rs7359397 near *SH2B1* (Src homology 2 [SH2] domain-containing putative adaptor protein B1) not only associates with population-measured BMI but also associates with increased fasting levels of insulin and plasma glucose [96, 97] (Fig. 6.2). This variant is in perfect linkage disequilibrium with missense variant rs7498665 in *SH2B1*, [111] which affects amino acid polarity (Thr484Ala) and falls into a highly conserved protein segment of SH2B1 containing a class II SH3 domain-binding site. Carriers of a large, rare chromosomal deletion of *SH2B1* exhibit hyperphagia and severe insulin resistance disproportionate to the degree of obesity [9, 107]. Doche et al. [19] also identified *SH2B1* loss-of-function mutations in a large cohort of 300 patients with severe early-onset obesity. Mutation carriers exhibited hyperphagia, childhood-onset obesity, disproportionate insulin resistance, and reduced final height as adults. Behavioral abnormalities, including social isolation and aggression, were also reported. SH2B1 modulates signaling by a variety of ligands that bind to receptor tyrosine kinases or JAK-associated cytokine receptors, including leptin, insulin, growth hormone, and nerve growth factor [85]. In mice, targeted deletion of *SH2B1* results in increased food intake, obesity, and insulin resistance; heterozygous null mice fed a high-fat diet have an intermediate phenotype. SH2B1 can act as an insulin sensitizer because it directly binds to insulin receptor substrates IRS1 and IRS2, and enhances insulin sensitivity by promoting insulin receptor catalytic activity and inhibiting tyrosine dephosphorylation of IRS protein [56, 68]. Interestingly, the expression of only neuronal SH2B1 in these knockout mice rescues some of the phenotypes seen in knockout mice and this suggest that SH2B1 regulates energy balance, body weight, peripheral insulin sensitivity, and glucose homeostasis at least in part by enhancing hypothalamic leptin sensitivity [81].

These data suggest that *SH2B1* plays a critical role in the control of body weight, food intake, leptin-insulin signaling, and a putative role in maladaptive human behavior.

The lead SNP (rs10767664) identified near *BDNF* in a GWAS for BMI-susceptible loci in 249,796 individuals of European ancestry [96, 97] is in high linkage disequilibrium ($r^2 = 0.76$) with rs6265, which results in a Val66Met missense change in BDNF. The BDNF 196G/A (Val66Met) polymorphism is strongly associated with the restricting type of anorexia nervosa and binge-eating/purging type of bulimia nervosa [51, 81] (Fig. 6.2), suggesting that the Met allele may be a susceptibility factor for eating disorders. Variants in or near *BDNF*, including rs6265 (Val66Met), that are associated with an increased BMI have also been associated with substance-related disorders, alcohol dependence, and mood disorders [31]. These effects may be due to *BDNF* disruption causing interference with dopamine neurotransmission in pathways involved in reward effects, motivation, and decision making [31, 34, 96, 97, 103]. Further, heterozygous *BDNF* knockout mice exhibited aggressiveness and hyperphagia accompanied by significant weight gain in early adulthood [61], and abnormal locomotor activity and infusion with *BDNF* in these mice can transiently reverse the eating behavior and obesity [48]; supporting that altering BDNF function can mediate both obesity and psychiatric behavioral phenotypes.

The variant rs10146997 in neurexin-3-alpha (*NRXN3*) was significantly associated with waist circumference in a large-scale GWAS of a European population [35]. Although it is unlikely that a different gene accounts for this finding as there are no other genes within a distance of more than several hundred kilobases of this SNP, additional research is required to prove that rs10146997 acts through *NRXN3* [35]. The rs760288–rs8019381–rs2293847 haplotype near *NRXN3* associates with gender-specific alcohol dependence, cocaine addiction, and illegal substance abuse in 332 alcohol-dependent human participants [39]; however, these variants are in very low linkage disequilibrium with rs10146997. Weak association between rs10146697 and impulsivity in women has been reported [101] (Fig. 6.2). Therefore, although a direct link between variants that associate with obesity phenotypes and those that associate with substance abuse has not been shown or confirmed, these phenotypes may all result from perturbations of *NRXN3*. *NRXN3*, which belongs to a family of proteins that function as receptors and cell adhesion molecules in the nervous system, may play a role in the development and function of synapses [58]. How polymorphisms near *NRXN3* confer vulnerability to addictions and obesity remains to be determined.

The BMI-increasing genome-wide significantly associated variant near the gastric inhibitory polypeptide receptor (*GIPR*, also known as the glucose-dependent insulinotropic polypeptide receptor) [96, 97] is in strong linkage disequilibrium ($r^2 = 0.83$) with a missense SNP in *GIPR* (rs1800437, p.Glu354Gln) that has been shown to influence glucose and insulin responses to an oral glucose challenge [88]. The BMI-increasing allele is associated with increased fasting glucose levels and decreased 2-h glucose levels [96, 97] (Fig. 6.2). *GIPR* is widely distributed in peripheral organs, including the pancreas, gut, and adipose tissue, suggesting a role for peripheral biology in obesity. GIP, which is expressed in the K cells of the duodenum and intestine, is an incretin hormone that mediates insulin secretion in response to oral intake of glucose. Mice with disruption of *GIPR* are resistant to diet-induced obesity [67], further supporting a

role of this pathway in affecting BMI. Interestingly, GIP and its receptor may constitute a link between the consumption of energy-rich high-fat diets and the development of obesity (reviewed by Irwin and Flatt [45] and McIntosh et al. [65]).

A GWAS of early-onset and morbid adult obesity in Europeans identified a coding missense variant in the Niemann–Pick C1 gene *NPC1* (rs1805081 [H215R]) that was genome-widely significantly associated with this trait. This variant had a population attributable risk for obesity of 9.6 % in children and 13.6 % in adults [66]. NPC1 is a protein involved in endosomal cholesterol trafficking in the central nervous system, liver, and macrophages [3, 44, 106]. *NPC1* mRNA was significantly increased in obese individuals in subcutaneous white adipose tissue (scWAT) and omental WAT (omWAT) and downregulated by weight loss, implicating *NPC1* in adipocyte biology [4]. Although rs1805081 has not been associated with the Niemann Pick C phenotype, *Npc1*-null mice exhibit late-onset weight loss and poor food intake, as well as neurological deficits and a cellular defect in cholesterol transport [20, 66, 108], suggesting that alteration of this gene results in pleiotropic effects.

The nonsynonymous variants rs6232[G] for *PCSK1* (prohormone convertase (PC) 1/3), encoding N221D, and rs6234-rs6235[C], encoding the Q665E–S690T changes, have been consistently associated with obesity in adults and children ($P = 7.27 \times 10^{-8}$ and $P = 2.31 \times 10^{-12}$, respectively) [6]. Rare mutations as well as common SNPs in *PCSK1* cause childhood obesity and abnormal glucose homeostasis with elevated proinsulin concentrations in Europeans [6, 23, 46, 84]. A large-scale GWAS in East Asians identified a common variant rs261967 near *PCSK1* that associates with BMI [109]. Precursor polypeptides, such as *POMC*, proglucagon, and proinsulin, which are involved in the regulation of energy metabolism, serve as substrates for *PCSK1* in various metabolic processes. The missense variant rs6235[G] coding for S690T in *PCSK1* is significantly associated with fasting proinsulin levels on a genome-wide scale [101], consistent with the function of *PCSK1* being the first enzymatic step in the insulin processing pathway. In addition, the obesity risk allele of SNP rs6232 (encoding missense variant N221D) was associated with reduced HOMA-IR, increased fasting glucose, and reduced 120-min glucose levels (Fig. 6.2), independently of BMI and proinsulin conversion in Europeans [38]. Functional analysis showed that the N221D-mutant PC1/3 protein has impaired catalytic activity [6]. Gjesing et al. [29] proposed that a direct or indirect consequence of having the C-allele of rs6235 for *PCSK1* is an increased GIP level that might lead to increased levels of circulating insulin, reduce glucose levels, and possibly protect against diabetes. Thus, *PCSK1* arbitrates its effect on obesity and related metabolic disorders through cleaving many precursor hormones in multiple tissues thus causing these pleiotropic effects in a parallel fashion.

The waist-to-hip ratio (WHR)-increasing allele at *GRB14* (rs10195252) shows a strong association with increased triglycerides ($P = 7.4 \times 10^{-9}$), fasting insulin levels ($P = 5.0 \times 10^{-6}$), and insulin resistance ($P = 1.9 \times 10^{-6}$) [36] (Fig. 6.2). Growth factor receptor-bound protein 14 (GRB14) is a member of a family of SH2-containing adaptor proteins and binds directly to the insulin receptor [18, 40]. *GRB14* expression is increased in the adipose tissue of insulin-resistant animal models and type 2 diabetic human patients, suggesting the effect of *GRB14* is by modulating insulin sensitivity [10] to exert its effects.

A meta-analysis of genome-wide association data for central adiposity identified rs2605100 (near LYPLAL1—lysophospholipase-like protein 1) as an association in females only for WHR [57]. These authors also found an association between the WHR-increasing G-allele of rs2605100 and increased fasting triglycerides. Although the subcutaneous and visceral adipose tissue of 16 obese subjects had elevated mRNA expression of LYPLAL1 [99], the functional and molecular details of how rs2605100 actually exerts its effects to result in increased WHR effect remain to be determined.

Body Fat Percentage

Variant rs2943650 was identified to be significantly associated at genome-wide levels with body fat percentage in a recent meta-analysis study. rs2943650 is near *IRS1* and shows an association with fat mass, greater in men than women [49]. The insulin receptor substrate protein *IRS1* is a key target of the insulin receptor tyrosine kinase and is required for hormonal control of metabolism. Variants in high linkage disequilibrium with the fat mass-associated variant at *IRS1* also associate with type 2 diabetes [30]; cardiovascular disease [87]; and plasma lipid levels [102]; and is in perfect linkage disequilibrium with a variant (rs2943641) identified to be associated with insulin resistance and hyperinsulinemia in European population-based cohorts [86]. The C-allele for rs2943641 was also associated with reduced basal levels of IRS1 protein and decreased insulin induction of IRS1-associated phosphatidylinositol-3-OH kinase activity in human skeletal muscle biopsies. Interestingly, the fat percentage decreasing allele was associated with increased risk for type 2 diabetes and cardiovascular disease, and with an impaired lipid profile [1, 91, 102] (Fig. 6.2). Further analyses showed that the body fat percentage decreasing allele lowered the subcutaneous fat—but not the more harmful visceral fat—and also lowered adiponectin levels. This suggests that this variant may act by affecting fat distribution and in particular it may promote metabolic disease development by increasing visceral versus subcutaneous fat [49, 59].

Visceral Adipose Tissue (VAT)

A higher ratio of visceral to subcutaneous adipose tissue is known to be associated with a higher risk for cardiovascular and metabolic diseases [25, 50]. A genome-wide association study of abdominal, subcutaneous, and visceral adipose tissue in individuals of European ancestry revealed a novel genome-wide significant variant rs1659258 near *THNSL2* (threonine synthase-like 2) that was significantly associated with visceral adipose tissue (VAT) only in women [24]. The VAT-increasing allele at *THNSL2* was associated with lower HDL and increased fasting glucose consistent (Fig. 6.2), and increased visceral adiposity promotes metabolic syndrome traits [24].

Liver Steatosis

A genome-wide association scan revealed a missense variant rs738409 (I148M) in *PNPLA3* (Patatin-like phospholipase domain-containing protein 3) to be significantly associated with hepatic fat accumulation and hepatic inflammation in Hispanics, European Americans, and African Americans [82]. PNPLA3 plays a role in the hydrolysis of glycerolipids and the I148M substitution causes a loss of function [42]. The rs738409 (I148M) in PNPLA3 is also associated with severe histologic complications of NAFLD, such as portal inflammation, lobular inflammation, Mallory-Denk bodies, and fibrosis [83, 96, 97]. Genome-wide association scans for hepatic steatosis measured using computed tomography scanning or magnetic resonance spectroscopy has revealed that variants in or near *PNPLA3*, *NCAN*, *GCKR*, *LYPLAL1*, and *PPP1R3B* associate with this trait [98]. Variants in or near *PNPLA3*, *NCAN*, *GCKR*, and *LYPLAL1* but not near *PPP1R3B* associate with NASH/fibrosis on a genome-wide significant level [98]. The hepatic steatosis-increasing allele at *NCAN* was associated with lower triglycerides and plasma LDL cholesterol levels [98] (Fig. 6.2) but how this variant exerts these effects is unclear. The nearest gene *NCAN* is a brain chondroitin sulfate proteoglycan that acts as an adhesion molecule [79] but it is not clear that the variant rs2228603 acts through this gene. The hepatic steatosis-increasing allele at *GCKR* (rs780094) was associated with higher levels of plasma LDL cholesterol and triglycerides, lower fasting glucose, lower HOMA-IR [98] (Fig. 6.2). *GCKR* inhibits glucokinase in the liver, pancreatic islet cells, and possibly other tissues and prevents the phosphorylation of glucose, which is required for its use as a substrate for de novo lipogenesis. Carriers of the common L446 allele for GCKR have higher triglyceride levels and lower fasting plasma glucose levels [76, 94]. The P446L coding change in the protein product of GCKR leads to a reduction in GCK-inhibition by the variant regulatory protein. This is predicted to increase glycolytic flux and hence glucose uptake by the liver. This enhanced rate of glycolysis may increase other liver metabolites such as malonyl-CoA, increasing triglyceride levels by two mechanisms—acting as a substrate for de novo lipogenesis, and also by blocking fatty acid oxidation [5]. This perturbation of hepatic metabolism may account for lowered glucose and raised triglycerides levels seen in L446-carriers of GCKR. In this way it may possibly promote hepatic steatosis. Similarly, since *PPP1R3B* encodes a protein that promotes the breakdown of liver glycogen to phosphorylated glucose [2], variants near this gene may increase its activity and predispose carriers to increased hepatic steatosis by increasing substrates for de novo lipogenesis. Indeed, the hepatic steatosis associated variants (rs4240624) at this locus increase PPP1R3B expression in the liver which is consistent with this model [98]. How these hepatic steatosis variants at PPP1R3B also cause increased HDL and LDL cholesterol and decreased fasting glucose levels (Fig. 6.2) remains to be determined. Finally, variants associated with hepatic steatosis in or near *PNPLA3* (rs738409) (encoded protein is a triacylglycerol lipase that mediates triacylglycerol hydrolysis in adipocytes) and *LYPLAL1* (rs12137855) (lysophospholipase-like 1 protein) show association with NASH/fibrosis but do not show significant

association for several lipid and glycemic traits tested. Thus, *PNPLA3* and *LYPLAL1* may be involved in lipid metabolism and exert their effects within the liver in ways that are not well reflected in serum glucose or lipid measurements [99]. These results were some of the first to show that genetics can dissociate epidemiologically correlated traits. Targeting the genes through which associated variants act may yield diverse outcomes, leading to personalized disease treatments.

Future Elucidation of More Obesity-Susceptible Loci Having Pleiotropic Association with Other Complex Diseases

Genome-wide association studies of many human phenotypes are beginning to uncover the unique and shared genetic basis of human traits. This information can complement studies of obesity syndromes to uncover the mechanisms by which genetic defects promote adiposity. Already we have learned that some obesity pleiotropisms may be caused by genetic defects that are affecting more than one gene, as occurs in Prader–Willi syndrome. Other pleiotropisms may arise from affected genes being expressed and acting in multiple tissues, as occurs with the BBS genes. A gene product may also have different effects in different tissues based on the expression of downstream mediators, as is seen for *POMC*; the expression of PC1 or PC2 determines how it is cleaved into different peptides with disparate effects, only some of which relate to the development of obesity. The mechanism by which many of the new GWAS-associated variants exert their pleiotropic effects remains to be determined.

Conclusion

With the increased prevalence of obesity worldwide, there has been a corresponding increase in many obesity-related diseases, with resultant morbidity and mortality, and for which there are few treatments and no cures. Genetic studies suggest that the development of obesity comorbidities may be influenced through different metabolic pathways that may represent distinct therapeutic targets. A better understanding of the unique and common genetic bases of these diseases may help us to improve personalized treatment based on individual needs. The integration of genetic data across phenotypes with data that include but are not limited to metabolomics, gene expression, or protein–protein interaction information can help us to better understand the mechanisms by which these genetic variants exert their effects and in this way help us to develop much needed new therapeutics for these conditions.

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Chapter 7
Functional Follow-up of Genetic Variants Using *FTO* as the Prime Example

Stefanie Seehaus and Ulrich Rüther

Abstract Obesity is the result of lifestyle and genetic predisposition. Tens of genes have been found to be associated with obesity. Among those, *FTO* showed the highest effect on body weight, but the function of the gene product was unknown at the time of discovering this association. As a consequence, several hundreds of studies have now been performed in just the last 6 years to unravel the biological role of *FTO* as an obesity gene. Although FTO is very likely to act as a RNA demethylase, the process by which FTO influences body weight is still unknown. In this review we have collected and evaluated most of the recent results which contribute to the relatively small but nevertheless important progress in FTO research with respect to obesity.

Abbreviations

BMI	Body mass index
CUX1	Cut-like homeobox 1
FTO	Fat mass and obesity associated
GWAS	Genome-wide association studies
m^6A	*N*6-methyladenosine
SNP	Single nucleotide polymorphism
alkB	Alpha-ketoglutarate-dependent dioxygenase
ALKBH	Mammalian alkB homolog

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Introduction

As soon as genome-wide association studies (GWAS) were efficiently possible, several groups screened for predispositions of increased body mass index (BMI). Up to date, tens of loci have been identified [1]. The locus with the best association was found to be the "*fat mass and obesity associated*" (*FTO*) gene [1]. Subsequently, this association could be reproduced worldwide for populations of different ethnicities [2–5]. Since the function of FTO was unknown, this review highlights the *FTO* gene as an example to illustrate the complex investigations necessary after the initial identification using GWAS. In principle, several fundamental questions have to be addressed which are equally valid for any other GWAS detected genes of unknown function:

- Is *FTO* really the obesity gene?
- What is the function of the gene product?
- What is causing the difference between the risk and the non-risk allele?
- Is FTO at all a potential drug target for common obesity?

On the History of *Fto*

In mice, *Fto* was identified via positional cloning as far back as 1999 [6]. At that time there was little clue that *FTO* would eventually become of immense interest to the obesity field. It is one of the six genes affected by the mouse mutation, Fused toes (*Ft*) [7]. Mice heterozygous for this 1.6 megabase deletion showed partial syndactyly of their forelimbs with fused toes and tremendous thymic hyperplasia, both caused most likely through impaired apoptosis. Homozygous *Ft*/*Ft* embryos died between embryonic day 10.5 and 12.5 due to severe developmental malformations.

Fto encodes a protein of 502 amino acid residues with a bipartite nuclear localization signal at the N terminus, suggesting a role in the nucleus. However, the 58-kDa protein showed no similarities to known proteins [6]. It is a large gene of nine exons present in all vertebrates and, surprisingly, also in marine algae [8, 9].

When the era of GWAS began, *FTO* was found to be associated with increased body mass index (BMI). Carriers homozygous for the risk allele weighted 3 kg more on average and had a 1.67-fold higher risk to become obese in comparison to non-risk allele carriers [10]. Of the several single nucleotide polymorphisms (SNPs) in the first intron identified so far, most are correlated with increased body weight [10–13]. Thus, all these data suggest *FTO* is indeed an important gene for obesity.

Originally *Fto* was given the name "Fatso", being the biggest gene affected by the *Ft* mutation [6]. However, after the discovery of *FTO* being associated with obesity [10, 13], *FTO* was renamed to the more appropriate designation "*fat mass and obesity associated*".

Is *FTO* Really the Obesity Gene?

Since most GWAS only indicate a loci and not a specific gene, the discovery raised the question: Is *FTO* really the obesity gene at this location or is it a neighbour of *FTO*? A possible candidate would be *RPGRIP1L* located in very close proximity 5′ of *FTO*, and transcribed in the opposite direction. RPGRIP1L has been shown to be essential for embryonic development through its involvement in cilia function [14]. Just a few publications attempted to address this question, which are summarized here.

Ex vivo and in vitro analyses studied the expression of *FTO* and *RPGRIP1L* in subcutaneous fat of lean and obese females, as well as preadipocyte differentiation. *FTO* was found to correlate positively with BMI but the same was not the case with *RPGRIP1L*. However, both *FTO* and *RPGRIP1L* were down-regulated with progression in adipocyte differentiation, suggesting that both might be involved in adipocyte differentiation [15].

Stratigopoulos and colleagues identified the transcription factor cut-like homeobox 1 (CUX1) as a potential regulator of *FTO* and *RPGRIP1L* [16, 17]. In vitro, CUX1 was found to bind to SNP rs8050136 of *FTO* and regulate the expression of *FTO* and *RPGRIP1L* using isolated human fibroblasts [17]. This SNP is part of the linkage disequilibrium region that harbours the first intronic SNP set found to be associated with obesity. This regulation of *FTO* and *RPGRIP1L* expression is suggested to be controlled via two isoforms of CUX1: P110 and P200. The authors suggested a regulatory role for CUX1 in modifying *FTO* and *RPGRIP1L* expression. P110 might operate as an activator of *FTO* and *RPGRIP1L* transcription, whereas P200 might act as a repressor of *FTO* transcription [16]. However, analysis of human *FTO* and *RPGRIP1L* mRNA expression levels from subcutaneous and visceral fat could not support the findings of Stratigopoulos and colleagues. There, expression of both *FTO* and *RPGRIP1L* in adipose tissue was not associated with rs8050136 genotypic status [18].

Fischer and colleagues addressed changes in regulation of *Rpgrip1l* due to structural alterations of the *Fto* gene in the course of its inactivation [19]. Within *Fto*-deficient mice, *Rpgrip1l* mRNA levels were unaltered in the hypothalamus, liver and white and brown adipose tissue. This was accomplished by data of Berulava and Horsthemke, showing that intronic SNPs in *FTO* did not affect *RPGRIP1L* expression [20], which is in line with data from a large association study showing no link between *RPGRIP1L* variation and BMI [21]. Hence, *RPGRIP1L* being the gene responsible for obesity in carriers of *FTO* susceptibility-conferring variants is unlikely.

In principle, even the gene at the 3′ site of *FTO*, the transcription factor *Iroquois 3* (*IRX3*) has to be considered as a potential candidate. This, however, has not been investigated by any researcher to date.

FTO Expression Profile in Relation to Nutritional States

As the function of FTO was initially unknown, expression characteristics of *Fto*/*FTO* had to be established first, in particular in the context of energy metabolism. Several studies showed ubiquitous expression of murine and human Fto/FTO

within nuclei of various cell types and tissues, revealing a generalized function for FTO. The highest expression of *Fto*/*FTO* was found in the brain, especially in the hypothalamus and cerebellum [6, 10, 22]. These results, together with the association of *FTO* with obesity, led researchers to focus on a possible role of FTO in regulating energy metabolism [23].

Fto expression in the hypothalamus has been shown to be concentrated in the dorsomedial (DMN), ventromedial (VMN), paraventricular (PVN) and arcuate (ARC) nuclei [22]. Furthermore, *Fto* mRNA has been reported to be localized in neuropeptide-regulating neurons guiding to a control of food intake [24]. Unfortunately, experiments altering nutritional states delivered controversial results in mammals regarding *Fto* expression within the hypothalamus. *Fto* mRNA expression of fasted rodents was found to be reduced [17, 22, 25], unaltered [26, 27] and even increased [24, 28]. In rats, fed on a high fat diet for several weeks, *Fto* mRNA appeared to be differentially expressed as well. In one study, *Fto* expression was up-regulated [29], whereas it was down-regulated in another [30]. In addition, mice receiving a solvent sucrose or Intralipid (fat emulsion) diet to their standard chow for 48 h exhibited unaltered *Fto* expression within the hypothalamus [28]. Olszewski and colleagues distinguished between mice partaking less calories (eating less) and mice taking in more calories (eating more). "Small eaters" were associated with up-regulated *Fto* mRNA expression in comparison to "big eaters" [28].

The discrepancy between *Fto* mRNA expression levels within the hypothalamus of different mammals made it rather complicated to understand the presumptive role of FTO in regulating appetite and food intake. Several aspects have to be taken in account. First of all, comparing results between different mammals is relatively challenging. Mice are much more sensitive to long-term fasting of 48 h than rats, showing enormous loss of fat stores. Secondly, results are dependent on experimental procedure. Sacrificing fasted rodents at different time points of the circadian system can influence study results. Importantly, comparing *Fto* mRNA expression levels in the entire hypothalamus between fasted and standard chow fed mammals has proven relatively meaningless. Different orexic and anorexic neuropeptides within different hypothalamic nuclei are responsible for controlling food intake [31]; therefore, it is of importance to detect alterations in FTO protein expression within appetite stimulating or inhibiting neuropeptide-harbouring cells due to changes in nutritional state. To our knowledge only McTaggart and colleagues addressed this in part, where they compared the percentage of FTO-positive anorexic proopiomelanocortin (Pomc) cells between fasted and standard chow fed mice without finding a significant difference [26]. Further analyses regarding the number of FTO-positive orexic neuropeptide-harbouring cells remained elusive. Finally, the main question is: "Are FTO expression levels altered between orexic and anorexic neuropeptide-containing cells during changing the nutritional state?" However, due to limitations in the experimental procedure this still remains to be answered.

Nevertheless, other publications tried to investigate factors involved in *FTO* regulation. Guo and colleagues designed 5′ deletion constructs of the *FTO* promoter to identify transcription factors relevant to expression of *FTO* [32]. Forkhead box A2 (FOXA2) appeared to bind to the promoter of *FTO* and thereby inhibiting the

expression of *FTO*. Interestingly, FOXA2 can bind also to *melanin-concentrating hormone* (*MCH*) and *Orexin* promoters and therefore regulating the expression of neuropeptides which are responsible for controlling food intake [33].

Functional Studies In Vitro

The Demethylase FTO

The first evidence of FTO function was achieved by *in silico* analysis, which suggested FTO to be a member of the superfamily of non-heme dioxygenases, depending on both Fe(II) as a co-factor and 2-oxogluterate as a co-substrate [9, 22]. Members of this superfamily are involved in posttranslational modifications, DNA repair and histone modifications and act as cellular sensors for metabolism and oxygen [34, 35]. Analyzing the highly conserved N-terminal region and secondary structures, FTO shows high sequence similarity to alpha-ketoglutarate-dependent dioxygenase alkB of *E. coli* and its mammalian homologues ALKBH2 and ALKBH3 [9, 22]. Thus, FTO appears to be a member of mammalian alpha-ketoglutarate-dependent dioxygenase alkB homologues (ALKBH1-8). AlkB and ALKBH3 prefer single stranded DNA and RNA (ssDNA, ssRNA) as their substrates whereas ALKBH2 prefers double-stranded DNA (dsDNA) [36]. Analyzing substrate specificity, FTO seemed to demethylate 3-methylthymine (m^3T) in the ssDNA setting as being the most likely scenario [22]. At this point it was already postulated that FTO may acts as a RNA demethylase, like the mammalian homologue ALKBH3 [22, 37]. Shortly after, in vitro analysis showed demethylation of m^3T in ssDNA and 3-methyluracil (m^3U) in ssRNA using recombinant human and mouse FTO [37]. Therefore, FTO favoured demethylation of m^3U in ssRNA over m^3T in ssDNA. Of note, FTO catalyzed demethylation of m^3U and m^3T with low efficiency; furthermore, m^3T methylation of DNA in mammalian had not been described up to that point. Thus, FTO functioning as a DNA demethylase is very unlikely.

Nevertheless, while the N terminus of FTO (32–326 aa residues) exhibits high sequence similarity to alkB and mammalian homologues [9], the C-terminal domain (327–498 aa residues) shows no sequence similarity to any gene. Han et al. shed some light on the function of FTO revealing its crystal structure [38]. As a consequence, the group confirmed the ß sheet containing N terminus as the catalytic domain, by showing a core of six anti-parallel ß sheet structures forming the so-called jelly-roll motif with an extra loop around the motif. This extra loop, which does not exist in any other alkB member, was suggested to be responsible for FTO selection for ssDNA or ssRNA. Binding of FTO to double-stranded nucleic acids would not be impossible, as the extra loop of FTO would compete with the unmethylated strand of the DNA duplex. Furthermore, it was shown that the alpha sheet containing C terminus formed bulky hydrophobic contacts with the N terminus, suggesting a role for N terminus-stabilization and therefore involving a catalytic role as well.

Recent findings identified *N*6-methyladenosine (m^6A) as another substrate for FTO in ssRNA, with a 50-fold higher substrate specificity over m^3U under physiological conditions in vitro [39]. Furthermore, FTO was detected in nuclear speckles [40] and was shown to co-localize with nuclear speckle factors, thus providing a hint for FTO in pre-mRNA processing [39]. Overexpression of FTO led to decreased quantity of m^6A in mRNA, whereas 90 % knockdown of FTO led to an increase in m^6A quantity in mRNA. This discovery raised questions regarding the role of m^6A for biological processes. Mapping m^6A enabled researchers to uncover the human and mouse m^6A RNA methylomes. Furthermore, m^6A modifications appeared to be dynamically and reversibly regulated [41, 42]. Collectively, more than 12,000 highly conserved m^6A sites were identified in the transcripts of more than 7,000 genes, preferentially within 3′UTRs and near stop codons within mRNA. The corresponding genes were found to be involved in transcriptional regulation, RNA metabolism and intracellular signalling cascades. In addition, 67 % of m^6A-containing 3′UTRs also contained microRNA-binding sites, suggesting a role of m^6A in microRNA pathways [42]. In addition, m^6A around stop codons could affect translational efficiency [41]. However, the two RNA methylome publications came to different conclusions. Meyer et al. suggested a localization of m^6A only in mature mRNA [42]. They could not show an enrichment of m^6A at splice junctions so they asked if previous studies, which used non-specific methylation inhibitors, pointed to the wrong direction proposing a role of m^6A in mRNA processing. Meanwhile, Dominissini's group [41] based their analysis on a possible role of m^6A on RNA splicing. A knockdown of the only known m^6A methylase, methyltransferase like3 (METTL3), affected alternative splicing of pre-mRNA, resulting in altered signalling pathways and apoptosis via alteration of *P53* in a human hepatocellular carcinoma cell line. Therefore, the authors proposed a role of m^6A modulating pre-mRNA splicing which was supported by the notion that FTO and METTL3 co-localize with splicing proteins in the nucleus, in particular nuclear speckles [39, 43, 44]. Nevertheless, a role for m^6A in modulating pre-mRNA splicing remained unclear. Recently, a second m^6A demethylase, the mammalian alkB homolog 5 (ALKBH5), was identified with localization to splicing proteins [44]. Certainly, further studies regarding FTO and ALKBH5 will bring clarity to this issue. Finally, it cannot be excluded that demethylation of m^6A via FTO could also affect rRNA and tRNA [45, 46].

Role of FTO in Amino Acid Sensing?

Other in vitro studies using human and mouse cell lines suggest a role for FTO in amino acid sensing [47, 48]. Essential amino acid-deprived human and mouse cell lines exhibited down-regulation of *FTO* mRNA and protein levels [47]. In addition, analyses of *Fto*-deficient mouse embryonic fibroblasts (MEFs) and human HEK cells showed decreased cell growth, impaired mRNA translation, more autophagic flux and reduced protein levels of tRNA synthetases, especially leucyl tRNA synthetase [48], thus defining a role for FTO in mammalian target of rapamycin complex 1

(mTORC1) signalling, among others, as an important protein complex for controlling food intake [49]. Future experiments are needed to further investigate how FTO is affected by amino acid sensing and thereby controlling protein biosynthesis.

Loss- and Gain-of-function-analyses of *Fto* in Mice and Rats

In recent years, *Fto* mouse mutants with loss or gain of function were designed to enable a better understanding of the role of FTO *in vivo*. For such an approach we assigned mouse line numbers for the different *Fto*-deficient mouse mutants based on appearance (mouse lines 1–7). Concordance and differences between mouse lines are illustrated in Table 7.1. Interestingly, constitutive *Fto*-deficient mice (mouse line 1) appeared to be protected from obesity, with decreased body weight and an increase in energy expenditure, although exhibiting relative hyperphagia and somewhat decreased spontaneous locomotor activity [19].

Analyzing body composition using magnetic resonance imaging (MRI) revealed a reduction in fat as well as lean mass. In addition, male mice were more affected then female mice. Furthermore, increased serum adiponectin and decreased serum leptin levels, markers for body weight regulation, underlined the lean phenotype due to loss of FTO within mouse line 1. Even on a high fat diet, constitutive mice of mouse line 1 did not gain weight compared to their wild type littermates. Finally, mice were growth retarded and showed a higher frequency of postnatal death. These results led to the suggestion that FTO is involved in body weight regulation and energy homoeostasis.

Almost as expected, a mouse line overexpressing FTO revealed gain in body weight due to increased fat mass but not lean mass in comparison to their wild type counterparts [50]. A dose-dependent effect with the highest increase in body mass was verified in male and female mice with four *Fto*-copies (two additional copies), respectively weighting an additional 10 or 22 %. Furthermore, mice exhibited an increase in food intake and energy expenditure but no alterations in physical activity or circadian rhythm.

A second mouse line with partial loss of FTO function due to a dominant missense mutation (mouse line 2; Table 7.1) exhibited the lean phenotype as seen in mouse line 1 [19] but to a milder extend [51]. However, several differences persisted between these two *Fto* mouse mutants. For instance, mouse line 2 did not show postnatal death, growth retardation or alterations in food intake and physical activity.

Since then, several other *Fto* mouse mutants were generated. Another constitutive (mouse line 3) and a nervous system-specific *Fto*-deficient mouse mutant (mouse line 4) displayed very similar phenotypes as mouse lines 1 and 2 [19, 51] but with emphasis on postnatal growth retardation [52]. Surprisingly, the lower body weight in mouse line 3 and mouse line 4 (Table 7.1) was found to be due to decreased lean mass. Fat mass was even increased in females of mouse line 3 and both male and female mice of mouse line 4. The authors suggested that FTO exerts its function in the brain and therefore could regulate growth and presumably food intake over

Table 7.1 Overview of published *Fto*-deficient mouse lines

	Mouse line						
	1	2	3	4	5	6	7
	Fischer et al. 2009 [19]	Church et al. 2009 [51]	Gao et al. 2010 [52]	Gao et al. 2010 [52]	McMurray et al. 2013 [53]	McMurray et al. 2013 [53]	McMurray et al. 2013 [53]
Fto-deficiency:	Constitutive	Partial	Constitutive[a]	Neural-specific[a]	Constitutive	Adult onset constitutive	Adult onset hypothalamus-specific[b]
Body weight	↓	↓	↓	↓	↓	↓	↔
Lean mass	↓	↔	↓	↓	↓	↓	↔
Fat mass	↓	↓	↔ ♂; ↑ ♀	↑	↓	↔ young; ↑ old	↔
Postnatal death	↑	↔	↑	n. a.	↑	↔	n. a.
Growth retarded	Yes	No	Yes	Yes	Yes	No	n. a.
Food intake	↑	↔	↑	↑	↔	↔	↓
Energy expenditure	↑	↑	↑	↑	↔	↔	↔

Compared to wild type situation: ↓ = decrease, ↑ = increase or appearance more often, ↔ = no difference; n.a. not addressed

[a]Efficiency of *Fto*-inactivation could not be shown

[b]Only partial inactivation of *Fto* due to delivery of Cre by adenoviral infection

energy expenditure [52]. However, taking a closer look at the study of Gao and colleagues, in both mouse lines (3 and 4) FTO was still found to be expressed within the hypothalamus, especially in neural-specific *Fto*-deficient mouse mutants [52].

Another publication presented three more *Fto* mouse lines (Table 7.1), namely, constitutive (mouse line 5), adult onset (mouse line 6) and adult onset hypothalamic (mouse line 7) *Fto*-deficient mouse mutants [53]. Comparing the new constitutive mouse line 5 with previously published constitutive *Fto*-deficient mice (mouse line 1 and 3), a very similar phenotype was found, pointing to a role of FTO in body weight and growth regulation. Adult onset *Fto*-deficient mice (mouse line 6) were protected from postnatal death, which was not the case for constitutive *Fto*-deficient mice (mouse line 1, 3 and 5) [19, 52, 53], suggesting FTO as being essential soon after birth. Surprisingly, fat mass of older adult onset *Fto*-deficient mice (mouse line 6) was found to be increased. It would be of interest to see if fat mass of young adult onset mice (mouse line 6) was increased as well by taking the lower body weight into account (percent fat). Another interesting observation was the decrease in food intake of adult onset hypothalamic *Fto*-deficient mice (mouse line 7). In this case the authors injected adeno-associated viral vectors encoding Cre recombinase into the hypothalamus of *Fto* floxed mice to regionally delete FTO expression and found decreased food intake. Using the same virus system to knock down FTO expression via short hairpin RNA in the hypothalamus, the same authors reported an increase in food intake in rats. Furthermore, in both rodent lines protein levels of FTO within the hypothalamus or ARC and PVN were found to be reduced by less than 50 % in comparison to sham operated rodents [29, 53]. Thus, the interpretation of these results is extremely complicated and challenging.

Finally, 4 years and 7 *Fto*-deficient mouse mutants later, the role of FTO in obesity is still unclear, especially after the report that an adult onset loss of FTO led to a gain in fat mass. Whether loss or gain of FTO function is involved in obesity still needs to be clarified.

Analysis of Individuals Carrying Genetic Variants of *FTO*

Several studies have tried to unravel the differences of the risk and non-risk allele of *FTO* for developing obesity. Whereas association between *FTO* variants and increased BMI and body weight were clearly identified [10, 11, 13], the question whether increased, decreased or deregulated FTO expression is responsible for the effect is still open and cannot yet be answered by the use of *Fto* mouse mutants. In addition, variants of the protein could attribute to the gain of body weight. While trying to address this question, most investigators have concentrated on the link between *FTO* risk allele carriers and *FTO* expression levels in adipose tissue. As a consequence, no association between *FTO* genotype and *FTO* expression levels in adipose tissue was found [18, 54, 55]. Furthermore, *FTO* expression levels were found to be either increased or decreased in adipose tissue of obese patients [15, 55–57]. Moreover, inconsistency was found in regard to *FTO* expression in subcutaneous and visceral adipose tissue of obese individuals [18, 55, 56].

Berulava and Horsthemke were so far the only investigators trying to address whether an allele specific difference of *FTO* expression can be found. Instead of analyzing *FTO* mRNA levels they analyzed levels in unspliced nuclear RNA of heterozygous risk allele carriers. Risk allelic expression of *FTO* was found to be more abundant than non-risk allele expression [20]. However, this result was only shown for fibroblasts; nevertheless, this study suggests that *FTO* overexpression might lead to an increase in body weight.

One study addressed variants that may impact the FTO protein by screening the nine exons of *FTO* in 2,866 individuals and identifying heterozygous non-synonymous variants [58]. However, these were equally frequent between lean and obese individuals. Furthermore, loss-of-function mutations of *FTO* were found in lean and obese people. Thus, showing clearly that non-synonymous variants in the coding region of *FTO*, and therefore of the protein, are not linked to obesity.

FTO as a Therapeutic Target for Obesity?

Even with the fragmentary knowledge of FTO function as a demethylase, the first mechanistic study was published presenting an effective cell-active inhibitor for human FTO, called rhein [59]. Rhein binds FTO reversibly, thus blocking the recognition site of FTO for m^6A substrates. However, considering the little knowledge we still have of the biochemical role of FTO, its ubiquitous expression in every tissue and the severe phenotype documented in humans being deficient for FTO demethylase activity [60], application of any drug-based anti-obesity therapy is currently still extremely risky. In other words, elucidation of the role of FTO is indispensable to design a tissue or signalling pathway-specific drug for FTO targeting.

How to Continue Functional Studies on FTO

So overall, what is known about FTO today? FTO is a member of the alkB demethylase family. Loss of FTO function leads to reduced body weight and growth retardation. However, whether the demethylase activity of FTO is responsible for the effects seen in mutant mice still remains unclear. If the demethylase activity of FTO is responsible, what are the targets of FTO and are these targets organ specific? Further, if FTO is involved in epigenetic regulation of the mammalian transcriptome and therefore might demethylate a high number of targets, which of those targets are relevant for obesity? Answering these questions might help to develop a therapy against obesity which in turn reduces the side effects to a minimum.

Much effort was put into uncovering the function of FTO and how it may be involved in becoming obese. However, still little is known. But at the end of the day, independent of our genetic status we should not forget: Do not eat more than your body burns, otherwise you will gain weight.

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