Review

Developments in Obesity Genetics in the Era of Genome-Wide Association Studies

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Key Words

Body mass index • Gene-environment interactions • Genetics • Genome-wide association studies • Obesity • Waist-to-hip ratio

Abstract

Obesity is an important factor contributing to the global burden of morbidity and mortality. By identifying obesity susceptibility genes, scientists aim to elucidate some of its aetiology. Early studies used candidate gene and genome-wide linkage approaches to search for such genes with limited success. However, the advent of genome-wide association studies (GWAS) has dramatically increased the pace of gene discovery. So far, GWAS have identified at least 50 loci robustly associated with body mass index (BMI), waist-to-hip ratio, body fat percentage and extreme obesity. Some of these have been shown to replicate in non-white populations and in children and adolescents. Furthermore, for some loci interaction studies have shown that the BMI-increasing effect is attenuated in physically active individuals. Despite many successful discoveries, the effect sizes of the established loci are small, and combined they explain only a fraction of the inter-individual variation in BMI. The low predictive value means that their value in mainstream health care is limited. However, as most of these newly established loci were not previously linked to obesity, they may provide new insights into body weight regulation. Continued efforts in gene discovery, using a range of approaches, will be needed to increase our understanding of obesity.

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Introduction

Obesity is clearly implicated as a risk factor for some of the leading causes of mortality – diabetes [1–3], heart disease [4–6] and a number of cancers [7–9] – and there are consistent associations with a range of other conditions such as mental health issues [10, 11] and muscular-skeletal conditions [3]. The increase in the prevalence of overweight and obesity has been observed worldwide, and obesity is likely to continue to be an important risk factor for the health of all populations, in particular of those in developing countries, for decades ahead [12–15].

Understanding the genetic causes of obesity susceptibility may reveal some of the underlying biology and thus lead to advances in prevention and possible treatments. While twin and family studies have suggested that 40–70% of the inter-individual variation in obesity risk and body mass index (BMI) can be attributed to genetic factors, the search for obesity susceptibility loci has only recently started to be successful [16–19].

Genetics of Obesity before Genome-Wide Association Studies

The first studies that searched for genes that predispose to common obesity were based on candidate genes, where the focus is on genes with a suspected role in the regulation of metabolism and food intake, such as those implicated in the leptin-melanocortin pathway. Genetic variation in such candidate genes was subsequently examined for association with obesity and related traits in the general population. However, the candidate gene approach has suffered from some limitations, most notably the fact that many studies have been small ($n < 1,000$) and few results have been replicated [20, 21]. Furthermore, the selection of appropriate candidate genes requires a good understanding of the underlying biology and for many of the proposed candidate genes these pathways are still not fully understood.

With progress in high-throughput genotyping and the availability of data from the Human Genome Project, genome-wide linkage studies provided an alternative methodology to search for obesity susceptibility genes. In genome-wide linkage studies, the whole genome of related individuals is screened for linkage of chromosomal regions with obesity or a related trait. Unlike candidate gene studies, which is hypothesis driven, genome-wide linkage studies do not rely on an a priori hypothesis but hope to identify previously unknown genetic loci, which may lead to new insights in the biology. However, genome-wide linkage analysis has not been a powerful method for finding genetic loci with small effects as expected for common obesity [22, 23]. Similar to candidate gene studies, genome-wide linkage studies have suffered from lack of replication of results, mostly because many have small sample sizes [23, 24]. Furthermore, a meta-analysis of 37 studies, including 31,000 individuals from >10,000 families and thus sufficiently powered, did not identify any locus robustly linked to BMI or obesity [23].

The Genome-Wide Association Era

The sequencing of the human genome [25, 26] and the creation of databases of single nucleotide polymorphisms (SNPs), such as dbSNP [27] and the International HapMap [28], ushered in a revolution in the way genetic studies were carried out. Advances in technology that made genotyping individuals faster and cheaper [29–31] have meant that researchers can study the majority of the common variants in the genome and in more individuals using genome-wide association studies (GWAS). Genome-wide association is currently the most

Fig. 1. Minimum detectable effect size of loci for a given risk allele frequency for varying sample sizes, assuming an α level of 10^{-6} and 80% power. The curves represent, for various sample sizes, the lower limit of the effect size of loci across the risk allele frequency spectrum that can be identified with 80% power.

commonly used method for searching for loci associated with a trait or disease (box 1). Similar to genome-wide linkage studies, GWAS are hypothesis generating and aim to identify new loci to increase our understanding of the biology that underlies the susceptibility to obesity. GWAS have the advantage over genome-wide linkage studies that they do not require participants to be related, which allows for studies with larger sample sizes, thus increasing the power to detect true associations [22, 32]. Larger samples enable the discovery of new genes and smaller effects, and also provide more accurate effect size estimates.

The identification of any locus as part of a GWAS depends upon its effect size and prevalence of the risk allele. The loci that have smaller effect sizes or lower allele frequency in the population will require larger studies in order to detect them (fig. 1). Since 2007, several waves of GWAS have been performed for various obesity-related traits, each subsequent wave including a larger sample than the preceding one.

Box 1. The Genome-Wide Association Approach

GWAS are currently the most commonly used approach to identify loci associated with a particular phenotype and consist of two stages. The first stage is the discovery stage in which hundreds of thousands of SNPs across the whole genome are tested for association with the trait of interest. The SNPs that show the highest level of association with the phenotype (at present typically 30–40 SNPs are chosen) are taken forward into the follow-up stage of the study. The SNPs taken forward are tested for association in a new population, ideally one of at least similar size and design. The association results from the discovery and follow-up stages are meta-analysed. Since a very large number of association tests are performed in the discovery stage, there is a high likelihood of false-positive findings. Therefore, the significance levels used are very stringent. GWAS will in general only consider associations that reach a value of $p < 0.5 \times 10^{-8}$ as significant.

GWAS for Overall Adiposity

Much of the focus of GWAS for adiposity has been on finding genes associated with BMI. BMI is an inexpensive, non-invasive measure of overall adiposity that is available in most health investigations. To date there have been four waves of discovery for BMI, with each wave increasing the number of studies and number of loci identified.

The First Wave and *FTO*

The GWAS that discovered the first locus associated with BMI was part of the Wellcome Trust Case Control Consortium studies examining the genetics of type 2 diabetes [33]. In this GWAS, genetic variation in 1,924 individuals with type 2 diabetes was compared with that in 2,938 population-based controls. A SNP in the *FTO* (fat mass- and obesity-associated) gene was found to show strong association with type 2 diabetes, which was subsequently replicated at the second stage with 3,757 cases with type 2 diabetes and 5,346 controls. However, once analyses were adjusted for BMI, the association with type 2 diabetes was abolished, indicating that the effect of *FTO* on type 2 diabetes was on a pathway through BMI. The association with BMI was subsequently robustly replicated in a sample comprising 19,424 adults from seven studies and 10,172 children from two separate studies. Thus, it was concluded that the *FTO* locus affects diabetes through its effect on adiposity.

At the same time, a GWAS in 6,142 individuals from Sardinia examined specifically anthropometric traits – BMI, hip circumference and weight. SNPs in two loci (*FTO* and *PFKP*) were followed up in 3,467 individuals in the GenNet family-based cohort [34]. Only the SNP in the *FTO* locus was confirmed to be robustly associated with BMI, as well as with the other anthropometric traits.

Since the discovery of *FTO* as an obesity susceptibility locus, its associations with BMI and several related obesity traits have been consistently replicated in subsequent studies. To date, the *FTO* locus is still the locus with the largest effect on BMI, which together with its high minor allele frequency means that *FTO* is a so-called low-hanging fruit that could fairly easily be identified by moderately sized studies.

The Second Wave and *MC4R*

To detect variants with smaller effect sizes or with a lower minor allele frequency other than the *FTO* locus, larger sample sizes were needed. For the second round of GWAS for BMI, a meta-analysis of seven studies was performed with data from 16,876 individuals of which 11,012 were from four population-based cohorts, and 5,864 from three disease-specific case series [35]. This study confirmed the robust association found at the *FTO* locus. Most importantly, a SNP near the melanocortin 4 receptor (*MC4R*), a gene previously implicated in studies of early-onset obesity, was convincingly replicated in a series of 10 studies comprising 60,352 adults and 5,988 children.

At the same time, a GWAS of various obesity-related traits in 2,682 Indian Asians, of which 23 SNPs were followed up in a sample of 11,955 individuals of both Indian and European descent, identified the same locus near *MC4R* to be associated with BMI [36].

The Third Wave

To detect even more loci, larger studies had to be undertaken. As such, the GIANT (Genetic Investigation of Anthropometric Traits) consortium was founded to bring together GWAS with anthropometric traits. The first meta-analysis by the GIANT consortium combined 15 cohorts to provide a discovery stage of 32,387 individuals. SNPs in 35 loci were taken forward for replication in a further 14 studies for a second-stage sample of 59,082 individuals [37]. Besides confirming *FTO* and near-*MC4R*, loci in or near *TMEM18*, *GNPDA2*, *SH2B1*, *MTCH2*, *KCTD15* and *NEGR1* were found to show genome-wide significant association with BMI, of which the association of the SNP near *NEGR1* is thought to implicate a 45-bp deletion.

Concurrently, meta-analyses of GWAS for BMI and body weight were performed, combining data from five studies totalling 34,416 individuals principally from Iceland [38]. The 43 most significantly associated SNPs were followed up in 5,586 Danes, and their associations were also examined in the data available from the GWAS performed by the GIANT consor-

Fig. 2. Effect sizes of established BMI loci ordered by date of discovery and by effect size. The effect size represented is the increase in body weight per risk allele for an individual 170 cm tall. Effect sizes are derived from the most recent and largest GWAS for BMI [39].

tium [37]. Associations were confirmed for genetic variants at loci in or near *NEGR1*, *TMEM18*, *SH2B1*, *KCTD15*, *ETV5*, *BDNF* and *SEC16B*, as well as confirming the *FTO* and near-*MC4R* loci. A further locus, *FAIM2*, was very close to genome-wide significance for body weight.

Both studies confirmed the loci in *FTO* and near *MC4R*, and 4 of the newly identified loci (*SH2B1*, *KCTD15*, *TMEM18* and *NEGR1*) overlapped between the two studies. Taken together, by the end of the third wave, a total of 12 loci had been found to be incontrovertibly associated with BMI (fig. 2).

The Fourth Wave

In the fourth wave, the GIANT consortium expanded further to provide the large sample size required to discover variants with even smaller effect sizes or lower allele frequencies than those discovered in the third wave. As such, the discovery stage comprised a meta-analysis of 46 studies including 123,865 individuals of white European descent. SNPs in the 42 most significantly associated genetic variants were taken forward for follow-up in 18 additional studies comprising 125,931 individuals [39]. All 12 previously established loci were confirmed, and 18 novel loci associated with BMI were discovered, totalling to 32 BMI-associated loci (table 1; fig. 2).

Effect Sizes and Variance Explained in BMI

The *FTO* locus has the largest effect size of the 32 established BMI-associated loci; i.e. for each additional risk allele, BMI increases by 0.39 km/m² (equivalent to an increase of ~1.1 kg for someone 170 cm tall; table 1; fig. 2). It also has a comparatively high effect al-

Table 1. Currently established loci associated with BMI (sorted by effect size)

Loci	Paper in which this locus was first cited	Increase in BMI for each additional risk allele	Risk allele frequency in white Europeans, %
<i>FTO</i>	Frayling et al. [33] (2007)	0.39	42
	Scuteri et al. [34] (2007)		
Near <i>TMEM18</i>	Willer et al. [37] (2009)	0.31	83
	Thorleifsson et al. [38] (2009)		
Near <i>MC4R</i>	Loos et al. [35] (2008)	0.23	24
	Chambers et al. [36] (2008)		
<i>SEC16B</i>	Thorleifsson et al. [38] (2009)	0.22	19
<i>BDNF</i>	Thorleifsson et al. [38] (2009)	0.19	78
<i>SLC39A8</i>	Speliotes et al. [39] (2010)	0.19	7
Near <i>GNPDA2</i>	Willer et al. [37] (2009)	0.18	43
Near <i>GPRC5B</i>	Speliotes et al. [39] (2010)	0.17	87
Near <i>PRKD1</i>	Speliotes et al. [39] (2010)	0.17	4
<i>SH2B1</i>	Willer et al. [37] (2009)	0.15	40
	Thorleifsson et al. [38] (2009)		
<i>QPCTL</i>	Speliotes et al. [39] (2010)	0.15	80
Near <i>RBJ</i>	Speliotes et al. [39] (2010)	0.14	47
Near <i>ETV5</i>	Thorleifsson et al. [38] (2009)	0.14	82
Near <i>NEGR1</i>	Willer et al. [37] (2009)	0.13	61
	Thorleifsson et al. [38] (2009)		
<i>TFAP2B</i>	Speliotes et al. [39] (2010)	0.13	18
<i>MAP2K5</i>	Speliotes et al. [39] (2010)	0.13	78
<i>NRXN3</i>	Speliotes et al. [39] (2010)	0.13	21
<i>FAIM2</i>	Speliotes et al. [39] (2010)	0.12	38
<i>LRRN6C</i>	Speliotes et al. [39] (2010)	0.11	31
Near <i>FLJ35779</i>	Speliotes et al. [39] (2010)	0.10	63
Near <i>FANCL</i>	Speliotes et al. [39] (2010)	0.10	29
<i>CADM2</i>	Speliotes et al. [39] (2010)	0.10	20
Near <i>TMEM160</i>	Speliotes et al. [39] (2010)	0.09	67
Near <i>LRP1B</i>	Speliotes et al. [39] (2010)	0.09	18
<i>MTIF3</i>	Speliotes et al. [39] (2010)	0.09	24
<i>TNNI3K</i>	Speliotes et al. [39] (2010)	0.07	43
Near <i>ZNF608</i>	Speliotes et al. [39] (2010)	0.07	48
<i>MTCH2</i>	Willer et al. [37] (2009)	0.06	41
Near <i>PTBP2</i>	Speliotes et al. [39] (2010)	0.06	59
Near <i>RPL27A</i>	Speliotes et al. [39] (2010)	0.06	52
Near <i>KCTD15</i>	Willer et al. [37] (2009)	0.06	67
	Thorleifsson et al. [38] (2009)		
<i>NUDT3</i>	Speliotes et al. [39] (2010)	0.06	21

Effect sizes and risk allele frequencies have been derived from the most recent and largest GWAS for BMI [39].

lele frequency of 42%. The discovery of the locus near *MC4R* required a much larger sample size, not only because its risk allele frequency is lower, but also because its effect size is smaller than that observed for the *FTO* locus. For each additional risk allele at the near-*MC4R* locus, BMI increases by 0.23 kg/m² (or ~0.6 kg for someone 170 cm tall; table 1; fig. 2). The effects of the subsequently indentified loci in the third and fourth wave range from 0.06 to 0.33 kg/m² (or ~0.2 to ~1.0 kg for someone 170 cm tall; table 1; fig. 2). In general, the effect sizes of the loci observed in the fourth wave tended to be smaller than

those observed in the third wave. While the effect size of the near-*TMEM18* locus (identified in the third wave) is larger than that of the near-*MC4R* locus (identified in the second wave; fig. 2), the near-*TMEM18* locus has a much lower minor allele frequency and thus required a substantially larger sample for it to be identified. For similar reasons, some loci with low minor allele frequency were only identified in the fourth wave despite relatively large effect sizes.

Despite strong evidence of association for each of the 32 loci, combined they explain only a fraction of the total variation in BMI (~1.45%). Therefore, it is not surprising that predicting obesity using the risk alleles of these BMI loci is not accurate. Genetic susceptibility scores using 8 [37], 12 [40] and most recently 32 loci [39] all confirmed their limited value to predict obesity. While including additional loci in the genetic scores somewhat improves the accuracy of the predictions, even a score including 32 loci is weak at discriminating between obese and non-obese individuals (ROC AUC = 0.575), and not sufficient to have any clinical application [39].

GWAS for Abdominal Obesity

While BMI is easy to obtain, it does not accurately reflect the distribution of body fat. To better capture abdominal obesity, waist circumference and waist-to-hip ratio (WHR) have been used in GWAS. So far, two waves of GWAS for abdominal obesity have been performed.

First Wave

The GIANT consortium performed a meta-analysis of 16 studies including 38,580 individuals examining waist circumference and WHR. A total of 26 SNPs were taken forward for follow-up in a sample of 70,689 individuals comprising 28 cohorts [41]. Associations with loci in *FTO* and near *MC4R* were confirmed, but novel associations with waist circumference were also observed near *TFAP2B* and near *MSRA*. Furthermore, a SNP near *LYPLAL1* showed association with WHR in women only.

At the same time, the CHARGE consortium performed a meta-analysis of 31,373 individuals from eight GWAS for waist circumference [42]. The 48 most significant loci were looked up in the data from the GIANT consortium study to confirm their observations. They identified association with a locus in the *NRXN3* gene, besides also observing association with the *FTO* and near-*MC4R* loci.

Taken together, this first wave identified 4 new loci for abdominal obesity, and confirmed the *FTO* and near-*MC4R* loci. Of interest is that the *FTO*, near-*MC4R*, near-*TFAP2B* and *NRXN3* loci have also been identified in the GWAS for BMI [39]. This suggests that, because of the high correlation between BMI and waist circumference, these loci associate more with overall obesity than with abdominal obesity.

Second Wave

Since the GIANT consortium had an interest in understanding the specific determinants of fat distribution, analyses in the second wave were adjusted for BMI, resulting in discoveries of loci that are more specific to abdominal obesity. As such, a large-scale meta-analysis of GWAS of WHR adjusted for BMI included data from 32 GWAS including up to 77,167 participants. SNPs in the 16 most significantly associated loci were followed up in 29 studies of 113,636 individuals [43]. The associations of 14 loci with WHR reached genome-wide significance, with effect sizes ranging from 0.042 to 0.020 per risk allele (table 2). Subsequent sex-specific analyses found that for 7 of the 14 loci the effects were significantly more pronounced in women than in men (fig. 3). The differences seen in the strength of association seem unsurprising given the observed sex-specific distributions of WHR.

Fig. 3. Effect sizes of 14 established WHR ratio loci in men and women separately (significant differences in size of effects between men and women: * $p < 0.01$). Data were derived from the most recent and largest GWAS for WHR [43].

Table 2. Currently established loci associated with WHR (sorted by effect size) for all individuals and for men and women separately

Loci	Per allele effect on WHR	Effect allele frequency in white Europeans, %	Per allele effect on WHR				p value for the difference between men and women
			men	p value in men	women	p value in women	
RSPO3	0.045	52	0.036	1.05×10^{-11}	0.053	1.93×10^{-32}	1.94×10^{-3}
Near NFE2L3	0.043	21	0.040	2.52×10^{-10}	0.046	1.41×10^{-16}	0.27
Near VEGFA	0.039	56	0.025	7.38×10^{-5}	0.051	2.27×10^{-26}	5.20×10^{-6}
TBX15-WARS2	0.031	37	0.029	9.41×10^{-13}	0.033	1.02×10^{-14}	0.951
Near GRB14	0.036	60	0.011	0.043	0.054	3.84×10^{-34}	1.41×10^{-11}
Near LYPLAL1	0.032	28	0.000	0.358	0.055	4.95×10^{-33}	1.18×10^{-13}
DNMT3-PIGC	0.026	57	0.022	7.81×10^{-9}	0.030	6.90×10^{-11}	0.855
Near ITPR2-SSPN	0.030	74	0.022	1.41×10^{-3}	0.038	2.41×10^{-17}	4.67×10^{-4}
Near HOXC13	0.030	24	0.024	9.45×10^{-4}	0.035	6.38×10^{-16}	2.23×10^{-3}
Near LY86	0.028	39	0.030	1.63×10^{-7}	0.026	2.40×10^{-12}	0.357
Near ADAMTS9	0.026	41	0.004	7.85×10^{-7}	0.042	1.92×10^{-16}	8.50×10^{-5}
ZNRF3-KREMEN1	0.019	57	0.012	1.94×10^{-3}	0.024	3.24×10^{-11}	0.032
NISCH-STAB1	0.036	94	0.032	1.68×10^{-4}	0.039	6.01×10^{-7}	0.574
CPEB4	0.019	34	0.015	3.03×10^{-4}	0.021	1.55×10^{-6}	0.555

Effect sizes, risk allele frequencies and p values have been derived from the most recent and largest GWAS for WHR [43].

GWAS for Body Fat Percentage

While BMI is a good estimate of overall body size, it does not differentiate between lean and fat mass. Body fat percentage is a more accurate estimate for adiposity, and the identification of loci associated with this trait might point towards pathways that would not have been highlighted with GWAS for BMI.

The first large-scale GWAS on body fat percentage consisted of a discovery stage combining 15 cohorts including 36,626 individuals. SNPs in 14 loci were followed up in 39,576 individuals from 11 studies [44]. Besides the *FTO* locus, two loci not previously implicated in any GWAS of adiposity were identified; one near *IRS1* and the other near *SPRY2*. The near-*IRS1* locus was found to associate with a range of metabolic traits, in particular in men. However, most interestingly, the fat percentage-decreasing allele associated with increased insulin resistance, lower HDL cholesterol levels, and increased risk of type 2 diabetes and cardiovascular disease. Further analyses suggested that this association pattern could be explained by the fact that the fat percentage-decreasing allele of the near-*IRS1* locus is associated with less subcutaneous fat, but not with visceral fat, which is metabolically more harmful.

GWAS for Extreme Obesity

It has been hypothesised that individuals who have extreme obese phenotypes, either very high BMI or early-onset obesity, might present more common risk alleles. Furthermore, case-control studies of these extreme phenotypes may have greater power than similar-sized studies that examine continuous traits.

The first study of extreme obesity considered 487 cases and 442 controls replicated in 644 families, but only identified the *FTO* locus [45]. A subsequent case-control GWAS included 1,380 individuals with extreme or early-onset obesity and 1,416 lean controls in the discovery stage. SNPs in 38 loci were followed up in 14,186 individuals from case-control and population-based studies, of which those in *FTO*, *NPC1* and near *MC4R*, *MAF* and *PTER* were found to show consistent association with risk of obesity and BMI [46].

A third study for extreme obesity combined data from 1,138 cases and 1,120 controls; parts of this sample overlapped with the two previous GWAS. A total of 44 SNPs were followed up in 1,181 obese and 1,960 normal- or underweight individuals [47]. Subsequently, 10 SNPs showing the strongest associations were tested for association in 31,182 individuals from population-based samples. Besides the confirmation of the *FTO* and near-*MC4R* loci, they identified one other locus between *MSRA* and *TNKS* to be robustly associated with extreme obesity.

Besides the *FTO* and near-*MC4R* loci, the other genes discovered in case-control settings appear to be different from those found in population-based settings, suggesting that there may be a specific set of genes that predisposes an individual to more extreme forms of obesity.

GWAS and Non-European Populations

Thus far, most GWAS have been performed in populations of white European descent, with the exception of one study that identified the locus near *MC4R*, which screened a South Asian population at the discovery stage [36]. However, examining associations in non-European populations can be informative for at least two reasons. Firstly, there may be obesity susceptibility loci that are particular to a specific ethnic group, while others might affect any ethnicity. Secondly, the genetic architecture differs across ethnicities, as illustrated by differences in the patterns of linkage disequilibrium, which allows for fine-mapping of obesity susceptibility loci identified in Europeans. More specifically, some loci span across a chromosomal region that in populations of European descent are part of the same haplotype

block, whereas this block may be more fragmented in populations of a different ethnicity. Association studies across such loci in different populations might indicate more precisely where the causal variant or gene might be located.

Replication of Obesity Susceptibility Loci in Non-Europeans

Of all obesity susceptibility loci identified so far, the *FTO* locus replicates consistently across populations of different ethnic backgrounds. In general, studies in populations of East Asian [48–62], South Asian [63–65] and African origin [66–73], in particular those with larger sample sizes, have found that the *FTO* locus associates convincingly with BMI and obesity risk, with effect sizes similar to those observed in white European populations. The risk allele frequency of the *FTO* locus, however, varies substantially: being ~20% in East Asian populations, ~30% in South Asians but ~45% in white Europeans. Populations of African origin show more diverse allele frequencies, while one study reported a range of ~7 to ~18% for two apparent effect alleles in *FTO* [71], frequencies outside this range in other populations of African descent have been observed [66–68]. As such, the contribution of *FTO* to the variation in BMI in obesity risk will vary across populations. By taking advantage of the population-specific genetic architecture, which in populations of African descent has lower levels of linkage disequilibrium, a study of African Americans and Afro-Caribbeans was able to fine-map the *FTO* locus and narrow down the potentially causal chromosomal region [71].

The near-*MC4R* locus was discovered by GWAS in a population of South Asian descent in the discovery stage. While the risk allele frequency is higher in Indian Asians (~40 vs. ~27% in white Europeans), the effect size on BMI is similar to that observed in white Europeans [36]. Subsequently, this locus has been replicated as an obesity susceptibility locus in other South Asian populations [65, 74] and in some studies of populations of African descent [38, 75]. Although the risk allele frequency (~16–20%) in populations of African ancestry was lower, the effect sizes tended to be similar or even somewhat larger than in white Europeans. Studies of populations of East Asian descent, where the risk allele is less frequent (~19%) than in white Europeans, have not yet found consistent replication of this locus [52, 56, 58, 60, 61, 76, 77]. A meta-analysis of all available data will be needed to confirm or refute association of this locus with obesity susceptibility in these populations.

Besides the *FTO* and the near-*MC4R* locus, obesity susceptibility loci identified in the earlier waves of discoveries have been examined in populations of other ethnicity, mainly in populations of East Asian [56, 58, 60, 61, 76, 77] and African origin [38, 75], but their replication has so far been rather unconvincing. However, as these loci were identified in meta-analyses of around 30,000 individuals, firm replication will require large sample sizes, too. Replication of loci identified in the most recent waves [39, 43] will also require very large replication samples, probably produced by meta-analysis, given how large the discovery sample sizes were.

GWAS for Obesity Susceptibility in Non-Europeans

So far, few GWAS for obesity susceptibility traits have been performed specifically in non-Europeans populations. Nevertheless, these types of GWAS are of particular interest to identify obesity susceptibility loci that are specific to certain ethnic groups.

A GWAS for BMI and WHR in a Korean population included 8,842 individuals, with a replication stage of 7,861 individuals [78]. This GWAS confirmed the association of *FTO* with BMI, and identified another locus on chr12q24 that was robustly associated with WHR. A GWAS for body fat percentage was performed in 413 Pima Indians with the most significant association being observed for a SNP in *A2BPI*, a promising biological candidate, which seemed specific to Pima Indians [79]. A study of 1,931 Africans and African Americans with

a follow-up sample of 3,700 did not identify any new genome-wide significant associations [75]. GWAS for BMI in 1,792 Filipino women confirmed the previously established loci in or near *FTO*, *MC4R* and *BDNF*, but identified no new loci either [62].

While the initial GWAS in non-Europeans have often been underpowered, they confirm that loci such as *FTO* and near-*MC4R* are associated with adiposity across populations of different ethnicity. Larger GWAS and meta-analyses will be needed for the discovery of loci that are specific to certain ethnic populations.

Genetic Associations in Children and Adolescents

To understand the broader nature of the established obesity susceptibility loci, some studies examined whether the associations seen in adults replicate in children and adolescents and how these loci affect life course changes in BMI in longitudinal studies [80–83].

A meta-analysis of three studies in 13,071 children and adolescents examined 13 of the established BMI loci [84]. All loci showed directionally consistent effects, 9 of which reached nominally significant associations. While there was no significant difference in the effect sizes observed in adults and children, the effects observed for the loci in or near *SEC16B*, *TMEM18* and *KCTD15* tended to be larger in children than in adults, whereas the opposite was observed for the *BDNF* locus. Of interest is that there is currently no evidence that any of the established BMI loci are associated with birth weight [85, 86].

Longitudinal studies, currently limited to the *FTO* and near-*MC4R* loci, have been performed to assess the timing of effect of obesity susceptibility loci during the life course. A study in 1,629 adult Danes showed that the *FTO* locus was consistently associated with BMI throughout adulthood [87]. A longitudinal analysis of >7,000 children found that during childhood and adolescence the association between *FTO* and BMI was most pronounced after age 11 years [88]. Another longitudinal study, with life course data from childhood up to the age of 53 years for 2,479 individuals, examined the effects of SNPs in *FTO* and near *MC4R* on BMI and weight [89]. The pattern of association was similar for both loci, showing an increase in the effect on BMI up to age 20 years after which the association weakened. A meta-analysis of eight longitudinal studies, with an average of 9,143 individuals per age stratum, was consistent with the previous study, showing an increase in the effect of *FTO* on BMI after the age of 5 years [90]. Interestingly, however, before age 2.5 years, the BMI-increasing *FTO* allele was associated with lower BMI. These changing patterns of association may be indicative of changing environmental influences throughout the life course.

Gene-Environment Interaction Studies

Common traits such as body composition are the result of both genetic and environmental effects that not only act in an additive but also interactive manner. The sample sizes required to detect interaction effects are very large [91], which are further compounded by the difficulty in obtaining accurate measures of lifestyle, such as diet [92, 93] or physical activity [94]. While in the past interaction studies were hampered by uncertain genetic association, since the discovery of *FTO* many studies have examined whether its effect on BMI and obesity risk is attenuated by a physically active lifestyle or a healthy diet.

A study in 6,104 Danes [95] showed that in more physically active individuals the effect of *FTO* was attenuated by ~30% compared to inactive individuals. Other large-scale studies confirmed this interaction between *FTO* and physical activity [96–98], although some did not [99]. It is not yet clear whether this observed interaction is due to specific effects of

physical activity on the function of *FTO*. Studies that examined the interaction with dietary factors showed that the effect of *FTO* on BMI was less pronounced in individuals with a low calorie intake [100] or with a healthier diet [101] than in those with less healthy dietary habits. The fact that similar interaction has been observed with diet and physical activity suggests that *FTO* might be sensitive to a healthy lifestyle in general.

In a large-scale population-based study of >20,000 men and women from the UK, the interaction between the overall genetic susceptibility and physical activity on BMI was examined [102]. To estimate the overall genetic susceptibility, a genetic score was calculated by adding the number of risk alleles an individual carried. Each additional risk allele increased the BMI by 0.154 kg/m² (or 0.44 kg for a person 170 cm tall). However, the effect was 40% less pronounced in individuals who were physically active (0.131 kg/m²/risk allele) compared to individuals who were inactive (0.205 kg/m²/risk allele).

The results of these interaction studies carry an important public health message as they challenge the deterministic view often held by the public that a genetic susceptibility cannot be attenuated by a healthy lifestyle.

Genetic Contributions to Understanding the Biology of Obesity

The identification of new loci is only the starting point of a new series of investigations to increase our understanding of the biological pathways they are involved in. A better understanding of these pathways may eventually lead to more targeted preventive strategies and may potentially result in novel drug targets. Since *FTO* was the firstly established obesity susceptibility locus, much of the experimental work has focused on trying to understand the biological function of this gene. The gene product of *FTO* is an AlkB-like 2-oxoglutarate-dependent nucleic acid demethylase [103]. Experiments in mice have shown that *Fto* expression is abundant in the hypothalamus and influenced by the animal's nutritional state suggesting that *FTO* increases risk of obesity through a central regulation of food intake [103]. This neuronal hypothesis was supported by a transgenic mouse model [104]. Mice that carry one or two extra copies of the gene display increased *Fto* expression in all tissues and also show increased energy intake and adiposity [104]. Furthermore, a growing body of evidence suggests that the *FTO* locus is associated with increased appetite, energy intake and with reduced satiety in humans [105–110].

MC4R has been known as a gene in which mutations lead to extreme and early-onset obesity long before GWAS identified the near-*MC4R* locus in which common variants showed association with obesity in the general population [111, 112]. It is currently unclear whether the near-*MC4R* locus associates with common obesity through *MC4R* itself. However, the fact that the risk allele of the common locus is not only associated with increased BMI but also with increased height is consistent with the observation that individuals with mutations in *MC4R* exhibit accelerated linear growth and thus suggests that this locus might indeed affect the function of *MC4R* [113].

Similar to the near-*MC4R* locus, there are at least 3 additional loci (near *POMC*, *SH2B1* and *BDNF*) that harbour genes that have been shown to carry monogenic mutations that cause extreme obesity [114–118]. Furthermore, one of the loci harbours *GIPR*, known for its role in glucose and insulin metabolism [119], whereas another locus harbours *HMG-CoA reductase*, which encodes the rate-limiting enzyme for cholesterol synthesis [120], each providing the first insights into potential pathways implicated in obesity. Nevertheless, for most of the recently discovered loci, the physiological role in relation to obesity risk is not or still poorly understood, and more experimental research will be needed to unravel the underlying physiological pathways.

Future Avenues

For the majority of the loci identified it is not clear which variant or gene is on the causal pathway towards obesity. Some loci harbour multiple genes, whereas other loci harbour no genes at all. Therefore, a lot of work remains to be done to fine-map these loci and identify the causal gene, such that they can be followed up in experimental research. In particular, in genetic association studies in individuals with different genetic background, the linkage disequilibrium structure might help with the fine-mapping.

Furthermore, more GWAS for obesity susceptibility traits in non-white populations are needed to identify loci that might be specific to or have a more pronounced effect in certain populations. Also, GWAS in children and adolescents might identify loci that may have more important effects early in life rather than later in adulthood.

There is no doubt that more genes and genetic loci associated with obesity remain to be discovered given that currently only a fraction of the heritability is explained by the established loci. It has been speculated that low-frequency variants with larger effect sizes might account for some of the unexplained heritability. An inexpensive way to search for such low-frequency variants is implementing the data made available by the 1000 Genomes Project into a GWAS [25] or taking advantage of custom-built arrays that aim to identify low-frequency variants nearby established loci. Alternatively, one can consider the more expensive route of deep-sequencing of the exomes or the whole genomes. The UK10K project is one of such efforts that aims to sequence thousands of individuals, including cases of extreme and early-onset obesity [121].

New technologies may provide further biological knowledge about genes already discovered, and, in some cases, may help to uncover new loci. Scans of histone modification and methylation can be used to examine if there are patterns of differential expression associated with BMI. This can either be done by using the established loci in a candidate approach, or by applying a hypothesis-free genome-wide approach that may uncover loci where changes in expression, rather than mutations in the DNA, result in different patterns of adiposity [122, 123]. Furthermore, systems biology, a pathway-driven analysis, links the genes harboured by the established obesity susceptibility loci to biological knowledge of how these genes act, as applied in the recent GWAS for BMI [39]. At least one study has used this approach as a starting point for new genome-wide studies where genes that are known to act in concert are tested for associations as a group [124].

Conclusions

GWAS have provided an extremely valuable contribution to the field of obesity genetics with the discovery of a large number of genetic loci robustly associated with various obesity-related traits, mainly in white European populations, in a short space of time. Studies in non-European populations have replicated the associations of some of the loci, mainly those of the *FTO* and near-*MC4R* loci. Studies in children, adolescents and individuals throughout their life course have provided the first evidence that loci identified in adults also affect adiposity in childhood and that the effects might be most pronounced towards the end of adolescence, just before the start of adulthood. While the established loci so far only explain a fraction of the total variation in the obesity-related traits and have a very limited predictive value, their main contribution lies in the fact that each of the loci may point towards a new biological pathway that was not previously linked to obesity susceptibility. Unravelling these pathways and translating them to mainstream health care will take many years, but it is speculated that this increased understanding will eventually lead to more targeted preventive strategies and potentially the development of more effective treatments.

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