Recent progress in the genetics of common obesity

Ruth J. F. Loos

MRC Epidemiology Unit, Institute of Metabolic Science, Cambridge, UK

Correspondence

Dr Ruth J. F. Loos, MRC Epidemiology Unit,
Institute of Metabolic Science,
Addenbrooke's Hospital – Box 285, Hills
Road, Cambridge CB2 0QQ, UK.
Tel: + 44 0 12 2376 9139
Fax: + 44 0 12 2333 0316
E-mail: ruth.loos@mrc-epid.cam.ac.uk

Keywords

body mass index, common obesity,
genetic epidemiology, genome-wide
association studies

Received

9 April 2009

Accepted

27 July 2009

The genetic contribution to interindividual variation in common obesity has been estimated at 40–70%. Yet, despite a relatively high heritability, the search for obesity susceptibility genes has been an arduous task. This paper reviews recent progress made in the obesity genetics field with an emphasis on established obesity susceptibility loci identified through candidate gene as well as genome-wide studies. For the last 15 years, candidate gene and genome-wide linkage studies have been the two main genetic epidemiological approaches to identify genetic loci for common traits, yet progress has been slow and success limited. Only recently have candidate gene studies started to succeed; by means of large-scale studies and meta-analyses at least five variants in four candidate genes have been found to be robustly associated with obesity-related traits. Genome-wide linkage studies, however, have so far not been able to pinpoint genetic loci for common obesity. The genome-wide association approach, which has become available in recent years, has dramatically changed the pace of gene discoveries for common disease, including obesity. Three waves of large-scale high-density genome-wide association studies have already discovered at least 15 previously unanticipated genetic loci incontrovertibly associated with body mass index and extreme obesity risk. Although the combined contribution of these loci to the variation in obesity risk at the population level is small and their predictive value is typically low, these recently discovered loci are set to improve fundamentally our insights into the pathophysiology of obesity.

The prevalence of obesity and overweight continues to rise worldwide, not only causing serious personal health problems but also imposing a substantial economic burden on societies [1]. By current estimates, nearly 70% of adults in the USA and >60% in the UK are overweight; half of these are obese [2, 3].

It is well established that rapid globalization of the westernized lifestyle is fuelling this emerging obesity epidemic. Yet, not everyone in the present-day obesogenic environment develops obesity, highlighting the multifactorial nature of the condition. Indeed, obesity arises through the joint actions of multiple genetic and environmental factors, i.e. the obesogenic environment increases the risk of obesity, in particular in those who are already genetically susceptible.

Family and twin studies have shown that genetic factors contribute 40–70% to the interindividual variation in common obesity [4]. Despite a relatively high heritability, the search for obesity susceptibility genes has not been trivial. For the last 15 years, candidate gene and genome-wide linkage studies have reported a multitude of genetic loci, but only a handful have been robustly confirmed in subsequent studies. In recent years, however, significant progress has been made through genome-wide associa-

tion studies with the discovery of at least 15 genetic loci incontrovertibly associated with obesity.

This paper reviews recent advances made in the obesity genetics field with an emphasis on established obesity susceptibility loci identified through candidate gene as well as genome-wide studies. The significant progress made by genome-wide association studies warrants specific and more extensive attention. I will therefore discuss the chronological sequence of discoveries, their impact on public health and clinical practice, their potential to unravel the underlying pathophysiology, and the ways ahead to find more obesity susceptibility loci.

Gene identification before the genome-wide association era

Although recent success of genome-wide association studies has drawn a lot of attention, gene identification for the last 15 years has been based on two broad genetic epidemiological approaches, i.e. candidate gene and genome-wide linkage studies.

For over a decade, the scientific community interested in obesity genetics was privileged to have a Human

Obesity Gene Map that catalogued all genetic variants and chromosomal loci ever associated with or linked to obesity-related traits [5]. Its first edition was published in 1996, followed by 10 yearly updates, the latest in 2006 covering the literature available as of the end of 2005. The map continues to be a useful resource, in print and web-based versions (<http://obesitygene.pbrc.edu>), providing a comprehensive and easily accessible overview of the literature on candidate gene and genome-wide linkage studies published before 2006.

Candidate gene studies

Candidate gene studies are hypothesis-driven and rely on the current understanding of the biology and pathophysiology that underlies the susceptibility to obesity. Genes, for which there is evidence for a role in regulation of the energy balance in animal models or in extreme/monogenic forms of obesity, are tested for association with obesity-related traits at the population level. In the mid 1990s, when genotyping was still an expensive and tedious task, candidate gene studies typically examined only one or a few variants per gene, with a focus on nonsynonymous variants because of their potential functional implications. Over time, genotyping costs have come down substantially, and publicly available datasets, such as dbSNP and the International HapMap, have provided deeper insight into genetic variation in genes. This knowledge has led to more comprehensive studies that systematically examine the association of all common variation in a gene of interest by means of carefully selected tagSNPs and their haplotypes under the assumption that a causal variant would be in high linkage disequilibrium with one of the tagSNPs or at least captured by the haplotypes.

Since the first candidate gene studies for obesity-related traits, >15 years ago, the number of proposed obesity susceptibility genes has grown steadily. The latest update of the Human Obesity Gene Map reported 127 candidate genes for which at least one study reported a positive association with obesity-related traits [5]. This rapid expansion reflects, at least in part, the availability of cost-effective genotyping technologies to a wider scientific community.

However, for many of the proposed candidate genes replication in successive studies has been inconsistent, so that the overall conclusion on association remains ambiguous. The limited success of the candidate gene approach can mainly be ascribed to small sample sizes ($n < 1000$) that are insufficiently powered to identify modest effects that are expected for common obesity. However, recent years have seen a change as an increasing number of studies have tested for associations in larger populations ($n > 5000$) and more often the initiative has been taken to meta-analyse all available published (and unpublished) data. The results of these large-scale studies are summarized in Tables 1 and 2.

Robust associations have been observed for nonsynonymous variants in the melanocortin 4 receptor (*MC4R*), prohormone convertase 1/3 (*PCSK1*), brain-derived neurotrophic factor (*BDNF*), and β -adrenergic receptor 3 (*ADRB3*) genes.

The *MC4R* gene has a strong biological candidacy; *MC4R* is widely expressed in the central nervous system and plays a key role in the regulation of food intake and energy homeostasis [6]. Rare functional mutations in *MC4R* are the commonest monogenic cause of severe early-onset obesity [7]. However, until recently its role as a common obesity susceptibility gene has been unconvincing. The two most common *MC4R* variants, V103I and I251L, each result in a nonsynonymous change with potential functional implications [8]. Numerous studies have examined these *MC4R* variants, but none has found significant association with obesity-related traits, apart from one sizeable population-based study that observed a significant protective effect of the 103I-allele (frequency: 2–3% of the population) on obesity risk [9] (Table 1). Subsequently, three large-scale meta-analyses confirmed that 103I-allele carriers have a 20% lower risk of obesity than V103V homozygotes [10–12] (Table 2). In addition, a meta-analysis of data on the I251L *MC4R* variant provided strong evidence for a protective effect with a nearly 50% reduced risk of obesity for carriers of the 251L-allele (frequency: 1–2%) [12] (Table 2).

The *PCSK1* gene is another strong candidate, as it encodes an enzyme that converts pro-hormones into hormones involved in energy metabolism regulation. Individuals with rare mutations in *PCSK1* are born with a PC1/3 deficiency resulting in a syndrome characterized by extreme childhood obesity [13–15]. In a comprehensive large-scale study, the role of common variants in the *PCSK1* gene was studied in relation to the risk of obesity [16]. After sequencing coding regions in a small sample of obese individuals, nine variants that captured the common genetic variation in *PCSK1* were genotyped in 13 659 individuals of European ancestry. Two nonsynonymous variants, N221D and the Q665E-S690T pair, were consistently associated with obesity in adults and children (Table 1). Each additional minor allele (frequency: 4–7%) of the N221D variant increased the risk of obesity 1.34-fold, while each additional minor allele (frequency: 25–30%) of the Q665E-S690T pair increased the risk 1.22-fold. Functional characterization of these variants suggested a modest deleterious effect of the N221D variant, but no significant functional role of the Q665E-S690T amino acid substitutions [16].

While *BDNF* has been mainly studied for its presumed role in the regulation of development, stress response, survival, and mood disorders, rodent studies have found *BDNF* to be implicated in eating behaviour, body weight regulation and hyperactivity [17, 18]. Similar to *MC4R* and *PCSK1*, a rare mutation in *BDNF* probably causes severe obesity and hyperphagia [19]. A recent large-scale study, including

Table 1Overview of large-scale ($n > 5000$) individual candidate gene studies for obesity-related traits (in alphabetical order)

Gene	Design	Variants	Individuals, <i>n</i>	Main outcome	Reference
ADRB1		Arg389Gly	7 677	No association with obesity or BMI	Gjesing <i>et al.</i> (Diabet Med 2007) [91]
ADRB2		Arg16Gly	7 808	No association with obesity	Gjesing <i>et al.</i> (Diabetologia 2007) [92]
Gln27Glu		No association with obesity			
ADRB3		Trp64Arg	7 605	No association with obesity	Gjesing <i>et al.</i> (Mol Genet Metab 2008) [93]
BDNF		Val66Met	10 109	Val-allele carriers have a higher BMI (+0.76 kg m ⁻² [95CI 0.036, 1.16]; $P < 0.001$) than Met-allele homozygotes	Shugart <i>et al.</i> (EJHG 2009) [20]
CNR1	Systematic screen of gene	26 SNPs	5 750	The rs806381 ($P = 1.1 \times 10^{-6}$) and rs2023239 (4.7×10^{-4}) are associated with increased obesity risk and BMI	Benzinou <i>et al.</i> (HMG 2008) [94]
DIO2		Thr92Ala	7 342	No association with obesity	Grarup <i>et al.</i> (JCEM 2007) [95]
ENPP1		Lys121Gln	5 863	No association with BMI or waist	Grarup <i>et al.</i> (Diabetologia 2006) [28]
ENPP1		Lys121Gln + 3 marker haplotype	8 676	No association with BMI	Lyon <i>et al.</i> (Diabetes 2006) [25]
ENPP1		Lys121Gln + 3 marker haplotype	8 089	No association with obesity	Weedon <i>et al.</i> (Diabetes 2006) [26]
ENPP1		Lys121Gln + 3 marker haplotype	5 153	No association with BMI, overweight, or obesity. Gln-allele nominally associated with morbid obesity ($P = 0.02$)	Meyre <i>et al.</i> (Diabetologia 2007) [96]
ERRalpha		Pro116Pro and ESRRA23	6 365	No association with obesity or BMI	Larsen <i>et al.</i> (IJO 2006) [97]
FAAH		Pro129Thr	5 801	No association with obesity or BMI	Jensen <i>et al.</i> (J Mol Medical 2007) [98]
IGF2	Systematic screen of gene	3 SNPs	5 000	No association with BMI	Heude <i>et al.</i> (JCEM 2007) [99]
KLF7	Systematic screen of gene	4 SNPs	14 818	Minor allele of rs7568369 protected against obesity (OR = 0.90 [0.84, 0.96], $P = 0.001$), and is associated with lower BMI ($P = 0.002$) and waist circumference ($P = 0.003$)	Zobel <i>et al.</i> (EJE 2009) [100]
LTA		Thr60Asn	5 630	N-allele carriers have increased waist circumference ($P = 0.009$), but not BMI	Hamid <i>et al.</i> (Diabetologia 2005) [101]
MC4R		V103I	7 937	Ile-allele carriers have a reduced risk of obesity (OR 0.69 [95% 0.50, 0.96], $P = 0.03$)	Heid <i>et al.</i> (J Medical Genetics 2005) [9]
NOS1AP		rs7538490	16 913	No association with obesity	Andreasen <i>et al.</i> (BMC Med Genetics 2008) [102]
NPY2R	Systematic screen of gene	3 SNPs	5 971	Minor-allele carriers of rs12649641 have increased risk of obesity ($P = 0.02$)	Torekov <i>et al.</i> (Diabetologia 2006) [103]
NPY2R	Systematic screen of gene	5 SNPs	>8 000	No association with BMI	Campbell <i>et al.</i> (Diabetes 2007) [104]
PCSK1	Systematic screen of gene	N221D	13 659	Minor allele is associated with increased risk of obesity (OR 1.34 [95% 1.20, 1.49], $P = 7.27 \times 10^{-8}$)	Benzinou <i>et al.</i> (Nature Genetics 2008) [16]
Q665E-S690T		Minor allele is associated with increased risk of obesity (OR 1.22 [95% 1.15, 1.29], $P = 2.31 \times 10^{-12}$)			
PKLR		rs3020781	16 801	No association with obesity	Andreasen <i>et al.</i> (BMC Med Genetics 2008) [102]
PPARA		Leu162Val	5 799	No association with obesity	Sparso <i>et al.</i> (Mol Genet Metab 2007) [105]
PPARD	Systematic screen of gene	12 SNPs	5 971	No association with BMI	Grarup <i>et al.</i> (Diabetologia 2007) [106]
PPARGC1B	Systematic screen of gene	Ala203Pro	7 790	203Pro allele is associated with a reduced obesity risk ($P = 0.004$)	Andersen <i>et al.</i> (J Medical Genet 2005) [107]
PYY		Arg72Thr	6 022	Thr-allele carriers have increased risk of overweight ($P = 0.02$)	Torekov <i>et al.</i> (Diabetes 2005) [108]

BMI, body mass index.

Table 2

Overview of meta-analyses of candidate gene loci for obesity-related traits (in alphabetical order)

Gene	Variants	Studies, <i>n</i>	Individuals, <i>n</i>	Outcome	Reference
ADRB2	Arg16Gly	11	4 328	No association with obesity	Jalba <i>et al.</i> (Obesity 2008) [109]
ADRB2	Glu27Gln	23	10 404	Glu27-allele carriers have an increased risk of obesity (OR 1.566; [95% CI 1.07, 2.29]; $P = 0.02$) in Asians, Pacific Islanders, and American Indians only. No association in Whites	Jalba <i>et al.</i> (Obesity 2008) [109]
ADRB3	Arg64Trp	36	7 399	Arg-carriers tend to have an increased BMI (+0.19 kg m ⁻² [95% CI -0.026, 0.41], $P = 0.07$)	Allison <i>et al.</i> (IJO 1998) [110]
ADRB3	Arg64Trp	48	9 236	Arg-carriers have an increased BMI (+0.30 kg m ⁻² [95% CI 0.13, 0.47])	Fujisawa <i>et al.</i> (Clin Endocrinol & Metab 1998) [111]
ADRB3	Arg64Trp	35	6 582	Arg-carriers have an increased BMI (+0.26 kg m ⁻² [95% CI 0.18, 0.42]; $P = 0.001$) in Japanese	Kurokawa <i>et al.</i> (Obes Res 2001) [112]
ADRB3	Arg64Trp	97	44 833	Arg-carriers have an increased BMI (+0.24 kg m ⁻² [95% CI 0.12, 0.37]; $P = 0.0002$) in East Asians only. No association in Whites	Kurokawa <i>et al.</i> (IJO 2008) [24]
GRL	Asn363Ser	12	4 792	No association with BMI or obesity	Marti <i>et al.</i> (BMC Med Genetics 2006) [113]
IL6	-174G→C	25	26 944	No association with BMI	Qi <i>et al.</i> (JCEM 2007) [114]
LEPR	K109R	8	3 012	No association with BMI	Heo <i>et al.</i> (IJO 2002) [115]
LEPR	Q223R	9	3 263	No association with BMI	Heo <i>et al.</i> (IJO 2002) [115]
LEPR	K656N	9	3 263	No association with BMI	Heo <i>et al.</i> (IJO 2002) [115]
MC4R	Val103Ile	12	7 713	Ile-allele carriers have a reduced risk of obesity (OR 0.69; [95% CI 0.50, 0.96]; $P = 0.03$)	Geller <i>et al.</i> (AJHG 2004) [10]
MC4R	Val103Ile	25	29 563	Ile-allele carriers have a reduced risk of obesity (OR 0.82; [95% CI 0.70, 0.96]; $P = 0.015$)	Young <i>et al.</i> (IJO 2007) [11]
MC4R	Val103Ile	29	39 879	Ile-allele carriers have a reduced risk of obesity (OR 0.80; [95% CI 0.70, 0.92]; $P = 0.002$)	Stutzmann <i>et al.</i> (HMG 2007) [12]
MC4R	Ile251Leu	12	11 435	Leu-allele carriers have a reduced risk of obesity (OR 0.52; [95% CI 0.38, 0.71]; $P = 3.58 \times 10^{-5}$)	Stutzmann <i>et al.</i> (HMG 2007) [12]
PPARG	Pro12Ala	50	29 424	Ala-allele carriers have an increased BMI in Whites ($P = 0.015$)	Tonjes <i>et al.</i> (Diabetes Care 2006) [116]

BMI, body mass index.

10 109 women, found that minor-allele homozygotes (Met66Met; frequency: 4.5%) of the Val66Met variant have a significantly lower body mass index (BMI) (-0.76 kg m⁻²) than Val66-allele carriers [20] (Table 1).

The Arg64Trp *ADRB3* variant is one of the first genetic variants for which association with obesity was reported [21–23]. *ADRB3* is an obvious candidate gene given its involvement in the regulation of lipolysis and thermogenesis. Following the first reports in 1995, >100 studies have been published on the association between the Arg64Trp variant and obesity-related traits, but results have been inconsistent. However, a recent meta-analysis that combined data of 44 833 individuals found significant association between the Arg64Trp variant and BMI in East Asians, with Arg64-allele carriers having a 0.31 kg m⁻² higher BMI compared with the Arg64Arg homozygotes [24] (Table 2). No associations were observed in Whites.

Large-scale studies are also powered to prove that an association is truly negative, such as, for example, for the Lys121Gln *ENPP1* variant. Four studies, each with >5000 participants and a combined sample size of 27 781 individuals, found no association between the Lys121Gln variant and obesity-related traits [25–28] (Table 1). For other candidate genes tested in large-scale studies or in meta-analyses and for most of the other 127 candidate

genes reported in the Human Obesity Gene Map, further follow-up is required to prove or refute unambiguously their role in obesity susceptibility.

In summary, the last 15 years of candidate gene studies have only recently starting to succeed; by means of large-scale studies and meta-analyses at least five variants in four candidate genes have been found to be robustly associated with obesity-related traits.

Genome-wide linkage studies

Genome-wide linkage studies are hypothesis-generating and, through surveying the whole genome, aim to identify new, unanticipated genetic variants associated with a disease or trait of interest. Genome-wide linkage studies rely on the relatedness of study participants and test whether certain chromosomal regions co-segregate with a disease or trait across generations. A whole-genome linkage survey requires 400–600 highly polymorphic markers, genotyped at 10-cM intervals. Genome-wide linkage studies have a rather coarse resolution and typically identify broad intervals that require follow-up genotyping to pinpoint the genes that underlie the linkage signal.

Since the first genome-wide linkage study was published in 1997, the number of chromosomal loci linked to

obesity-related traits has grown exponentially. The latest Human Obesity Gene Map update reported 253 loci from 61 genome-wide linkage scans, of which 15 loci have been replicated in at least three studies [5]. Yet none of these replicated loci could be narrowed down sufficiently to pinpoint the genes or variants that underlie the linkage signal. Despite substantial power, a meta-analysis of 37 genome-wide linkage studies with data on >31 000 individuals from 10 000 families of European origin could not locate a single obesity or BMI locus with convincing evidence [29]. This meta-analysis indicates that genome-wide linkage might not be an effective approach for identifying genetic variants for common obesity.

Genome-wide association studies

Similar to genome-wide linkage, the genome-wide association approach interrogates the entire genome, unconstrained by prior assumptions. It aims to identify previously unsuspected genetic loci associated with a disease or trait of interest and, thereby, to expand our understanding of the underlying physiology. Genome-wide association studies screen the whole genome at higher resolution levels than genome-wide linkage studies and are thus able to narrow-down the associated locus more accurately. Genome-wide association does not rely on familial relatedness and can therefore achieve larger sample sizes than typical family-based studies.

Two major advances, i.e. the rapid expansion of our knowledge of the human genome in concert with substantial progress in high-throughput genotyping technology, have led the way to the genome-wide association. Together, these two new advances have given rise to the production of smartly designed chips that permit interrogation of the entire genome in one single experiment.

Genome-wide association has revolutionized the field of genetic epidemiology and has already resulted in an unprecedented chain of discoveries with >300 replicated associations for >70 common diseases and traits.

The genome-wide association study design

Genome-wide association studies typically comprise two (or more) stages: a discovery stage, followed by at least one replication stage.

The discovery stage entails high-density genotyping of hundreds of thousands of genetic variants, typically single nucleotide polymorphisms (SNPs), across the genome. Each SNP is tested for association with a trait or disease of interest. Studies with large sample sizes at this stage tend to be more successful, in particular for common traits with moderate heritability, as they are better powered to detect associations of small effect size. Collective efforts have led to genome-wide meta-analyses, which combine summary statistics of a series of individual genome-wide association studies in one analysis. Because individual studies often

use different genotyping platforms, meta-analyses using genotyped data only would be limited to the subset of SNPs that is common to all platforms, which is a rather inefficient use of the available data. To facilitate genome-wide meta-analyses imputation of genotypes, identical across all studies involved in the meta-analysis, is now routinely done. In brief, based on the observed haplotype structure of the genotyped SNPs and that of a reference panel (e.g. the CEU HapMap population), the genotypes of ~2.5 million untyped HapMap SNPs are inferred for each of the individuals of each study separately using one of the publicly available imputation software programs. Imputation is not perfect and this uncertainty is accounted for in the association analyses. The genome-wide meta-analysis requires only the association summary statistics of each SNP, directly genotyped and imputed, for each of the individual genome-wide association studies to calculate the overall significance of the associations.

Associations that meet the genome-wide significance threshold are taken forward for replication in subsequent independent studies to validate the initial observation. While it has been recommended to use a nominal P -value threshold of $<5.0 \times 10^{-8}$ [30], which corresponds to a 5% genome-wide type I error rate, more liberal thresholds have been used by early genome-wide association studies. Ideally, study design, ancestry of replication samples, and trait or disease of discovery and replication stages are the same or at least very similar. The sample size of the replication stage is preferably at least as large as that of the discovery stage to ensure sufficient power to overcome the so-called winner's curse (i.e. the fact that effect sizes at discovery stage may have been inflated). Only SNPs or loci for which the association observed at the discovery stage is confirmed at the replication stage are considered 'true hits'.

The discoveries

Genome-wide association studies have led to a rapid expansion in the number of loci implicated in predisposition of various polygenic diseases, including obesity. Since the introduction of the genome-wide association approach, we have witnessed three fruitful waves of discoveries based on large-scale high-density genome-wide association studies for obesity-related traits (Table 3). Most studies have tested for association with BMI as a continuous trait [31–35]. BMI is considered a good proxy-measure of adiposity in adults that is easy to obtain and available in many studies [36]. Others have examined association with risk of early-onset or adult extreme obesity [37, 38], under the assumption that morbidly obese individuals might be enriched for variants that predispose to obesity in the general population. Studies have been carried out predominantly in individuals of European ancestry, apart from one, which was based on an Indian Asian population [33].

The first wave took place in 2007 and comprised three high-density genome-wide association studies that each

Table 3

Summary of three waves of large-scale high-density genome-wide association studies for BMI and extreme obesity risk (ordered chronologically)

Study	Frayling <i>et al.</i> (Science 2007) [39]	Scuteri <i>et al.</i> (PLoS Genetics 2007) [31]	Hinney <i>et al.</i> (PLoS ONE 2007) [37]	Loos <i>et al.</i> (Nature Genetics 2008) [32]	Chambers <i>et al.</i> (Nature Genetics 2008) [33]	Willer <i>et al.</i> (Nature Genetics 2009) [34]	Thorleifsson <i>et al.</i> (Nature Genetics 2009) [35]	Meyre <i>et al.</i> (Nature Genetics 2009) [38]
Discovery stage Study design	Case-control study	Population-based study	Case-control study	Meta-analysis of 4 population-based studies and 3 disease-case series	Population-based study	Meta-analysis of 10 population-based cohorts and 5 disease-case series	Meta-analysis of 4 population-based cohorts	Case-control study
Ethnicity	White Europeans from the UK	White Europeans from Sardinia (Italy)	White Europeans from Germany	White Europeans from the UK and Switzerland	Indian Asians	Whites from the UK, USA, Sweden, Finland, Italy, Switzerland and Germany	Whites from Iceland, the USA and the Netherlands, and African-Americans	White Europeans from France
Traits	Type 2 diabetes	BMI, hip and weight	Early-onset extreme obesity	BMI	BMI, waist circumference and waist-hip ratio, weight, HOMA-IR, diastolic blood pressure, triglycerides, HDL-cholesterol, Type 2 diabetes and composite metabolic syndrome	BMI	BMI and weight	Early-onset and morbid adult obesity
n	1924 Type 2 diabetes patients; 2938 population controls	4741	487 extremely obese young individuals; 442 healthy lean controls	16 876	2684	32 387	34 416	1380 with early-onset and morbid adult obesity; 1416 age-matched normal-weight controls
SNPs tested	N/A	362 129	440 794	344 883	318 237	2399 588	305 846	308 846 SNPs
Significance level	N/A	FDR Q-value <0.20	15 most significant SNPs (corresponding to $P < 3.6 \times 10^{-6}$)	$P < 10^{-5}$	$P < 10^{-5}$	35 SNPs drawn from among the most strongly associated loci	$P < 10^{-5}$	FDR Q-value <0.20
SNPs for replication	N/A	2	11	10	23	35	43	38
Replication stage Study design	Type 2 diabetes case-series, case-series and population-based series. Adults and children	Family studies. Adults	Obesity family study (families with at least one extremely obese child or adolescent). Adults and children	Population-based studies, case-series, obesity case-control studies, family study. Adults and children	Population-based studies. Adults	Population-based studies, case-series, obesity case-control studies. Adults and children	GIANT meta-analysis (Willer <i>et al.</i>) and population-based study. Adults	Four case-control studies and 2 population-based studies
Ethnicity	White Europeans from the UK, Italy	European Americans, Hispanic Americans, and African Americans	White Europeans from Germany	White Europeans from the UK, Italy, Germany, USA, Sweden and Finland	Indian Asians and White Europeans from the UK	Whites from the UK, USA, Sweden, Finland, Italy, and the Netherlands	Whites from the UK, USA, Sweden, Finland, Italy, the Netherlands and Denmark	White Europeans from France, Germany, Finland, and Switzerland
Traits	BMI and obesity	BMI, hip and weight	Extreme obesity	BMI and obesity	BMI, waist circumference and waist-hip ratio, weight, HOMA-IR, diastolic blood pressure, triglycerides, HDL-cholesterol, Type 2 diabetes and composite metabolic syndrome	BMI and obesity	BMI and weight	Obesity and BMI
n	38 759	3205	2309	75 991	11 955	59 082	37 973	14 186
Replicated loci/genes	In FTO	In FTO	In FTO	In FTO near MC4R	Near MC4R	In FTO Near MC4R Near NEGR1 Near TMEM18 Near KCTD15 & CHST8 In SH2B1 Near GNPDA2, GUF1 and YPF7 in MITCH2	In FTO Near MC4R Near NEGR1 Near TMEM18 Near KCTD15 & CHST8 In SH2B1 In SEC16B, near RASAL2 Near SRSF10, ETV5, DGKG In BDNF and near LGR4 and LINC01, near BCDIN3D, FAIM2	In FTO Near MC4R In NPC1 Near NAF Near PTER

BMI, body mass index; SNP, single nucleotide polymorphism; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance.

confirmed fat mass and obesity associated gene (*FTO*) as the first gene incontrovertibly associated with common obesity and related traits (Tables 3 and 4). Each of the three studies examined a different trait of interest, yet they all identified the same locus, reflecting the robustness of the observation. The first study was a genome-wide association study for Type 2 diabetes [39], identifying a cluster of SNPs in the first intron of *FTO* that were highly significantly associated with Type 2 diabetes. After adjusting for BMI, the association with Type 2 diabetes was completely abolished, suggesting that the *FTO*–Type 2 diabetes association was mediated through BMI. The replication stage, including 13 cohorts with 38 759 adults and children, firmly validated *FTO* as an obesity susceptibility locus as each study confirmed association with BMI and obesity risk. Virtually at the same time, the study by Scuteri *et al.* [31] was published, which was the first large-scale high-density genome-wide association study of BMI in >4000 Sardinians. In their discovery stage, variants in the *FTO* and *PFKFB* (platelet-type phosphofructokinase) gene showed the strongest association, but only those in *FTO* replicated in 1496 European Americans and 839 Hispanic Americans. A third genome-wide association study examined the risk of early-onset extreme obesity by comparing 487 extremely obese young individuals and 442 healthy lean controls [37]. Of the 15 most significant SNPs that were taken forward for replication, six were located in the *FTO* gene, which were the only ones for which association with obesity was confirmed in 644 nuclear families with at least one obese offspring.

Despite the sample sizes of the first genome-wide association studies being relatively small, they were sufficiently powered to harvest low-hanging fruit. It was clear that for a second wave of discoveries, however, collaborative efforts would be required combining individual genome-wide association studies to increase sample size and thus power to identify common variants with small effects. The Genomic Investigation of Anthropometric Traits (GIANT) consortium is such an international collaborative initiative that brings together research groups from across Europe and the USA and that specifically focuses on anthropometric traits. In their first meta-analysis, data of seven genome-wide association scans for BMI including 16 876 individuals were combined [32]. Despite a quadrupling increase in sample size compared with first-wave studies, only *FTO* and one new locus out of 10 loci that were taken forward for replication were unequivocally confirmed (Tables 3 and 4). The newly identified locus mapped at 188 kb downstream of *MC4R*, an established biological candidate, rare coding mutations of which result in monogenic forms of obesity [7] and common variants associate with a reduced risk of obesity [11, 12]. The same locus was also identified by a genome-wide association study in 2684 Indian Asians and confirmed in 11 955 individuals of Indian Asian and European ancestry [33]. Although the effect size is the same in both ethnic groups, the frequency of the risk-allele

is higher in Indian Asians (36%) than in White Europeans (27%), which might in part explain why this locus could be identified with a relatively small sample of Indian Asians in the discovery stage.

For the third wave of discoveries, the GIANT consortium doubled its discovery stage sample size to 32 387 adults of European ancestry from 15 cohorts [34]. Of the 35 loci that were taken forward for follow-up in an independent series of 59 082 individuals, eight loci were firmly replicated. These include the previously established *FTO* and *near-MC4R* loci and six new loci, i.e. near neuronal growth regulator 1 (*NEGR1*), near transmembrane protein 18 (*TMEM18*), in SH2B adaptor protein 1 (*SH2B1*), near potassium channel tetramerization domain containing 15 (*KCTD15*), near glucosamine-6-phosphate deaminase 2 (*GNPDA2*), and in mitochondrial carrier homologue 2 (*MTCH2*). In parallel with the analyses of the GIANT consortium, deCODE genetics performed a meta-analysis of four genome-wide association studies for BMI, including 30 232 individuals of European descent and 1160 African-Americans [35]. A total of 43 SNPs in 19 chromosomal regions were taken forward for replication genotyping in 5586 Danish individuals and for confirmation in discovery stage data of the GIANT consortium. Besides the *FTO* and *near-MC4R* loci, eight additional loci reach genome-wide significance (Tables 3 and 4). Of these, four loci (near *NEGR1*, near *TMEM18*, in *SH2B1*, near *KCTD15*) had also been identified by the GIANT consortium, whereas four loci were new, i.e. in SEC16 homologue B (*SEC16B*), between ets variant gene 5 (*ETV5*) and diacylglycerol kinase (*DGKG*), in *BDNF*, and between BCDIN3 domain containing (*BCDIN3D*) and Fas apoptotic inhibitory molecule 2 (*FAIM2*). Variation in the HLA-B associated transcript 2 (*BAT2*) gene was consistently associated with weight, but not BMI, suggesting that this locus might contribute to overall size rather than adiposity. While the studies by the GIANT consortium and deCODE genetics focused on BMI as the main outcome, a third genome-wide association study examined association with the risk of early-onset and morbid adult obesity in 1380 cases and 1416 controls [38]. A total of 38 highly significant markers were taken forward for genotyping in 14 186 adults and children to test for replication with BMI and obesity risk. In addition to *FTO* and *near-MC4R*, three new markers were identified; in Niemann–Pick disease, type C1 (*NPC1*), near v-maf musculoaponeurotic fibrosarcoma oncogene homologue (*MAF*) and near phosphotriesterase related (*PTER*) (Tables 3 and 4).

For the *FTO*, *near-MC4R* and the *BDNF* loci it has been suggested that there might be two signals that each independently associate with BMI [32, 35]. However, further follow-up studies will be required to confirm this observation.

In this review, I have focused on large-scale high-density genome-wide association studies. However, other genome-wide association studies have been published for which findings were less robust. These studies were usually

Table 4

Risk-allele frequencies and effect sizes reported for the established obesity-susceptibility loci discovered through three waves of large-scale high-density genome-wide association studied for BMI and extreme obesity risk

(Nearby) gene(s)	Chromosome	Frayling <i>et al.</i> (Science 2007) [39]			Scuteri <i>et al.</i> (PLoS Genetics 2007) [31]			Loos <i>et al.</i> (Nature Genetics 2008) [32]		
		SNP (rs-number & position)	BMI-increasing allele	Effect size (per risk-allele)	SNP (rs-number & position)	BMI-increasing allele	Effect size (per risk-allele)	SNP (rs-number & position)	BMI-increasing allele	Effect size (per risk-allele)
FTO	16q12	rs9939609 (52378028)	A	CEU: 46% CHB: 15% JPT: 19% YRI: 51% 0.10 z-score or -0.40 kg m ⁻²	rs9930506 (52387966)	G	CEU: 48% CHB: 21% JPT: 23% YRI: 20% 0.13 z-score or -0.66 kg m ⁻²	rs1121980 (52366748)	A	CEU: 48% CHB: 20% JPT: 22% YRI: 47% 0.06 z-score or 0.26 kg m ⁻²
Near MC4R	18q21							rs17782313 (56002077)	C	CEU: 27% CHB: 19% JPT: 24% YRI: 31% 0.05 z-score or -0.22 kg m ⁻²
Near NEGR1	1p31									
Near TMEM18	2p25									
SH2B1, ATP2A1	16p11									
Near KCTD15 & CHST8	19q13									
Near GNPDA2, GUF1 and YPF7	4p13									
MTCH2	11p11									
In SEC16B, near RASAL2	1q25									
Near SFRS10, ETV5 and DGKG	3q27									
In BDNF and near LGR4 and LIN7C,	11p14									
Near BCDIN3D and FAIM2	12q13									
Near PTER	10p12									
Near MAF	16q23									
NPC1	18q11									

Effect sizes represent the increase in body mass index (BMI) for each additional risk allele. Effect sizes were derived from replication association studies, except for Frayling *et al.* [39], Scuteri *et al.* [31] and Loos *et al.* [32] *Additive effect size inferred from effect sizes reported for dominant model for comparison. †Absolute BMI values inferred from z-scores assuming a SD of 4.3 kg m⁻².

based on smaller samples, using genotyping platform with lower resolution, or did not include a replication stage. For example, the very first genome-wide association study for BMI, published in 2006, was performed in 694 individuals

from the Framingham Heart Study using a 100 K SNP chip [40]. A SNP (rs7566605) 10 kb upstream of the *INSIG2* was identified and replicated in four separate samples. However, results from subsequent large-scale studies have

Chambers <i>et al.</i> (Nature Genetics 2008) [33]				Willer <i>et al.</i> (Nature Genetics 2009) [34]				Thorleifsson <i>et al.</i> (Nature Genetics 2009) [35]				Meyre <i>et al.</i> (Nature Genetics 2009) [38]			
BMI-increasing allele				BMI-increasing allele				BMI-increasing allele				BMI-increasing allele			
SNP (rs-number & position)	Allele	Freq (%) (HapMap Phase III)	Effect size (per risk- allele)	SNP (rs-number & position)	Allele	Freq (%) (HapMap Phase III)	Effect size (per risk- allele)	SNP	Allele	Freq (%) (HapMap Phase III)	Effect size (per risk allele) [†]	SNP	allele	Freq (%) (HapMap Phase III)	Effect size (per risk allele)
rs12970134 (56035730)	A	CEU: 28% CHB: 18% JPT: 19% YRI: 17%	0.25 kg m ⁻²	rs9939609 (52378028)	A	CEU: 46% CHB: 15% JPT: 19% YRI: 51%	0.33 kg m ⁻²	rs8050136 (52373776)	A	CEU: 46% CHB: 14% JPT: 18% YRI: 46%	0.080 z-score or -0.35 kg/m ²	rs1421085 (52358455)	C	CEU: 46% CHB: 14% JPT: 19% YRI: 7%	0.080 z-score or -0.35 kg/m ²
				rs17782313 (56002077)	C	CEU: 27% CHB: 19% JPT: 24% YRI: 31%	0.20 kg m ⁻²	rs12970134 (56035730)	A	CEU: 28% CHB: 18% JPT: 19% YRI: 17%	0.044 z-score or -0.19 kg/m ²	rs17782313 (56002077)	C	CEU: 27% CHB: 19% JPT: 24% YRI: 31%	0.074 z-score or -0.32 kg/m ²
				rs2815752 (72585028)	A	CEU: 64% CHB: 92% JPT: 92% YRI: 53%	0.10 kg m ⁻²	rs2568958 (72537704)	A	CEU: 64% CHB: 92% JPT: 92% YRI: 53%	0.030 z-score or -0.13 kg/m ²				
				rs6548238 (624905)	C	CEU: 85% CHB: 93% JPT: 82% YRI: 90%	0.26 kg m ⁻²	rs7561317 (634953)	G	CEU: 85% CHB: 92% JPT: 88% YRI: 79%	0.043 z-score or -0.19 kg/m ²				
				rs7498665 (28790742)	G	CEU: 38% CHB: 17% JPT: 13% YRI: 21%	0.15 kg m ⁻²	rs8049439 (28745016)	C	CEU: 39% CHB: 24% JPT: 22% YRI: 41%	0.034 z-score or -0.15 kg/m ²				
				rs11084753 (39013977)	G	CEU: 69% CHB: 38% JPT: 33% YRI: 69%	0.06 kg m ⁻²	rs29941 (39001372)	C	CEU: 68% CHB: 28% JPT: 26% YRI: 86%	0.040 z-score or -0.17 kg/m ²				
				rs10938397 (44877284)	G	CEU: 45% CHB: 25% JPT: 38% YRI: 21%	0.19 kg m ⁻²								
				rs10838738 (47619625)	G	CEU: 36% CHB: 32% JPT: 35% YRI: 5%	0.07 kg m ⁻²								
								rs10913469 (176180142)	C	CEU: 25% CHB: 24% JPT: 24% YRI: 34%	0.026 z-score or -0.11 kg/m ²				
								rs7647305 (187316984)	C	CEU: 80% CHB: 93% JPT: 94% YRI: 61%	0.043 z-score or -0.19 kg/m ²				
								rs4923461 (27613486)	A	CEU: 77% CHB: 55% JPT: 63% YRI: 86%	0.043 z-score or -0.19 kg/m ²				
								rs7138803 (48533735)	A	CEU: 35% CHB: 30% JPT: 34% YRI: 17%	0.020 z-score or -0.09 kg/m ²				
												rs10508503 (16339957)	C	CEU: 91% CHB: 100% JPT: 100% YRI: 100%	0.017 z-score or -0.07 kg/m ²
												rs1424233 (78240252)	A	CEU: 56% CHB: 70% JPT: 76% YRI: 65%	0.030 z-score or -0.13 kg/m ²
												rs1805081 (19394430)	A	CEU: 53% CHB: 77% JPT: 73% YRI: 100%	0.032 z-score* or -0.14 kg/m ²

been inconclusive [41–45]. The 100 K genome-wide association study in the Framingham Heart Study was extended to a larger sample ($n = 1341$), but no replication data were available to confirm potential hits [46]. A genome-wide association study in 1000 White Americans

using a 500 K SNP chip found replicated evidence for common variants in the catenin (cadherin-associated protein), β -like 1 (*CTNBL1*) gene to be associated with BMI [47]. However, subsequent studies reported inconsistent support for this observation [48, 49] and also recent

large-scale genome-wide association meta-analyses did not validate this locus [34, 38].

Taken together, three waves of high-density multistage genome-wide association analyses have so far discovered 15 new loci consistently associated with obesity-related traits. It should be noted, however, that the exact identity of the susceptibility variants at each locus is often uncertain. Pinpointing the causal variants will be a prime task before research towards novel biology and new therapies can take place.

Implications for public health

A major challenge is the translation of this new knowledge into public health and clinical practice. The flurry of discoveries has raised hopes of the development of genetic risk profiles that, based on the obesity-susceptibility variants, would predict early in life who would be at risk to develop obesity.

Effect sizes, risk and predication Despite highly significant associations and consistent and repeated replication, each of the recently identified loci have only small effects on BMI (Table 4) and obesity risk (Figure 1). As discovery is the first aim of genome-wide association studies, significance of association and replication has been the main focus. For a better understanding of the importance of the susceptibility loci towards variation in obesity risk and BMI at the population level, effect size and prevalence of the risk-alleles need to be taken into account. Table 4 and Figure 1 summarize these key parameters for each of the 15 established obesity susceptibility loci for BMI and obesity risk, respectively. It should be noted that discoveries are often

made thanks to the 'winner's curse' effect that typically overestimates effect sizes. True effect sizes are more likely to be derived from the replication stage.

Up to now, genetic variation in the *FTO* gene still has the largest, yet small, effect on BMI in individuals of European descent. Each risk-allele increases BMI by 0.26–0.66 kg m⁻² (Table 4), which is equivalent to ~0.84–2.1 kg in body weight for a person 1.8 m tall. Accordingly, the risk of obesity increases by 1.25–1.32 odds, for each additional risk-allele (Figure 1). Although results were initially inconsistent in East Asian populations [50, 51], there is increasing evidence that effect sizes are similar to those observed for the European population [52–58]. Genetic variation in *FTO* does not seem to affect BMI in an African population [59], in African-Americans [31, 60], in South Asian Indians [61] or in Oceanic populations [62].

The effect sizes for the other susceptibility loci identified through genome-wide association studies for BMI are smaller than for *FTO*, i.e. effect sizes for BMI range between 0.06 and 0.26 kg m⁻² (Table 4) and for risk of obesity between 1.03 and 1.19 odds (Figure 1). The smallest effects were observed for the *MTCH2*, *KCTD15* and *FAIM2* loci, whereas effects were more pronounced for the near-*MC4R*, *TMEM18* and *BDNF* loci. Of interest, the three loci identified through a genome-wide association study for extreme early onset and adult obesity were not identified by studies on BMI. Their effect sizes for common obesity risk seem in general more pronounced [~1.20 to ~1.40 odds ratio (OR)] than of those discovered through genome-wide association studies of BMI. This observation suggests that differences in study design at the discovery stage might lead to the identification of a different series of suscepti-

Figure 1

Effect sizes [odds ratios (OR)] for risk of obesity reported for the established obesity-susceptibility loci discovered through three waves of large-scale high-density genome-wide association studies for body mass index (BMI) and extreme obesity risk. Effect sizes represent the increased odds of obesity for each additional risk-allele. ORs for PTER and NPC1 were inferred from the OR reported for the dominant model. Frayling *et al.* (2007) (X); Loos *et al.* (2008) (▲); Willer *et al.* (2009) (▲); Thorleifsson *et al.* (2009) (○); Meyre *et al.* (2009) (◆)

bility variants. The locus near *MC4R* has a similar effect on BMI in Indian Asians and Europeans [33]. So far, no data on effect sizes in different ethnicities of the other 13 obesity-susceptibility loci have been reported.

The prevalence of risk-alleles in the population is another important determinant of their contribution toward public health and varies between 27 and 91% for the 15 loci in Europeans (Table 4 and Figure 1). For example, the frequency of the *FTO* risk-allele is high in Europeans; 63% carry at least one risk-allele and 16% are homozygous. As such, the population attributable risk (PAR) of the *FTO* locus for obesity was estimated at ~20% [39], implying that from a population perspective, 20% of obese cases could be prevented if the negative effects of the *FTO* risk-allele were eliminated. However, it is well-recognized that the PAR does not indicate how many individuals need treatment to obtain a 20% reduction in obesity prevalence, nor does it provide clinically useful risk predictions. More valuable risk estimates for clinical practice can be derived from prospective cohort studies. A 9-year follow-up study that examined the influence of the *FTO* and near-*MC4R* loci found that each additional risk-allele increases the incidence of obesity by 24% [63]. Such information is currently lacking for the more recently identified loci.

In view of applying genetic profiles to identify high-risk individuals, data on the combined influence of all established loci will be needed. In the paper by Willer *et al.* [34], SNPs of eight obesity susceptibility loci were genotyped in 20 000 individuals of the population-based EPIC-Norfolk cohort. The average BMI of individuals carrying 13 or more risk-alleles (<2% of the population) was 1.46 kg m⁻² (or ~3.7–4.7 kg in body weight) higher compared with those carrying three or fewer risk-alleles (<2% of the population). Although this is a respectable difference due to genetic variation, it should be noted that this compares only the two extreme 1–2% of the population. Overall, the eight variants explained only a sobering 0.84% of the variance in BMI [34]. Furthermore, the area under the curve of the receiver–operating characteristic curve was only 0.548, indicating that the eight loci combined have a rather limited predictive value [34].

Thus, despite overwhelming significances and repeated replications, the explained variance and predictive value of the currently identified obesity susceptibility loci is too low to be clinically useful.

Influence in childhood and adolescence Only one genome-wide association study for the risk of early-onset extreme obesity has been reported so far, confirming *FTO* as an obesity susceptibility locus [37]. Collaborative efforts to increase the sample size and thus the power will be needed to allow examination of whether genome-wide association scans in children and adolescents identifies different loci from those identified in adults. It is thought that early-onset obesity is a genetically different trait from common adult obesity.

Loci identified through genome-wide association studies in adults have been tested for association with BMI and risk of obesity in children. For the *FTO* locus, similar effect sizes were observed in childhood that persist into adolescence [39, 60, 64, 65]. Although no association was observed with birth weight [39, 66–68], some found that already at the age of 2 weeks *FTO* SNPs were associated with increased weight and ponderal index [67]. The effect size of the near-*MC4R* locus on childhood BMI was twice as large as in adults and of similar size as the effect size observed for the *FTO* locus [32]. No associations were observed with body size at birth or during the first 3 years of life [32]. Association between the near-*MC4R* locus and childhood obesity (OR ~1.4) was confirmed in European American children and adolescents, which is more than twice the effect size for adult obesity risk, whereas no such associations were observed in African-American children [69]. Of the more recently identified loci, significant associations with BMI were observed for the *TMEM18*, *GNPDA2* and *KCTD15* loci in a population-based sample of 11-year-old children [34]. The *TMEM18* and *GNPDA2* loci as well as the *NEGR1*, *NPC1* and *PTER* loci were significantly associated with extreme childhood obesity [34, 38]. No associations with childhood BMI or obesity risk were observed for the *MAF*, *SH2B1* or *MTCH2* loci, while childhood data for other loci were not available [34, 38].

Gene–lifestyle interaction It is well-recognized that a westernized lifestyle is the major driving force behind the obesity epidemic. However, genetically susceptible individuals will gain more weight in this obesogenic environment than those who are genetically protected. Recent studies have reported on such a *FTO*–environment interaction, showing that the association between *FTO* variants and BMI is more pronounced in individuals who are sedentary, whereas the association is attenuated in those who have a physically active lifestyle [63, 66, 70]. These observations suggest that genetic susceptibility towards obesity induced by variation in *FTO* can be overcome, at least in part, by adopting a physically active lifestyle. Yet, lifestyle intervention studies in individuals with increased risk for Type 2 diabetes could not confirm interaction with *FTO* in relation to weight loss [71–73]. There are multiple factors that can explain the discrepancy between the results from cross-sectional studies and intervention trials, including study design, study population, outcome, and lifestyle factors studied. For the more recently identified obesity susceptibility loci, no interactions with lifestyle have been reported so far, apart from one study in 5807 Danish that reported absence of interaction between the near-*MC4R* locus and physical activity in relation to BMI [74].

As gene–environment interaction studies require large sample sizes [75, 76], large-scale studies, ideally with objective measurement of lifestyle, using a prospective (intervention) design, will be needed to test the hypothesis that

living a healthy lifestyle can overcome a genetic susceptibility to obesity. Meta-analysis of gene–lifestyle data is hampered by heterogeneity in measurement of lifestyle factors and outcomes. Also, publication bias might cause inaccurate results as negative interaction results often remain unpublished. Yet gene–lifestyle interaction data might hold important public health messages. They may allow identifying environments in which the currently established susceptibility loci explain a larger part of the variance in BMI and obesity risk. Furthermore, gene–lifestyle interaction studies may help remove the deterministic picture of obesity susceptibility genes, as they could show that a healthy lifestyle can overcome a genetic predisposition to become obese.

Implications for the aetiology of obesity

It is anticipated that the newly identified genetic loci will shed new light on the complex physiology governing the regulation of energy balance. This has raised expectations for the development of more effective preventive and therapeutic interventions.

New insights based on animal and humans studies have begun to accumulate, in particular on the biology of the *FTO* gene. *FTO* is a member of the nonhaeme dioxygenase superfamily that encodes a 2-oxoglutarate-dependent nucleic acid demethylase and catalyses the demethylation of 3-methylthymine in single-stranded DNA [77, 78]. Studies in rodents indicated that *Fto* mRNA expression is abundant in the brain, particularly in the hypothalamic nuclei governing energy balance, and dependent on the energy state [77, 79, 80]. Studies in humans have supported a central neuronal role for *FTO* as its risk-alleles have been associated with increased appetite and energy intake, and reduced satiety [81–85]. A recent study in mice, however, has challenged the neuronal hypothesis and has provided strong evidence for a peripheral role of *FTO* [86]. Fischer *et al.* [86] found that the loss of *Fto* in mice leads to a significant reduction in adipose tissue and lean body mass, which is a consequence of increased energy expenditure and systemic sympathetic activation. Interestingly, spontaneous locomotor activity is decreased, whereas relative food intake is increased. Thus, these experiments suggest that *FTO* might influence body composition through control of energy expenditure [86]. A peripheral role for *FTO* was also proposed by a study in healthy women showing that carriers of the risk-allele had reduced lipolytic activity, independent of BMI [87]. However, other studies in humans could not confirm association with resting or physical activity energy expenditure [65, 70, 83–85, 88]. Adding to the complexity is the observation that the *Ftm* gene, that lies in opposite orientation to *Fto*, has a similar hypothalamic expression pattern as *Fto* suggesting that the gene might be co-regulated, so that it is not completely clear yet which of these two genes (or both) functionally relevant [79].

The near-*MC4R* locus, identified in the second wave of genome-wide association studies, is located at 188 kb downstream of *MC4R*, which is an obvious candidate gene given its role in monogenic early-onset obesity. Although *MC4R* is the gene nearest to the association signal and the phenotypic associations are consistent with effects mediated through *MC4R* function, it has not yet been firmly established whether this locus indeed reflects *MC4R* function.

For most of the 13 loci discovered during the third wave of genome-wide association studies, the physiological role in relation to obesity risk is not or poorly understood [34, 35, 38]. In fact, many of these loci harbour multiple genes, sometimes located within a recombination interval with high linkage disequilibrium, which hampers pinpointing the causal variants. Yet two loci contain genes with a strong candidacy. *SH2B1* is implicated in leptin signalling and *Sh2b1*-null mice are obese [89]. Although the *SH2B1* variant with the most significant association is a nonsynonymous SNP (Thr484Ala), it is in strong linkage disequilibrium with variants in neighbouring genes [34]. Thus it remains to be confirmed whether the signal is indeed representing *SH2B1* function. Also, the *BDNF* locus is a strong prior candidate and one of the SNPs in this locus is the nonsynonymous Val66Met that has previously been examined in candidate gene studies of eating behaviour and BMI (Table 1) [20].

Of interest is that many of the currently identified obesity susceptibility loci locate near genes that are highly expressed in the brain and hypothalamus, favouring a role for the nervous system in body weight control [34, 35].

Comprehensive resequencing and fine mapping will be required to identify unambiguously the causal variant before physiologists can start exploring the functional relevance of the locus in relation to the risk of obesity, which will be key for translation into clinical practice.

Ways ahead

Despite the enormous success of genome-wide association studies, the established loci in combination explain <2% of interindividual variation in BMI. Given that the heritability of BMI is estimated at 40–70%, one should conclude that many more susceptibility loci remain to be uncovered. Various approaches have been proposed to identify more genetic loci, to pinpoint the causal variants, and to explore the physiological mechanisms and pathways that underlie the observed association [90].

One approach is to initiate a fourth wave of genome-wide association studies that further increases the sample size of the discovery stage. For example, the GIANT consortium has extended its meta-analyses, including >100 000 individuals with genome-wide association data. This will further improve power to uncover common variants with

even smaller effect sizes. Alternatively, less stringent significance levels for taking variants forward for replication could identify true loci that are currently hidden amid false-positive results.

As power to reveal new loci might vary across populations because of differences in effect sizes and allele frequencies, the study of ethnicities other than White European might provide new gene discovery opportunities. For example, Chambers *et al.* needed only a relatively small discovery sample of Indian Asians to identify the near-*MC4R* because the frequency of the risk-allele was substantially higher than in Europeans [32, 33].

So far, most studies have used BMI as a simple and inexpensive proxy-measure of adiposity, which is easy to collect in large samples. More accurate measures of adiposity might further improve power, yet these are often more expensive and harder to collect. While the power of a study will depend on the balance between sample size and accuracy of measurement, emphasis on one or the other might yield different loci. Genome-wide association studies for body fat percentage, waist circumference, extreme obesity risk, for mediating traits that underlie obesity, such as food intake and energy expenditure, might reveal new obesity-susceptibility loci that are currently hidden in studies on BMI.

Risk-allele frequencies of the newly identified obesity susceptibility loci vary between 27 and 91% (Figure 1) in European populations. It is unlikely that within this range of allele frequencies there are variants with larger effect sizes than those already observed, as one would expect that at least the most recent genome-wide association studies would have had sufficient power to identify those. However, less frequent variants with larger effects may have remained undetected, as the current genome-wide scans have only limited potential to capture rarer variants. The 1000 Genomes Project, which aims also to catalogue variants of lower frequency, might lead to new genome-wide genotyping chips that more fully capture the genetic variation in humans.

The contribution of copy number variants to the predisposition of obesity has so far been unexplored. Further advances in technology and analytical modelling will be required before analyses of copy number variants can be implemented on a larger scale. Yet the observation that the *NEGR1* locus might represent a copy number variant indicates the potential importance of this source of variation [34].

Besides aiming to identify more susceptibility loci, follow-up of the established loci in molecular and physiological studies will be key to determine the mechanisms through which the loci confer obesity. A major challenge before loci can be passed on to physiologists is pinpointing the causal variant or gene. This will require high-throughput sequencing of the region of interest in extreme cases and control or in different ethnicities. It is only when the causal

locus is identified and its modes of action are fully understood that this knowledge can be translated into mainstream healthcare and clinical practice.

Conclusions

With genome-wide association, we have entered a new era of gene discovery for common obesity. While over the past 15 years candidate gene studies have identified a handful of genetic variants convincingly associated with obesity-related traits, genome-wide association studies have changed the pace of discoveries with the discovery of at least 15 loci in less than 3 years.

Recent progress in obesity genetics has already provided valuable new insights into pathophysiological mechanisms and pathways that underlie obesity development. Although these advances have raised hopes for genetic risk profiling and therapeutic intervention, the implementation of such approaches in mainstream healthcare remains distant, as we stand to learn much more about the causal variants and their functional implications.

Expectations are high but many challenges remain. Among the latter, translating new advances on the genetic predisposition to obesity into useful guidelines for prevention and treatment will be the most demanding.

Competing interests

None to declare.

REFERENCES

- 1 World Health Organisation. Obesity and overweight – Fact sheet N°311. Geneva: World Health Organisation, 2006.
- 2 National Center for Health Statistics. Health, United States, 2008 – with Chartbook on Trends in the Health of Americans. Bethesda, MD: Centers for Disease Control and Prevention, 2008.
- 3 International Association for the Study of Obesity. 2009. Available at <http://www.iaotf.org/> (last accessed March 2009).
- 4 Maes HH, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human obesity. *Behav Genet* 1997; 27: 325–51.
- 5 Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, Perusse L, Bouchard C. The human obesity gene map: the 2005 update. *Obes Res* 2006; 14: 529–644.
- 6 Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F. Targeted

- disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 1997; 88: 131–41.
- 7** Farooqi IS, Keogh JM, Yeo GSH, Lank EJ, Cheetham T, O'Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med* 2003; 348: 1085–95.
- 8** Xiang Z, Litherland SA, Sorensen NB, Proneth B, Wood MS, Shaw AM, Millard WJ, Haskell-Luevano C. Pharmacological characterization of 40 human melanocortin-4 receptor polymorphisms with the endogenous proopiomelanocortin-derived agonists and the agouti-related protein (AGRP) antagonist. *Biochemistry* 2006; 45: 7277–88.
- 9** Heid IM, Vollmert C, Hinney A, Doring A, Geller F, Lowel H, Wichmann HE, Illig T, Hebebrand J, Kronenberg F, the KORA Group. Association of the 103I MC4R allele with decreased body mass in 7937 participants of two population based surveys. *J Med Genet* 2005; 42: e21.
- 10** Geller F, Reichwald K, Dempfle A, Illig T, Vollmert C, Herpertz S, Siffert W, Platzer M, Hess C, Gudermann T, Biebermann H, Wichmann HE, Schafer H, Hinney A, Hebebrand J. Melanocortin 4 receptor gene variant i103 is negatively associated with obesity. *Am J Hum Genet* 2004; 74: 572–81.
- 11** Young EH, Wareham NJ, Farooqi S, Hinney A, Hebebrand J, Scherag A, O'Rahilly S, Barroso I, Sandhu MS. The V103I polymorphism of the MC4R gene and obesity: population based studies and meta-analysis of 29,563 individuals. *Int J Obes* 2007; 31: 1437–41.
- 12** Stutzmann F, Vatin V, Cauchi S, Morandi A, Jouret B, Landt O, Tounian P, Levy-Marchal C, Buzzetti R, Pinelli L, Balkau B, Horber F, Bougneres P, Froguel P, Meyre D. Non-synonymous polymorphisms in melanocortin-4 receptor protect against obesity: the two facets of a Janus obesity gene. *Hum Mol Genet* 2007; 16: 1837–44.
- 13** Jackson RS, Creemers JWM, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, Hutton JC, O'Rahilly S. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet* 1997; 16: 303–6.
- 14** Farooqi IS, Volders K, Stanhope R, Heuschkel R, White A, Lank E, Keogh J, O'Rahilly S, Creemers JWM. Hyperphagia and early-onset obesity due to a novel homozygous missense mutation in prohormone convertase 1/3. *J Clin Endocrinol Metab* 2007; 92: 3369–73.
- 15** Jackson RS, Creemers JW, Farooqi IS, Raffin-Sanson ML, Varro A, Dockray GJ, Holst JJ, Brubaker PL, Corvol P, Polonsky KS, Ostrega D, Becker KL, Bertagna X, Hutton JC, White A, Dattani MT, Hussain K, Middleton SJ, Nicole TM, Milla PJ, Lindley KJ, O'Rahilly S. Small-intestinal dysfunction accompanies the complex endocrinopathy of human proprotein convertase 1 deficiency. *J Clin Invest* 2003; 112: 1550–60.
- 16** Benzinou M, Creemers JW, Choquet H, Lobbens S, Dina C, Durand E, Guerardel A, Boutin P, Jouret B, Heude B, Balkau B, Tichet J, Marre M, Potoczna N, Horber F, Le Stunff C, Czernichow S, Sandbaek A, Lauritzen T, Borch-Johnsen K, Andersen G, Kiess W, Korner A, Kovacs P, Jacobson P, Carlsson LM, Walley AJ, Jorgensen T, Hansen T, Pedersen O, Meyre D, Froguel P. Common nonsynonymous variants in PCSK1 confer risk of obesity. *Nat Genet* 2008; 40: 943–5.
- 17** Kernie SG, Liebl DJ, Parada LF. BDNF regulates eating behavior and locomotor activity in mice. *EMBO J* 2000; 19: 1290–300.
- 18** Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, Lechan RM, Jaenisch R. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol Endocrinol* 2001; 15: 1748–57.
- 19** Gray J, Yeo GSH, Cox JJ, Morton J, Adlam AL, Keogh JM, Yanovski JA, El Gharbawy A, Han JC, Tung YCL, Hodges JR, Raymond FL, O'Rahilly S, Farooqi IS. Hyperphagia, severe obesity impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. *Diabetes* 2006; 55: 3366–71.
- 20** Shugart YY, Chen L, Day INM, Lewis SJ, Timpson NJ, Yuan W, Abdollahi MR, Ring SM, Ebrahim S, Golding J, Lawlor DA, Davey-Smith G. Two British women studies replicated the association between the Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) and BMI. *Eur J Hum Genet* 2009; 17: 1050–5.
- 21** Clement K, Vaisse C, Manning BS, Basdevant A, Guy-Grand B, Ruiz J, Silver KD, Shuldiner AR, Froguel P, Strosberg AD. Genetic variation in the β 3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med* 1995; 333: 352–4.
- 22** Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC. Association of a polymorphism in the {beta}3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. *N Engl J Med* 1995; 333: 348–52.
- 23** Walston J, Silver K, Bogardus C, Knowler WC, Celi FS, Austin S, Manning B, Strosberg AD, Stern MP, Raben N, Sorkin JD, Roth J, Shuldiner AR. Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the {beta}3-adrenergic-receptor gene. *N Engl J Med* 1995; 333: 343–7.
- 24** Kurokawa N, Young EH, Oka Y, Satoh H, Wareham NJ, Sandhu MS, Loos RJ. The ADRB3 Trp64Arg variant and BMI: a meta-analysis of 44 833 individuals. *Int J Obes (Lond)* 2008; 32: 1240–9.
- 25** Lyon HN, Florez JC, Bersaglieri T, Saxena R, Winckler W, Almgren P, Lindblad U, Tuomi T, Gaudet D, Zhu X, Cooper R, Ardlie KG, Daly MJ, Altshuler D, Groop L, Hirschhorn JN. Common variants in the ENPP1 gene are not reproducibly associated with diabetes or obesity. *Diabetes* 2006; 55: 3180–4.
- 26** Weedon MN, Shields B, Hitman G, Walker M, McCarthy MI, Hattersley AT, Frayling TM. No evidence of association of ENPP1 variants with type 2 diabetes or obesity in a study of 8,089 U.K. Caucasians. *Diabetes* 2006; 55: 3175–9.

- 27 Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoœur C, Vatin V, Ghoussaini M, Wachter C, Hercberg S, Charpentier G, Patsch W, Pattou F, Charles MA, Tounian P, Clement K, Jouret B, Weill J, Maddux BA, Goldfine ID, Walley A, Boutin P, Dina C, Froguel P. Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. *Nat Genet* 2005; 37: 863–7.
- 28 Grarup N, Urhammer SA, Ek J, Albrechtsen A, Glumer C, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O. Studies of the relationship between the ENPP1 K121Q polymorphism and type 2 diabetes, insulin resistance and obesity in 7,333 Danish white subjects. *Diabetologia* 2006; 49: 2097–104.
- 29 Saunders CL, Chiodini BD, Sham P, Lewis CM, Abkevich V, Adeyemo AA, de Andrade M, Arya R, Berenson GS, Blangero J, Boehnke M, Borecki IB, Chagnon YC, Chen W, Comuzzie AG, Deng HW, Duggirala R, Feitosa MF, Froguel P, Hanson RL, Hebebrand J, Huezo-Dias P, Kissebah AH, Li W, Luke A, Martin LJ, Nash M, Ohman M, Palmer LJ, Peltonen L, Perola M, Price RA, Redline S, Srinivasan SR, Stern MP, Stone S, Stringham H, Turner S, Wijmenga C, Collier D. Meta-analysis of genome-wide linkage studies in BMI and obesity. *Obesity (Silver Spring)* 2007; 15: 2263–75.
- 30 de Bakker PIW, Ferreira MAR, Jia X, Neale BM, Raychaudhuri S, Voight BF. Practical aspects of imputation-driven meta-analysis of genome-wide association studies. *Hum Mol Genet* 2008; 17: R122–8.
- 31 Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orru M, Usala G, Dei M, Lai S, Maschio A, Busonero F, Mulas A, Ehret GB, Fink AA, Weder AB, Cooper RS, Galan P, Chakravarti A, Schlessinger D, Cao A, Lakatta E, Abecasis GR. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* 2007; 3: e115.
- 32 Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, Inouye M, Freathy RM, Abecasis GR, Albai G, Attwood AP, Beckmann JS, Berndt SI, Boehnke M, Chanock SJ, Hayes RB, Bergmann S, Bennett AJ, Bingham SA, Bochud M, Brown M, Cauchi S, Chanock SJ, Collins FS, Connell JM, Cooper C, Smith GD, Day I, Dina C, De S, Dermizakis ET, Doney AS, Elliott KS, Elliott P, Evans DM, Sadaf FI, Froguel P, Ghorri J, Gieger C, Groop L, Groves CJ, Gwilliam R, Hadley D, Hall AS, Hankinson SE, Hattersley AT, Hayes RB, Hebebrand J, Heid IM, Illig T, Lamina C, Gieger C, Illig T, Kraft P, Meitinger T, Wichmann HE, Herrera B, Hinney A, Hu FB, Hunt SE, Hunter DJ, Jackson AU, Jacobs KB, Jarvelin MR, Johnson T, Jolley JD, Karpe F, Keniry A, Khaw KT, Lamina C, Luben RN, Lyon HN, Mangino M, Marchini J, McArdle WL, McGinnis R, Meitinger T, Meyre D, Munroe PB, Mohlke KL, Morris AD, Nagaraja R, Ness AR, Neville MJ, Nica AC, Ong KK, O'Rahilly S, Owen KR, Palmer CN, Papadakis K, Potter S, Pouta A, Qi L, Randall JC, Rayner NW, Ridderstrale M, Ring SM, Sandhu MS, Sanna S, Scherag A, Scheet P, Sims MA, Schlessinger D, Song K, Soranzo N, Speliotes EK, Syddall HE, Teichmann SA, Timpson NJ, Tobias JH, Tuomilehto J, Uda M, Vogel CI, Voight BF, Wallace C, Waterworth DM, Weedon MN, Wichmann HE, Willer CJ, Wraight VL, Yuan X, Zeggini E, Hirschhorn JN, Strachan DP, Ouwehand WH, Caulfield MJ, Samani NJ, Frayling TM, Vollenweider P, Waeber G, Mooser V, Deloukas P, McCarthy MI, Wareham NJ, Barroso I. Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nat Genet* 2008; 40: 768–75.
- 33 Chambers JC, Elliott P, Zabaneh D, Zhang W, Li Y, Froguel P, Balding D, Scott J, Kooner JS. Common genetic variation near MC4R is associated with waist circumference and insulin resistance. *Nat Genet* 2008; 40: 716–8.
- 34 Willer CJ, Speliotes EK, Loos RJF, Li S, Lindgren CM, Heid IM, Berndt SI. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 2009; 41: 25–34.
- 35 Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadóttir A, Styrkarsdóttir U, Gretarsdóttir S, Thorlacius S, Jonsdóttir I, Jonsdóttir T, Olafsdóttir EJ, Olafsdóttir GH, Jonsson T, Jonsson F, Borch-Johnsen K, Hansen T, Andersen G, Jorgensen T, Lauritzen T, Aben KK, Verbeek AL, Roeleveld N, Kampman E, Yanek LR, Becker LC, Tryggvadóttir L, Rafnar T, Becker DM, Gulcher J, Kiemeneý LA, Pedersen O, Kong A, Thorsteinsdóttir U, Stefansson K. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 2009; 41: 18–24.
- 36 Bouchard C. BMI, fat mass, abdominal adiposity and visceral fat: where is the 'beef'? *Int J Obes* 2007; 31: 1552–3.
- 37 Hinney A, Nguyen TT, Scherag A, Friedel S, Bronner G, Muller TD, Grallert H, Illig T, Wichmann HE, Rief W, Schafer H, Hebebrand J. Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants. *PLoS ONE* 2007; 2: e1361.
- 38 Meyre D, Delplanque J, Chevre JC, Lecoœur C, Lobben S, Gallina S, Durand E, Vatin V, Degraeve F, Proenca C, Gaget S, Korner A, Kovacs P, Kiess W, Tichet J, Marre M, Hartikainen AL, Horber F, Potoczna N, Hercberg S, Levy-Marchal C, Pattou F, Heude B, Tauber M, McCarthy MI, Blakemore AIF, Montpetit A, Polychronakos C, Weill J, Coin LJM, Asher J, Elliott P, Jarvelin MR, Visvikis-Siest S, Balkau B, Sladek R, Balding D, Walley A, Dina C, Froguel P. Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat Genet* 2009; 41: 157–9.
- 39 Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007; 316: 889–94.
- 40 Herbert A, Gerry NP, McQueen MB, Heid IM, Pfeuffer A, Illig T, Wichmann HE, Meitinger T, Hunter D, Hu FB, Colditz G, Hinney A, Hebebrand J, Koberwitz K, Zhu X, Cooper R, Ardlie K, Lyon H, Hirschhorn JN, Laird NM, Lenburg ME, Lange C, Christman MF. A common genetic variant is associated with adult and childhood obesity. *Science* 2006; 312: 279–83.

- 41 Loos RJF, Barroso I, O'Rahilly S, Wareham NJ. Comment on 'A common genetic variant is associated with adult and childhood obesity'. *Science* 2007; 315: 187c.
- 42 Dina C, Meyre D, Samson C, Tichet J, Marre M, Jouret B, Charles MA, Balkau B, Froguel P. Comment on 'A common genetic variant is associated with adult and childhood obesity'. *Science* 2007; 315: 187b.
- 43 Roszkopf D, Bornhorst A, Rimbach C, Schwahn C, Kayser A, Kruger A, Tessmann G, Geissler I, Kroemer HK, Volzke H. Comment on 'A common genetic variant is associated with adult and childhood obesity'. *Science* 2007; 315: 187d.
- 44 Lyon HN, Emilsson V, Hinney A, Heid IM, Lasky-Su J, Zhu X, Thorleifsson G, Gunnarsdottir S, Walters GB, Thorsteinsdottir U, Kong A, Gulcher J, Nguyen TT, Scherag A, Pfeufer A, Meitinger T, Brönner G, Rief W, Soto-Quiros ME, Avila L, Klanderma B, Raby BA, Silverman EK, Weiss ST, Laird N, Ding X, Groop L, Tuomi T, Isomaa B, Bengtsson K, Butler JL, Cooper RS, Fox CS, Donnell CJ, Vollmert C, Juan C, Wichmann HE, Hebebrand J, Stefansson K, Lange C, Hirschhorn JN. The association of a SNP upstream of INSIG2 with body mass index is reproduced in several but not all cohorts. *PLoS Genet* 2007; 3: e61.
- 45 Andreasen CH, Mogensen MS, Borch-Johnsen K, Sandbak A, Lauritzen T, Sorensen TIA, Hansen L, Almind K, Jørgensen T, Pedersen O, Hansen T. Non-replication of genome-wide based associations between common variants in INSIG2 and PFKF and obesity in studies of 18,014 Danes. *PLoS ONE* 2008; 3: e2872.
- 46 Fox C, Heard-Costa N, Cupples LA, Dupuis J, Vasan R, Atwood L. Genome-wide association to body mass index and waist circumference: the Framingham Heart Study 100 K project. *BMC Med Genet* 2007; 8: S18.
- 47 Liu YJ, Liu XG, Wang L, Dina C, Yan H, Liu JF, Levy S, Papasian CJ, Drees BM, Hamilton JJ, Meyre D, Delplanque J, Pei YF, Zhang L, Recker RR, Froguel P, Deng HW. Genome-wide association scans identified CTNBL1 as a novel gene for obesity. *Hum Mol Genet* 2008; 17: 1803–13.
- 48 Andreasen CH, Mogensen MS, Borch-Johnsen K, Sandbaek A, Lauritzen T, Almind K, Hansen L, Jørgensen T, Pedersen O, Hansen T. Studies of CTNBL1 and FDF1 variants and measures of obesity: analyses of quantitative traits and case-control studies in 18,014 Danes. *BMC Med Genet* 2009; 10: 17.
- 49 Vogel C, Greene B, Scherag A, Muller T, Friedel S, Grallert H, Heid I, Illig T, Wichmann HE, Schafer H, Hebebrand J, Hinney A. Non-replication of an association of CTNBL1 polymorphisms and obesity in a population of Central European ancestry. *BMC Med Genet* 2009; 10: 14.
- 50 Li H, Wu Y, Loos RJF, Hu FB, Liu Y, Wang J, Yu Z, Lin X. Variants in FTO gene are not associated with obesity in a Chinese Han population. *Diabetes* 2008; 57: 264–8.
- 51 Horikoshi M, Hara K, Ito C, Shojima N, Nagai R, Ueki K, Froguel P, Kadowaki T. Variations in the HHEX gene are associated with increased risk of type 2 diabetes in the Japanese population. *Diabetologia* 2007; 50: 2461–6.
- 52 Hotta K, Nakata Y, Matsuo T, Kamohara S, Kotani K, Komatsu R, Itoh N, Mineo I, Wada J, Masuzaki H, Yoneda M, Nakajima A, Miyazaki S, Tokunaga K, Kawamoto M, Funahashi T, Hamaguchi K, Yamada K, Hanafusa T, Oikawa S, Yoshimatsu H, Nakao K, Sakata T, Matsuzawa Y, Tanaka K, Kamatani N, Nakamura Y. Variations in the FTO gene are associated with severe obesity in the Japanese. *J Hum Genet* 2008; 53: 546–53.
- 53 Cha SW, Choi SM, Kim KS, Park BL, Kim JR, Kim JY, Shin HD. Replication of genetic effects of FTO polymorphisms on BMI in a Korean population. *Obesity (Silver Spring)* 2008; 16: 2187–9.
- 54 Chang YC, Liu PH, Lee WJ, Chang TJ, Jiang YD, Li HY, Kuo SS, Lee KC, Chuang LM. Common variation in the fat mass and obesity-associated (FTO) gene confers risk of obesity and modulates BMI in the Chinese population. *Diabetes* 2008; 57: 2245–52.
- 55 Tan JT, Dorajoo R, Seielstad M, Sim XL, Ong RT, Chia KS, Wong TY, Saw SM, Chew SK, Aung T, Tai ES. FTO variants are associated with obesity in the Chinese and Malay populations in Singapore. *Diabetes* 2008; 57: 2851–7.
- 56 Omori S, Tanaka Y, Takahashi A, Hirose H, Kashiwagi A, Kaku K, Kawamori R, Nakamura Y, Maeda S. Association of CDKAL1, IGF2BP2, CDKN2A/B, HHEX, SLC30A8, and KCNJ11 with susceptibility to type 2 diabetes in a Japanese population. *Diabetes* 2008; 57: 791–5.
- 57 Ng MC, Park KS, Oh B, Tam CH, Cho YM, Shin HD, Lam VK, Ma RC, So WY, Cho YS, Kim HL, Lee HK, Chan JC, Cho NH. Implication of genetic variants near TCF7L2, SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2, and FTO in type 2 diabetes and obesity in 6,719 Asians. *Diabetes* 2008; 57: 2226–33.
- 58 Al-Attar SA, Pollex RL, Ban MR, Young TK, Bjerregaard P, Anand SS, Yusuf S, Zinman B, Harris SB, Hanley AJ, Connelly PW, Huff MW, Hegele RA. Association between the FTO rs9939609 polymorphism and the metabolic syndrome in a non-Caucasian multi-ethnic sample. *Cardiovasc Diabetol* 2008; 7: 5.
- 59 Hennig B, Fulford A, Sirugo G, Rayco-Solon P, Hattersley A, Frayling T, Prentice A. FTO gene variation and measures of body mass in an African population. *BMC Med Genet* 2009; 10: 21.
- 60 Grant SFA, Li M, Bradfield JP, Kim CE, Annaiah K, Santa E, Glessner JT, Casalunovo T, Frackelton EC, Otieno FG, Shaner JL, Smith RM, Imielinski M, Eckert AW, Chiavacci RM, Berkowitz RI, Hakonarson H. Association analysis of the FTO gene with obesity in children of Caucasian and African ancestry reveals a common tagging SNP. *PLoS ONE* 2008; 3: e1746.
- 61 Yajnik C, Janipalli C, Bhaskar S, Kulkarni S, Freathy R, Prakash S, Mani K, Weedon M, Kale S, Deshpande J, Krishnaveni G, Veena S, Fall C, McCarthy M, Frayling T, Hattersley A, Chandak G. FTO gene variants are strongly associated with type 2 diabetes in South Asian Indians. *Diabetologia* 2009; 52: 247–52.
- 62 Ohashi J, Naka I, Kimura R, Natsuhara K, Yamauchi T, Furusawa T, Nakazawa M, Ataka Y, Patarapotikul J,

- Nuchnoi P, Tokunaga K, Ishida T, Inaoka T, Matsumura Y, Ohtsuka R. FTO polymorphisms in oceanic populations. *J Hum Genet* 2007; 52: 1031–5.
- 63** Cauchi S, Stutzmann F, Cavalcanti-Proena C, Durand E, Pouta A, Hartikainen AL, Marre M, Vol S, Tammelin T, Laitinen J, Gonzalez-Izquierdo A, Blakemore A, Elliott P, Meyre D, Balkau B, Jarvelin MR, Froguel P. Combined effects of MC4R and FTO common genetic variants on obesity in European general populations. *J Mol Med* 2009; 87: 537–46.
- 64** Haworth CMA, Carnell S, Meaburn EL, Davis OSP, Plomin R, Wardle J. Increasing heritability of BMI and stronger associations with the FTO gene over childhood. *Obes Res* 2008; 16: 2663–8.
- 65** Hakanen M, Raitakari OT, Lehtimäki T, Peltonen N, Pakkala K, Sillanmäki L, Lagstrom H, Viikari J, Simell O, Ronnemaa T. FTO genotype is associated with body mass index after the age of 7 years but not with energy intake or leisure-time physical activity. *J Clin Endocrinol Metab* 2009; 94: 1281–7.
- 66** Andreasen CH, Stender-Petersen KL, Mogensen MS, Torekov SS, Wegner L, Andersen G, Nielsen AL, Albrechtsen A, Borch-Johnsen K, Rasmussen SS, Clausen JO, Sandbaek A, Lauritzen T, Hansen L, Jorgensen T, Pedersen O, Hansen T. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes* 2008; 57: 264–8.
- 67** Lopez-Bermejo A, Petry CJ, Diaz M, Sebastiani G, de Zegher F, Dunger DB, Ibanez L. The association between the FTO gene and fat mass in humans develops by the postnatal age of two weeks. *J Clin Endocrinol Metab* 2008; 93: 1501–5.
- 68** Jess T, Zimmermann E, Kring SII, Berentzen T, Holst C, Toubro S, Astrup A, Hansen T, Pedersen O, Sorensen TIA. Impact on weight dynamics and general growth of the common FTO rs9939609: a longitudinal Danish cohort study. *Int J Obes* 2008; 32: 1388–94.
- 69** Grant SFA, Bradfield JP, Zhang H, Wang K, Kim CE, Annaiah K, Santa E, Glessner JT, Thomas K, Garriss M, Frackelton EC, Otieno FG, Shaner JL, Smith RM, Imielinski M, Chiavacci RM, Li M, Berkowitz RI, Hakonarson H. Investigation of the locus near MC4R with childhood obesity in Americans of European and African ancestry. *Obesity (Silver Spring)* 2009; 17: 1461–5.
- 70** Rampersaud E, Mitchell BD, Pollin TI, Fu M, Shen H, O'Connell JR, Ducharme JL, Hines S, Sack P, Naglieri R, Shuldiner AR, Snitker S. Physical activity and the association of common FTO gene variants with body mass index and obesity. *Arch Intern Med* 2008; 168: 1791–7.
- 71** Haupt A, Thamer C, Machann J, Kirchhoff K, Stefan N, Tschritter O, Machicao F, Schick F, Haring HU, Fritsche A. Impact of variation in the FTO gene on whole body fat distribution, ectopic fat, and weight loss. *Obes Res* 2008; 16: 1969–72.
- 72** Franks PW, Jablonski K, Delahanty L, McAteer J, Kahn S, Knowler W, Florez J, for the Diabetes Prevention Program Research Group. Assessing gene-treatment interactions at the FTO and INSIG2 loci on obesity-related traits in the Diabetes Prevention Program. *Diabetologia* 2008; 51: 2214–23.
- 73** Lappalainen TJ, Tolppanen AM, Kolehmainen M, Schwab U, Lindstrom J, Tuomilehto J, Pulkkinen L, Eriksson JG, Laakso M, Gylling H, Uusitupa M. The common variant in the FTO gene did not modify the effect of lifestyle changes on body weight: the Finnish Diabetes Prevention Study. *Obes Res* 2009; 17: 832–6.
- 74** Zobel DP, Andreasen CH, Grarup N, Eiberg H, Sorensen TIA, Sandbaek A, Lauritzen T, Borch-Johnsen K, Jorgensen T, Pedersen O, Hansen T. Variants near MC4R are associated with obesity and influence obesity-related quantitative traits in a population of middle-aged people: studies of 14,940 Danes. *Diabetes* 2009; 58: 757–64.
- 75** Luan JA, Wong MY, Day NE, Wareham NJ. Sample size determination for studies of gene–environment interaction. *Int J Epidemiol* 2001; 30: 1035–40.
- 76** Wong MY, Day NE, Luan JA, Chan KP, Wareham NJ. The detection of gene–environment interaction for continuous traits: should we deal with measurement error by bigger studies or better measurement? *Int J Epidemiol* 2003; 32: 51–7.
- 77** Gerken T, Girard CA, Tung YCL, Webby CJ, Saudek V, Hewitson KS, Yeo GSH, McDonough MA, Cunliffe S, McNeill LA, Galvanovskis J, Rorsman P, Robins P, Prieur X, Coll AP, Ma M, Jovanovic Z, Farooqi IS, Sedgwick B, Barroso I, Lindahl T, Ponting CP, Ashcroft FM, O'Rahilly S, Schofield CJ. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science* 2007; 318: 1469–72.
- 78** Sanchez-Pulido L, Andrade-Navarro MA. The FTO (fat mass and obesity associated) gene codes for a novel member of the non-heme dioxygenase superfamily. *BMC Biochem* 2007; 8: 23.
- 79** Stratigopoulos G, Padilla S, LeDuc CA, Watson E, Hattersley AT, McCarthy MI, Zeltser LM, Chung WK, Leibel RL. Regulation of Fto/Ftm gene expression in mice and humans. *Am J Physiol Regul Integr Comp Physiol* 2008; 294: R1185–96.
- 80** Fredriksson R, Hagglund M, Olszewski PK, Stephansson O, Jacobsson JA, Olszewska AM, Levine AS, Lindblom J, Schiöth HB. The obesity gene, FTO, is of ancient origin, upregulated during food deprivation and expressed in neurons of feeding-related nuclei of the brain. *Endocrinology* 2008; 149: 2062–71.
- 81** Wardle J, Carnell S, Haworth CMA, Farooqi IS, O'Rahilly S, Plomin R. Obesity associated genetic variation in FTO is associated with diminished satiety. *J Clin Endocrinol Metab* 2008; 93: 3640–3.
- 82** Timpson NJ, Emmett PM, Frayling TM, Rogers I, Hattersley AT, McCarthy MI, Davey Smith G. The fat mass- and obesity-associated locus and dietary intake in children. *Am J Clin Nutr* 2008; 88: 971–8.
- 83** Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CNA. An obesity-associated FTO gene variant and increased energy intake in children. *N Engl J Med* 2008; 359: 2558–66.
- 84** Wardle J, Llewellyn C, Sanderson S, Plomin R. The FTO gene and measured food intake in children. *Int J Obes* 2008; 33: 42–5.

- 85** Haupt A, Thamer C, Staiger H, Tschritter O, Kirchhoff K, Machicao F, Haring HU, Stefan N, Fritsche A. Variation in the FTO gene influences food intake but not energy expenditure. *Exp Clin Endocrinol Diabetes* 2009; 117: 194–7.
- 86** Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Bruning JC, Ruther U. Inactivation of the Fto gene protects from obesity. *Nature* 2009; 458 (7240): 894–8.
- 87** Wahlen K, Sjolín E, Hoffstedt J. The common rs9939609 gene variant of the fat mass and obesity associated gene (FTO) is related to fat cell lipolysis. *J Lipid Res* 2007; 49: 607–11.
- 88** Berentzen T, Kring SII, Holst C, Zimmermann E, Jess T, Hansen T, Pedersen O, Toubro S, Astrup A, Sorensen TIA. Lack of association of fatness-related FTO gene variants with energy expenditure or physical activity. *J Clin Endocrinol Metab* 2008; 93: 2904–8.
- 89** Ren D, Zhou Y, Morris D, Li M, Li Z, Rui L. Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. *J Clin Invest* 2007; 117: 397–406.
- 90** McCarthy MI, Hirschhorn JN. Genome-wide association studies: potential next steps on a genetic journey. *Hum Mol Genet* 2008; 17: R156–65.
- 91** Gjesing AP, Andersen G, Albrechtsen A, Glumer C, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O. Studies of associations between the Arg389Gly polymorphism of the beta1-adrenergic receptor gene (ADRB1) and hypertension and obesity in 7677 Danish white subjects. *Diabet Med* 2007; 24: 392–7.
- 92** Gjesing AP, Andersen G, Burgdorf KS, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O. Studies of the associations between functional beta2-adrenergic receptor variants and obesity, hypertension and type 2 diabetes in 7,808 white subjects. *Diabetologia* 2007; 50: 563–8.
- 93** Gjesing AP, Andersen G, Borch-Johnsen K, Jørgensen T, Hansen T, Pedersen O. Association of the [beta]3-adrenergic receptor Trp64Arg polymorphism with common metabolic traits: studies of 7605 middle-aged white people. *Mol Genet Metab* 2008; 94: 90–7.
- 94** Benzinou M, Chevre JC, Ward KJ, Lecoœur C, Dina C, Lobbens S, Durand E, Delplanque J, Horber FF, Heude B, Balkau B, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O, Meyre D, Froguel P. Endocannabinoid receptor 1 gene variations increase risk for obesity and modulate body mass index in European populations. *Hum Mol Genet* 2008; 17: 1916–21.
- 95** Grarup N, Andersen MK, Andreasen CH, Albrechtsen A, Borch-Johnsen K, Jorgensen T, Auwerx J, Schmitz O, Hansen T, Pedersen O. Studies of the common DIO2 Thr92Ala polymorphism and metabolic phenotypes in 7342 Danish White Subjects. *J Clin Endocrinol Metab* 2007; 92: 363–6.
- 96** Meyre D, Bouatia-Naji N, Vatin V, Veslot J, Samson C, Tichet J, Marre M, Balkau B, Froguel P. ENPP1 K121Q polymorphism and obesity, hyperglycaemia and type 2 diabetes in the prospective DESIR Study. *Diabetologia* 2007; 50: 2090–6.
- 97** Larsen LH, Rose CS, Sparso T, Overgaard J, Torekov SS, Grarup N, Jensen DP, Albrechtsen A, Andersen G, Ek J, Glumer C, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O. Genetic analysis of the estrogen-related receptor [alpha] and studies of association with obesity and type 2 diabetes. *Int J Obes* 2006; 31: 365–70.
- 98** Jensen D, Andreasen C, Andersen M, Hansen L, Eiberg H, Borch-Johnsen K, Jørgensen T, Hansen T, Pedersen O. The functional Pro129Thr variant of the FAAH gene is not associated with various fat accumulation phenotypes in a population-based cohort of 5,801 whites. *J Mol Med* 2007; 85: 445–9.
- 99** Heude B, Ong KK, Luben R, Wareham NJ, Sandhu MS. Study of association between common variation in the insulin-like growth factor 2 gene and indices of obesity and body size in middle-aged men and women. *J Clin Endocrinol Metab* 2007; 92: 2734–8.
- 100** Zobel D, Andreasen C, Burgdorf K, Andersson E, Sandbaek A, Lauritzen T, Borch-Johnsen K, Jorgensen T, Maeda S, Nakamura Y, Eiberg H, Pedersen O, Hansen T. Variation in the gene encoding Kruppel-like factor 7 influences body fat: studies of 14,818 Danes. *Eur J Endocrinol* 2009; 160: 603–9.
- 101** Hamid YH, Urhammer SA, Glümer C, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O. The common T60N polymorphism of the lymphotoxin-alpha gene is associated with type 2 diabetes and other phenotypes of the metabolic syndrome. *Diabetologia* 2005; 48: 445–51.
- 102** Andreasen C, Mogensen M, Borch-Johnsen K, Sandbaek A, Lauritzen T, Almind K, Hansen L, Jorgensen T, Pedersen O, Hansen T. Lack of association between PKLR rs3020781 and NOS1AP rs7538490 and type 2 diabetes, overweight, obesity and related metabolic phenotypes in a Danish large-scale study: case-control studies and analyses of quantitative traits. *BMC Med Genet* 2008; 9: 118.
- 103** Torekov S, Larsen L, Andersen G, Albrechtsen A, Glumer C, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O. Variants in the 5' region of the neuropeptide Y receptor Y2 gene (NPY2R) are associated with obesity in 5,971 white subjects. *Diabetologia* 2006; 49: 2653–8.
- 104** Campbell CD, Lyon HN, Nemesh J, Drake JA, Tuomi T, Gaudet D, Zhu X, Cooper RS, Ardlie KG, Groop LC, Hirschhorn JN. Association studies of BMI and type 2 diabetes in the neuropeptide Y pathway: a possible role for NPY2R as a candidate gene for type 2 diabetes in men. *Diabetes* 2007; 56: 1460–7.
- 105** Sparso T, Hussain MS, Andersen G, Hainerova I, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O. Relationships between the functional PPAR[alpha] Leu162Val polymorphism and obesity, type 2 diabetes, dyslipidaemia, and related quantitative traits in studies of 5799 middle-aged white people. *Mol Genet Metab* 2007; 90: 205–9.
- 106** Grarup N, Albrechtsen A, Ek J, Borch-Johnsen K, Jorgensen T, Schmitz O, Hansen T, Pedersen O. Variation in the peroxisome proliferator-activated receptor delta gene in relation to common metabolic traits in 7,495 middle-aged white people. *Diabetologia* 2007; 50: 1201–8.

- 107** Andersen G, Wegner L, Yanagisawa K, Rose CS, Lin J, Glumer C, Drivsholm T, Borch-Johnsen K, Jorgensen T, Hansen T, Spiegelman BM, Pedersen O. Evidence of an association between genetic variation of the coactivator PGC-1{beta} and obesity. *J Med Genet* 2005; 42: 402–7.
- 108** Torekov SS, Larsen LH, Glumer C, Borch-Johnsen K, Jorgensen T, Holst JJ, Madsen OD, Hansen T, Pedersen O. Evidence of an association between the Arg72 allele of the peptide YY and increased risk of type 2 diabetes. *Diabetes* 2005; 54: 2261–5.
- 109** Jalba MS, Rhoads GG, Demissie K. Association of codon 16 and codon 27 [beta]2-adrenergic receptor gene polymorphisms with obesity: a meta-analysis. *Obes Res* 2008; 16: 2096–106.
- 110** Allison DB, Heo M, Faith MS, Pietrobelli A. Meta-analysis of the association of the Trp64Arg polymorphism in the beta3 adrenergic receptor with body mass index. *Int J Obes Relat Metab Disord* 1998; 22: 559–66.
- 111** Fujisawa T, Ikegami H, Kawaguchi Y, Ogihara T. Meta-analysis of the association of Trp64Arg polymorphism of {beta}3-adrenergic receptor gene with body mass index. *J Clin Endocrinol Metab* 1998; 83: 2441.
- 112** Kurokawa N, Nakai K, Kameo S, Liu ZM, Satoh H. Association of BMI with the {beta}3-adrenergic receptor gene polymorphism in Japanese: meta-analysis. *Obes Res* 2001; 9: 741.
- 113** Marti A, Ochoa MC, Sanchez-Villegas A, Martinez JA, Martinez-Gonzalez MA, Hebebrand J, Hinney A, Vedder H. Meta-analysis on the effect of the N363S polymorphism of the glucocorticoid receptor gene (GRL) on human obesity. *BMC Med Genet* 2006; 7: 50.
- 114** Qi L, Zhang C, van Dam RM, Hu FB. Interleukin-6 genetic variability and adiposity: associations in two prospective cohorts and systematic review in 26,944 individuals. *J Clin Endocrinol Metab* 2007; 92: 3618–25.
- 115** Heo M, Leibel R, Fontaine KR, Boyer BB, Chung WK, Koulu M, Karvonen MK, Pesonen U, Rissanen A, Laakso M, Uusitupa MJ, Chagnon YC, Bouchard C, Donohoue PA, Burns TL, Shuldiner AR, Silver K, Andersen RE, Pedersen O, Echwald SM, Sorensen TIA, Behn P, Permutt MA, Jacobs KB, Elston RC, Hoffman DJ, Gropp A, Allison DB. A meta-analytic investigation of linkage and association of common leptin receptor (LEPR) polymorphisms with body mass index and waist circumference. *Int J Obes Relat Metab Disord* 2002; 26: 640–6.
- 116** Tonjes A, Scholz M, Loeffler M, Stumvoll M. Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with Pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. *Diabetes Care* 2006; 29: 2489–97.