European Journal of Pharmacology 660 (2011) 156–164

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Review

The genetic epidemiology of *melanocortin 4 receptor* variants

Ruth J.F. Loos *

Medical Research Council Epidemiology Unit, Institute of Metabolic Science, Addenbrooke's Hospital, P.O. Box 285, Cambridge, CB5 8QD, United Kingdom

ARTICLE INFO

Article history:
Received 29 September 2010
Received in revised form 24 December 2010
Accepted 13 January 2011
Available online 2 February 2011

Keywords:
MC4R (*Melanocortin 4 receptor*)
Obesity
Body mass index
Genetic epidemiology
Candidate gene study
Genome-wide linkage study
Genome-wide association study

ABSTRACT

While rare *MC4R* mutations are the commonest cause of monogenic forms of extreme, early-onset obesity, growing evidence shows that common *MC4R* variants contribute to obesity in the general population. Candidate gene studies have focussed on the V103I and I251L *MC4R* variants that both affect MC_4 receptor function *in vitro*. Individual association studies, which are typically small and underpowered, have found no association between V103I (frequency of 103I-allele: ~4%) or I251L (251 L-allele: ~2%) and the risk of obesity in the general population. However, large-scale meta-analyses have confirmed that both variants reduce the risk of obesity by −21% in 103I-allele carriers ($P<10^{-4}$) and by −50% in 251 L-allele carriers ($P<10^{-4}$). Recently, genome-wide association studies have identified a common variant (minor allele frequency: ~27%) at ~188 kb downstream of *MC4R* showing robust association ($P<5\times10^{-8}$) with BMI and obesity in adults and children. Each additional minor allele increases BMI by 0.20 kg/m^2, body weight by 700–1000 g, and obesity risk by 14% in adults. Interestingly, this variant also showed association with increased height, consistent with the phenotype seen for rare *MC4R* mutations. Although *MC4R* is the nearest gene and phenotypic associations are consistent with those of *MC4R* mutations, it has not yet been established whether this variant indeed reflects MC_4 receptor function. Taken together, common *MC4R* variants contribute to variation in BMI and obesity risk in the general population. Of particular interest is the finding from genome-wide association studies that suggests that the region downstream of *MC4R* contributes to its regulation.

Contents

1. Introduction

The melanocortin pathway plays a pivotal role in the central regulation of energy homeostasis. Genetic variations and mutations in several of the peptides that constitute this pathway contribute to obesity susceptibility in humans. Here, the role of common genetic variation in and near the melanocortin 4 receptor (*MC4R*) gene, which encodes one of the key peptide of the melanocortin pathway, is reviewed.

The MC_4 receptor is a seven-transmembrane G-protein coupled receptor that is expressed predominantly in the brain. Its contribution

* Tel.: +44 1223 769139; fax: +44 1223 330316.
E-mail address: ruth.loos@mrc-epid.cam.ac.uk.

0014-2999/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2011.01.033

to body weight regulation was first demonstrated in 1997 with the observation that targeted disruption of the *Mc4r* gene resulted in hyperphagia, hyperinsulinemia, mature-onset obesity and increased linear growth in mice (Huszar et al., 1997). At the same time, a pharmacological study showed that activation of the MC_4 receptor by central administration of the synthetic agonist MTII suppressed food intake, while co-administration of the antagonist SHU9119 blocked this suppression (Fan et al., 1997). In the year following these two reports in mice, the first *MC4R* mutations in humans were identified by screening extremely obese individuals and their relatives (Yeo et al., 1998; Vaisse et al., 1998). Two studies each reported a heterozygous frameshift mutation in *MC4R*, one in the fifth and one between the sixth and seventh transmembrane domains, that co-segregated in a dominant manner with severe early-onset obesity (Yeo et al., 1998; Vaisse et al., 1998). Soon thereafter, targeted sequencing efforts of *MC4R* in extremely obese children and adults identified many more novel non-synonymous mutations. Currently, over a 100 different heterozygous and homozygous *MC4R* mutations have been reported in obese individuals from various ethnic backgrounds (Farooqi et al., 2003; Lubrano-Berthelier et al., 2006; Stutzmann et al., 2008; MacKenzie, 2006). Mutations occur throughout the coding sequence and many of the missense and frameshift mutations lead to a complete or partial loss of function (Stutzmann et al., 2008; Farooqi et al., 2003). Up to 6% of individuals with severe, early-onset obesity carry pathogenic mutations in *MC4R*, making *MC4R* deficiency the commonest form of monogenic obesity (Farooqi et al., 2003; Vaisse et al., 2000). Patients with *MC4R* deficiency exhibit hyperphagia, increased fat and lean mass, greater bone mineral density, and accelerated linear growth (Farooqi et al., 2003). The severity of the clinical phenotype is proportional to the functional implications of the mutation on the receptor; i.e. all aspects of the phenotype are more severe in those with complete *MC4R* deficiency as compared to those with partial loss of function (Farooqi et al., 2003). While the role of rare *MC4R* mutations in the development of extreme and early-onset obesity has been well-established for several years, convincing evidence that also common genetic variation in and near *MC4R* contributes to common obesity-susceptibility has only recently emerged.

2. The *MC4R* locus

The MC_4 receptor is encoded by a single exon gene located on chromosome 18q22 at 56.19 Mb (NCBI Assembly Build 36.3), which counts 1438 base pairs (bp) of which 999 bp are translated into 332 amino acids (Fig. 1). The 1 Mb-region surrounding *MC4R* harbours only one other gene, the *PMAIP1* (phorbol-12-myristate-13-acetate-induced protein 1), located 500 kb downstream (on Chr18q21.32 at 55.72 Mb) of *MC4R*. Genome-wide copy number variation (CNV) surveys have identified one insertion and two deletions in the 1 Mb-region (Redon et al., 2006; Conrad et al., 2006). The insertion is located at 400 kb downstream of *MC4R*, counts between 13–34 kb, and has an estimated prevalence of ~1% (Redon et al., 2006). One deletion is located at 130 kb downstream, is ~2 kb long, and has a prevalence of ~3% (Conrad et al., 2006). The other deletion is rare (prevalence ~0.5%), located at 65 kb upstream of *MC4R*, and counts ~27 kb (Redon et al., 2006).

3. Candidate gene studies

Since the identification of *MC4R* as a candidate gene for obesity in 1997, numerous studies have examined whether common *MC4R* variants are associated with obesity and related traits in populations of different ethnicity. Thorough sequence analyses of the *MC4R* gene have revealed only two common variants (Gotoda et al., 1997; Hinney et al., 1999), the V103I (rs2229616) and the I251L (rs52820871), which each result in a non-synonymous change with potential functional implications (Xiang et al., 2006).

3.1. The V103I MC4R variant and common obesity

In vitro characterization experiments have shown that the 103I-allele of the V103I variant exhibits normal receptor expression levels and normal endogenous and synthetic agonist ligand affinity. However, its AGRP antagonist potency showed a twofold decrease compared to that of V103V wild-types (Xiang et al., 2006). The 103I-allele is rather infrequent in the general population; i.e. around 2–4% of individuals of white European carry at least one 103I-allele. The reported carrier-frequency for other ethnic groups varies, but is consistently well below 10% (Geller et al., 2004; Wang et al., 2010).

So far, more than 35 candidate gene studies (Box 1) have tested for association between the V103I *MC4R* variant and obesity risk in populations of various ethnicity (Wang et al., 2010). Given the low prevalence of 103I-allele carriers, large sample sizes are required to provide sufficient power to identify the small genetic effects that are expected for common obesity. It is therefore no surprise that only four studies have reported nominally significant association of the V103I variant with obesity risk (Fig. 2); three of the studies found that 103I-allele carriers had a reduced risk of obesity (Heid et al., 2005; Hart Sailors et al., 2007; Wang et al., 2006), whereas in a Turkish sample the 103I-allele had the opposite effect (Yurtcu et al., 2009).

More conclusive results on the association between the V103I variant and obesity risk have been obtained through meta-analyses of the available data. In the past six years, four consecutive meta-analyses have been published, each including the growing number of data (Geller et al., 2004; Young et al., 2007; Stutzmann et al., 2007; Wang et al., 2010) (Table 1). These meta-analyses have been instrumental in confirming the protective effect of the V103I-allele on obesity risk. The first meta-analysis combined data from 12 studies, including 3631 obese and 4082 non-obese individuals, and found that the 103I-allele was associated with a 31% reduction of obesity risk (Geller et al., 2004). The second meta-analysis was performed three years later and included 29,563 individuals or almost four times as many as in the first meta-analysis (Young et al., 2007). This study further confirmed the significant association between the V103I variant and obesity risk, though the observed protective effect of the 103I-allele was attenuated to 18%. The third meta-analysis, which followed soon after the second, included an additional seven studies or 10,000 individuals more and found a very similar reduction (20%) in obesity risk for 103I-allele carriers (Stutzmann et al., 2007). The latest meta-analysis included data of 55,195 individuals from 37 cohorts and again confirmed that 103I-allele carriers have a ~20% reduced risk of obesity (Wang et al., 2010). In a sub-analysis that combined data of East Asians only, the risk of obesity was 31% lower in the 103I-allele carriers compared the V103V homozygotes (Wang et al., 2010) (Table 1).

While meta-analyses have established the association with obesity risk by combining all data from case–control studies, it remains uncertain whether the V103I variant is also associated with BMI as a continuous proxy-measure of overall adiposity. One population-based study, including 3861 French men and women, found that the 103I-allele was significantly associated with a 0.8 kg/m^2 lower BMI (equivalent to 2.3 kg for a person of 1.70 m tall) (Stutzmann et al., 2007). However, other large-scale population-based studies could not confirm such trend (Geller et al., 2004; Young et al., 2007). A meta-analysis of all available data on the association between the V103I variants and BMI will be needed to confirm or refute these preliminary observations.

Taken together, large-scale meta-analyses consistently show that 103I-allele carriers, who represent 3–4% of the population,

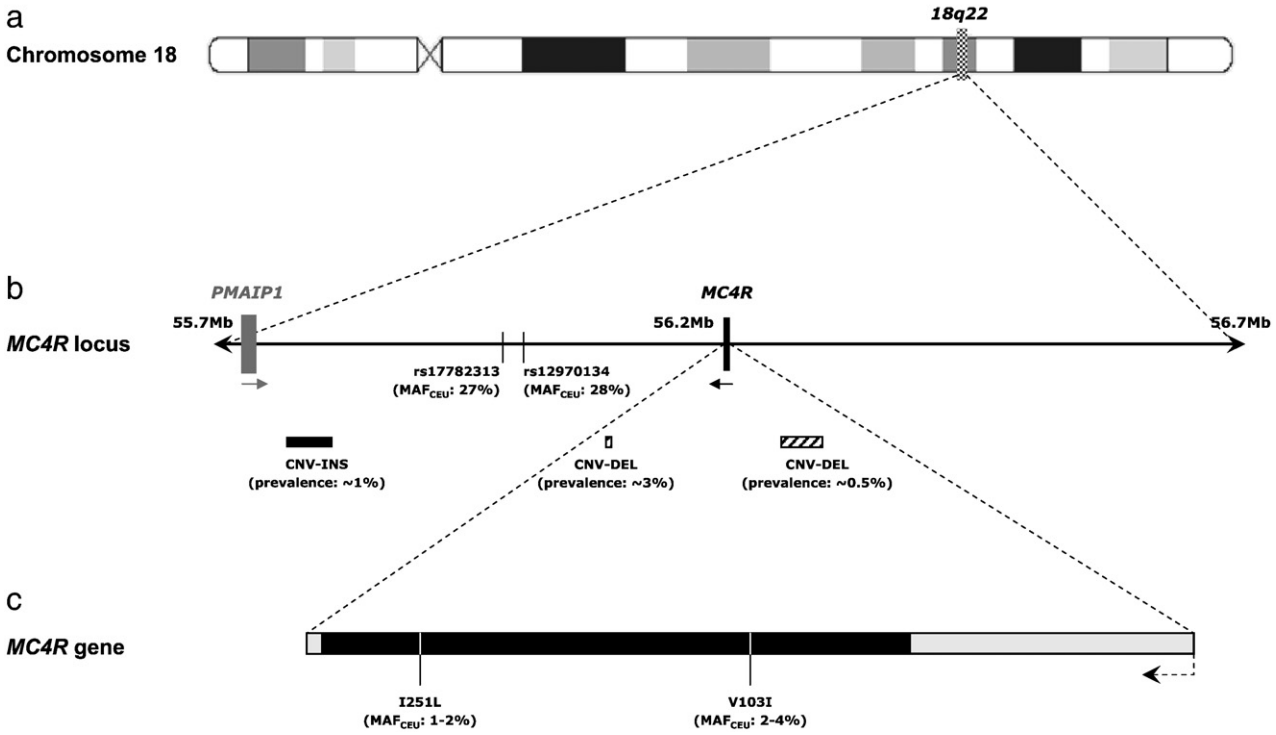

Fig. 1. The *MC4R* locus – (a) Chromosomal location – *MC4R* is located on chromosomal 18q22; (b) The 1 Mb sequence surrounding *MC4R* – *MC4R* is located at 56.2 Mb on chr18, with *PMAIP* being the nearest gene. rs17782313 and rs12970134, are the two variants that were first identified to be associated with BMI and waist circumference through the genome-wide association approach. The region also harbour three structural variants, of which two are deletions and on is an insertion; (c) The *MC4R* gene sequence consists of 1438 bp, of which 999 bp are coding, which harbours two non-synonymous polymorphisms (V103I and I251L).

have a 20% lower risk of obesity compared to V103V-homozygotes. This risk reduction seems more pronounced in individuals of East Asian Origin. None of the meta-analyses reported evidence for age- or sex-specific differences. The results on the association between the V103I variant and BMI have so far been inconclusive.

Box 1
Approaches used in genetic epidemiology.

Genetic epidemiologists have relied mainly on candidate gene and genome-wide screening approaches to identify genetic variants associated with (common) diseases or traits in the general population.

The candidate gene approach. The candidate gene approach is a *hypothesis-driven* approach and relies 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. Candidate gene studies have been performed since the early 1990's; i.e. as soon as technology allowed genotyping at a population level.

The genome-wide screening approach. The genome-wide screening approach is a *hypothesis-generating* method that, through surveying the whole genome, aims to identify new, unanticipated genetic variants associated with a disease or trait of interest. In the context of a strong biological candidate (such as *MC4R*), genome-wide screening studies can contribute to the confirmation of the role of the candidate gene in common obesity risk in the general population. They can also provide insight in the role of the DNA sequence surrounding the candidate gene as this might affect its function and/or expression. The genome-wide screening approach has been implemented in linkage and association studies.

Genome-wide linkage studies. 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 genome-wide linkage survey requires 400–600 highly polymorphic markers, genotyped at 10-cM intervals. Genome-wide linkage studies have a rather low resolution and typically identify broad intervals that require follow-up genotyping to pinpoint the genes that underlie the linkage signal. The genome-wide linkage approached has been available since the mid-1990's thanks to progress in genotyping technology and preliminary insights into genetic variation from the Human Genome Project.

Genome-wide association studies. Genome-wide association studies screen the whole genome at much 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. Genome-wide association studies consist of a discovery stage, which is the actual genome-wide association. SNPs that show significant association are taken forward to the follow-up stage to confirm (or refute) the association observed in the discovery stage. Associations are considered significant if *P*-values reach a significance threshold of $<5 \times 10^{-8}$. Substantial advances in high-throughput genotyping technology and a detailed knowledge of the human genetic architecture have enabled genome-wide association studies that have been available since 2005.

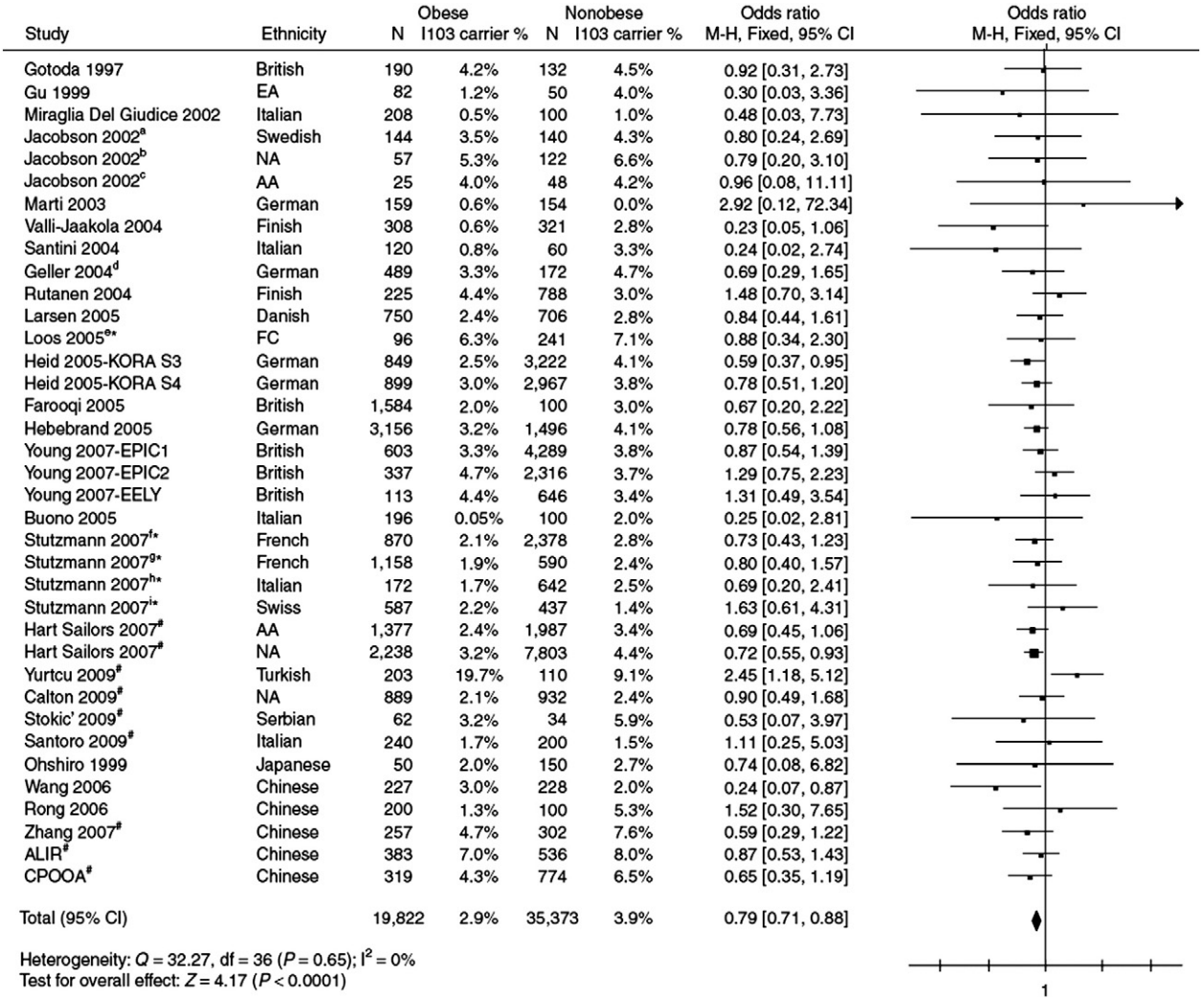

Fig. 2. Meta-analysis of 36 studies on the association between the V103I *MC4R* variant and obesity risk (adapted from Wang et al., 2010).

3.2. The I251L MC4R variant and common obesity

The I125L variant is the only other missense polymorphism in *MC4R* that, with a prevalence of 1–2% for the 251 L-allele, is less frequent than the V103I variant. There is evidence that this variant might have functional implications as *in vitro* experiments found that the 251 L-allele increased the basal activity of the MC_4 receptor without increasing the number of receptors expressed at the cell surface (Xiang et al., 2006).

So far, at least 15 case–control studies have examined the association between the I251L variant and risk of obesity. While the association reached significance in only three individual studies, the meta-analysis of all 15 studies combined, including 5463 cases and 5972 controls, unequivocally confirmed the strong protective effect of

Table 1
Meta-analyses of the associations between the V103I and I251L *MC4R* variants and risk of obesity.

Reference	Number of cohorts	Number of study participants		Prevalence of minor-allele carriers		OR	Association[a]	
		Obese	Non-obese	Obese	Non-obese		95%CI	*P*-value
V103I (rs2229616)								
Geller et al. (2004)	12	3631	4082	2.1%	3.5%	0.69	0.50–0.96	0.03
Young et al. (2007)	22	10,975	18,588	2.9%	3.8%	0.82	0.70–0.96	0.015
Stutzmann et al. (2007)	29	15,820	24,797	–	–	0.80	0.70–0.92	0.002
Wang et al. (2010)	37	19,822	35,373	2.9%	3.9%	0.79	0.71–0.88	<0.0001
Wang et al. (2010) — East Asian only	6	1436	2090	4.4%	6.5%	0.69	0.50–0.94	0.02
I251L (rs52820871)								
Stutzmann et al. (2007)	9	5463	5972	–	–	0.52	0.38–0.71	3.58×10^{-5}

95%CI: 95% confidence interval.
OR: odd ratio.
[a] Association between minor alleles of the V103I or I251L variant and risk of obesity, assuming a dominant model (i.e. minor allele carriers are compared to major-allele homozygotes).

the 251 L-allele (Table 1) (Stutzmann et al., 2007). Carriers of the 251 L-allele have a nearly 50% reduced risk of obesity (Odds ratio (OR): 0.52, *P*-value = 3.58×10^{-5}) compared to I251I homozygotes (Stutzmann et al., 2007). Only one large-scale population-based study (n = 3861) has reported on the association with BMI as a continuous trait and found a consistent protective trend (*P*-value = 0.05); i.e. the 251 L-allele was associated with a 0.7 kg/m^2 lower BMI (equivalent to ~2 kg for a person of 1.70 m tall) (Stutzmann et al., 2007). Additional studies will be needed to confirm the association with BMI.

Taken together, while mutations in *MC4R* often lead to extreme and early onset obesity, minor-allele carriers of the rather infrequent polymorphisms, V103I and I251L, have a 20% and 50% reduced risk of obesity, respectively. It should be noted that both meta-analyses are based mainly (for V103I) or exclusively (for I251L) on data from case–control studies for (extreme) obesity, which may result in effect sizes that are somewhat inflated than if data were obtained from population-based cohorts.

4. Genome-wide linkage studies

Over the past 12 years, genome-wide linkage studies have proposed a large number of chromosomal loci to be linked to common obesity and to related traits (Box 1). The last Human Obesity Gene Map update (Rankinen et al., 2006), which reviewed the literature up to October 2005, reported 253 loci from 61 genome-wide linkage scans, and more loci have been reported since. However, none of the genome-wide linkage studies so far have identified the chromosomal sequence at chr18q22, which harbours *MC4R*, to be significantly linked to any obesity-related trait. The two loci that have been mapped closest to *MC4R* by genome-wide linkage studies are located at chr18q21.32 and chr18q21.31, which is ~1 Mb and ~3 Mb downstream of the gene, respectively (Ohman et al., 2000; North et al., 2004). Yet, for both loci only suggestive evidence of linkage with obesity and BMI was reached (Ohman et al., 2000; North et al., 2004). Even a large-scale meta-analysis of 37 genome-wide linkage studies with data on more than 31,000 individuals from 10,000 families of European origin, which was sufficiently powered to identify loci with small effects, did not identify chr18q22 (or any other locus) to be linked to obesity or BMI locus with convincing evidence (Saunders et al., 2007).

Because of the large distance between these loci and *MC4R* and because of the absence of significant linkage, the downstream chromosomal area was never further explored for potential regulatory elements that may affect the function of *MC4R*.

Taken together, the current literature suggests that genome-wide linkage has not been an effective approach to increase our insights in the role of *MC4R* or its neighbouring genomic sequence at chr18q22 in common obesity and related traits.

5. Genome-wide association studies

The genome-wide association approach has accelerated the discovery of genetic loci for common disease and traits beyond expectations (Box 1). In the past five years, genome-wide association studies have identified more than 750 genetic loci robustly associated with at least 148 common traits (Hindorff et al., 2010). In the field of obesity genetics, the *FTO* locus was the firstly discovered locus to be highly significantly associated with BMI on obesity risk (Frayling et al., 2007; Scuteri et al., 2007).

5.1. The discovery of the near MC4R locus

One year after the discovery of the *FTO* locus, a meta-analysis of seven genome-wide association studies for BMI, including 16,876 individuals of white-European descent, unequivocally established the second obesity-susceptibility locus after replicating the association in an independent sample of 60,352 individuals (overall *P*-value $< 2.8 \times 10^{-15}$) (Loos et al., 2008). The newly identified locus, represented by the rs17782313 single nucleotide polymorphism (SNP), mapped at 188 kb downstream of *MC4R* and was not in linkage disequilibrium (LD) (i.e. did not 'correlate') with the V103I or I251L *MC4R* variants. At the same time, a genome-wide association study in 2684 Indian Asians identified a locus, represented by the rs129070134-SNP, at ~150 kb downstream of *MC4R*, which was confirmed in 11,955 individuals of Indian Asian and European ancestry (overall *P*-value $< 1.7 \times 10^{-9}$) (Chambers et al., 2008). Although the locus identified by Chamber et al. maps 38 kb closer to *MC4R* than the locus identified by Loos et al., both loci are part of the same cluster of genetic variants that are in high LD ($r^2 > 0.75$ in white Europeans) (Figs. 1 and 3). The frequency of the BMI-increasing 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.

Subsequent large-scale genome-wide association studies each found highly significant associations for SNPs located in the near-*MC4R* locus with BMI (Fig. 3) (Willer et al., 2009, Thorleifsson et al., 2009), waist circumference (Lindgren et al., 2009; Heard-Costa et al., 2009) and risk of obesity (Meyre et al., 2009; Scherag et al., 2010). Although the genome-wide association studies each may have identified different SNPs in the near-*MC4R* locus that show the most significant association with the obesity-related traits, all the identified SNPs are in high LD and thus represent the same association signal (Fig. 3).

5.2. The near-MC4R locus and association with anthropometric traits in adults

Despite highly significant associations and repeated replication, SNPs in the near-*MC4R* locus have only a small effect on body composition. Data from large-scale studies (n > 10,000) in adult individuals of white European descent, have shown that BMI increases by ~0.10–30 kg/m^2 (Loos, 2009; Li et al., 2010; Zobel et al., 2009; Holzapfel et al., 2010) and waist circumference by ~0.40–0.70 cm (Li et al., 2010; Zobel et al., 2009) for each additional minor (or risk-) allele an individual carries. Of interest is that the BMI-increasing allele is also significantly associated with a ~0.20–0.30 cm increase in height (Loos et al., 2008; Li et al., 2010; Zobel et al., 2009). Consequently, the association with weight (~700–1000 g/allele) is even stronger than that with BMI (Loos et al., 2008; Li et al., 2010; Zobel et al., 2009). Furthermore, each additional BMI-increasing allele increases the risk of being overweight by ~8% and the risk of obesity by ~14% (Li et al., 2010; Loos et al., 2008; Zobel et al., 2009).

Compared to the effect sizes observed for the *FTO* locus, which continues to have the largest effect on obesity-related traits, the effect sizes observed for the near-*MC4R* locus are nearly 40% smaller. Furthermore, the frequency of the BMI-increasing allele of the near-*MC4R* locus (~27% in white Europeans) is substantially lower than that of the *FTO* locus (~48%), such that the overall contribution of the near-*MC4R* locus to the inter-individual variation in adult BMI is low (~0.14% for near-*MC4R* vs ~0.35% for *FTO*) (Loos et al., 2008).

5.3. The near-MC4R locus and associations in individuals of different ethnicity

The role of the near-*MC4R* locus in BMI and obesity risk has been explored in populations with various ethnic backgrounds.

One study in Asian Sikhs confirmed that the rs12970134-SNP in the near-*MC4R* locus is associated with BMI and waist circumference in Indian Asians (Been et al., 2009). The effect sizes reported in this study, which had a type 2 diabetes case–control design, were twofold larger than those observed in the initial genome-wide association study of Indian Asians (Chambers et al., 2008), which found similar

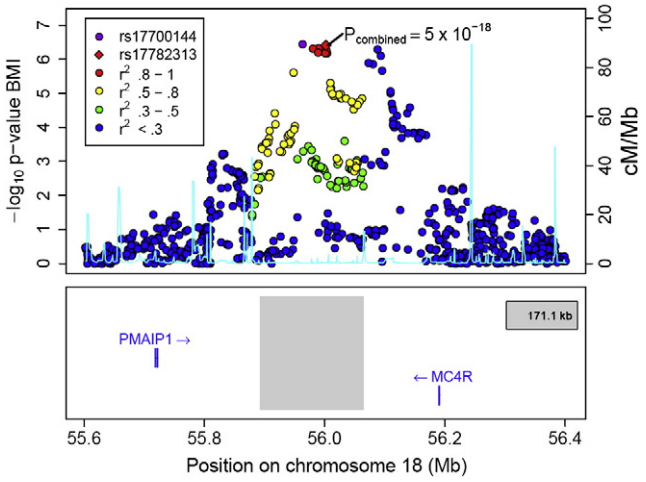

Fig. 3. Regional plot of association around the *MC4R* locus. SNPs are plotted by position on chromosome 18 against association with BMI (−log10 *P*-value). The figures highlight the most significant SNP (in purple). The linkage disequilibrium (LD) between the followed up SNP and the most significant SNP in the region is indicated by the colour of the diamond (adapted from Willer et al., 2009).

effect sizes as those seen for white-Europeans. As the BMI-increasing allele frequency in Indian Asians (36–40%) is higher than in white Europeans (~28%) and as the effect sizes in Indian Asians are at least as large as in white Europeans, the contribution of the near-*MC4R* locus to obesity susceptibility is likely larger in Indian Asians.

The frequency of the BMI-increasing allele in individuals of Chinese (~19%), Japanese (~24%), and Korean (25%) descent is only slightly lower than the frequency observed in individuals of white European descent. A genome-wide association meta-analysis of population-based cohorts including 8842 Koreans confirmed significant association between the rs17782313-SNP in the near-MC4R locus and BMI with an effect size (0.25 kg/m^2 per allele) similar to that observed in white Europeans (Cho et al., 2009). Significant and even more pronounced associations with BMI (0.37 kg/m2 per allele) and obesity risk (OR 1.43 per allele) were also observed in two studies in Chinese from Shanghai (n = 1170) (Shi et al., 2010) and Hong Kong (n = 1170) (Cheung et al., 2010) that both examined the rs17782313-SNP. Another study in 6681 Chinese adults from Hong Kong, mainly type 2 diabetes cases, observed directionally consistent trends between the rs12970134-SNP and BMI (Ng et al., 2010). However, the association did not reach significance and the effect size for BMI was much smaller (0.09 kg/m^2 per allele) than those reported previously for white Europeans and Chinese populations. Inconsistent results on the association between genetic variants in the near-*MC4R* locus and obesity-related traits have also been reported for Japanese (Hotta et al., 2009, 2010; Tabara et al., 2009). An obesity case–control study of 2865 Japanese individuals found a borderline significant association between the rs17782313-SNP and risk of obesity, with each BMI-increasing allele increasing the odds of obesity by 13%, which is similar to the effects observed in white Europeans (Hotta et al., 2009). The BMI-increasing allele of the same SNP tended to increase BMI by ~0.20 kg/m^2 in a population-based study of 2806 middle-aged to elderly Japanese, but the association did not reach significance ($P = 0.12$) (Tabara et al., 2009). A third study in 1228 overweight Japanese individuals found no evidence of association between the rs17700144-SNP and BMI (Hotta et al., 2010).

The reason for the inconsistent results among East Asians could be due to the fact that the minor allele frequency is somewhat lower, such that larger sample sizes are required to replicate the effects observed in white Europeans and Indian Asians. Furthermore, few studies have examined the association in population-based studies, which may provide more statistical power than case-only studies (of obesity or type 2 diabetes cases) that have often been used so far. As the genetic architecture differs between populations of different ethnicity, inconsistent association results could also have emerged because of the use of different SNPs across the various studies. However, the currently published studies in Chinese and Japanese have either used the SNPs originally reported (Loos et al., 2008; Chambers et al., 2008), or SNPs in high LD with the original SNPs ($r^2 > 0.90$ in HapMap CEU, JPT and CHB populations) such that differences in genetic architecture is an unlikely reason for the observed inconsistent findings. Taken together, more large-scale population-based studies in individuals of East Asian origin will be needed to more convincingly establish the association between genetic variation in the near-*MC4R* locus and obesity-related traits.

Examining genetic associations across different ethnicities is not only informative towards confirming the role of the respective genetic variants in other populations. They can also contribute to the fine-mapping of the locus to identify the causal variant by taking advantage of the known differences in the genetic architecture between ethnicities. The LD between SNPs in the near-*MC4R* locus is high ($r^2 > 0.80$) in individuals of white European origins, such that it is impossible to identify which of the SNPs in the locus is truly causing the association. While the LD patterns for this locus are similar in Indian and East Asians, they are less tight in individuals of African origin. In this context, a genome-wide association study in 1188 Nigerians and 743 African-Americans may be of particular use for narrowing down the near-*MC4R* locus (Kang et al., 2010). In this study, the minor allele (frequency = ~20%) of the rs6567160-SNP was found to be associated with a 0.14 kg/m^2 increase in BMI in individuals of African origin (Kang et al., 2010). Of interest is that in white Europeans this SNP is in high LD ($r^2 > 0.95$) with the two original SNPs and thus part of the same locus, whereas in Africans (HapMap YRI) the LD is much lower (rs6567160–rs17782313 $r^2 = 0.09$; rs6567160–rs12970134 $r^2 = 0.50$) indicating that the rs6567160-SNP is independent of the rs17782313 and rs12970134 SNPs. This preliminary observation suggests that the causal variant of the near-*MC4R* locus might be located more near the rs6567160 variant (i.e. 22 kb downstream of rs17782313) (Fig. 1). Deep-sequencing of the near-*MC4R* locus in extremely obese cases and in lean controls might elucidate the true causal variant that underlies the observed associations with BMI and obesity risk.

5.4. The near-MC4R locus and associations in infancy, childhood and adolescence

The near-*MC4R* locus was first identified in adults, but there is growing evidence that the locus already affects obesity susceptibility in early life.

While several studies, including large-scale (n > 5000) population-based studies, found no evidence of association between genetic variants in the near-*MC4R* locus and birth weight (Loos et al., 2008; Cauchi et al., 2009; Hardy et al., 2010; Petry et al., 2010; Wu et al., 2010), the locus seems to be associated with weight and BMI soon after birth. In a study in 278 healthy newborns, the BMI-increasing allele of the rs1772313-SNP was found to be associated with increased body weight already at age 2 weeks (Petry et al., 2010). However, such an early effect on body weight was not confirmed by two population-based longitudinal studies (n > 2400) with body weight measures throughout infancy and childhood (Loos et al., 2008; Hardy et al., 2010). In both studies, association between the rs1772313-SNP and body weight only emerged in childhood, i.e. around the age of 6–7 years (Loos et al., 2008; Hardy et al., 2010). Other cross-sectional studies confirmed the influence of the near-*MC4R* locus on weight, BMI and obesity risk in children and adolescents of white European descent (Cauchi et al., 2009; Liem et al., 2010; Zhao et al., 2009). A

recent meta-analysis of cross-sectional data for BMI in 13,004 children and adolescents found that each additional risk-allele of the near-*MC4R* locus increases BMI by 6.7% SD, an effect that is marginally larger than that seen in adults (4.7% SD/allele) (den Hoed et al., 2010). This is consistent with the observations of a recent study that examined the association between the rs1772313-SNP and body weight across the life course of 2479 men and women born in 1946 (Hardy et al., 2010). The associations between the rs1772313-SNP and body weight strengthened with age up to a peak at age 20 years and then weakened again with increasing adult age (Fig. 4) (Hardy et al., 2010). These changes might reflect age-dependent influences of lifestyle, behaviour and other environmental factors.

While studies among children and adolescents of white European descent consistently confirm association between the near-*MC4R* locus and obesity-susceptibility, results in Chinese children and adolescents are inconsistent (Wu et al., 2010; Ng et al., 2010), and no association with risk of obesity was observed in African American children and adolescents (Grant et al., 2009). More studies will be needed to confirm or refute the role of the near-*MC4R* locus in obesity susceptibility in children and adolescents of different ethnicity.

5.5. The near-MC4R locus and association with intermediate lifestyle factors

While the consistent and highly significant replication of the association signal firmly establishes the genomic sequence near *MC4R* as the second obesity-susceptibility locus, it remains elusive how this locus confers increased risk of obesity. Studies that examine associations with lifestyle factors that are known to affect BMI and obesity risk can provide insight into how genetic variation at the near-*MC4R* locus influences obesity susceptibility.

Several studies have tested the hypothesis that the near-*MC4R* locus increases the risk of obesity through its influence on dietary factors. Two studies found that the BMI-increasing allele of SNPs in the near-*MC4R* locus is associated with increased food intake (Qi et al., 2008), and unhealthy eating behaviour (Stutzmann et al., 2009). However, other studies, including the population-based MONICA-KORA study with more than 12,000 individuals (Holzapfel et al., 2010), could not confirm association with dietary factors (Tenesa et al., 2008; Bauer et al., 2009; Hasselbalch et al., 2010). Therefore, the hypothesis that the near-*MC4R* locus affects obesity risk through increased food intake remains currently unresolved.

The MONICA-KORA study also examined the role of other behaviours, such as physical activity, smoking and alcohol consumption, for which data was collected through standardised questionnaires (Holzapfel et al., 2010). However, no evidence of association between SNPs in the near-*MC4R* locus influence and any of these behaviours was observed (Holzapfel et al., 2010).

Intervention studies, which by design can control lifestyle and behaviour better than observational studies but which are often smaller, have found no evidence of association between variants in the near-*MC4R* locus and weight loss during a 9-month diet and exercise intervention in 242 overweight individuals (Haupt et al., 2008) or a 12-week resistance training programme in 785 women (Haupt et al., 2009). Also maximum weight loss and weight regain 6 years after bariatric surgery in 1443 patients of the Swedish Obese Subjects (SOS) intervention study was not different according genotype at the near-*MC4R* locus (Sarzynski et al., 2010).

Taken together, the current data on association between the near-*MC4R* locus and intermediate traits lacks consistency, such that it remains unclear through which pathways this locus confers increased risk of obesity.

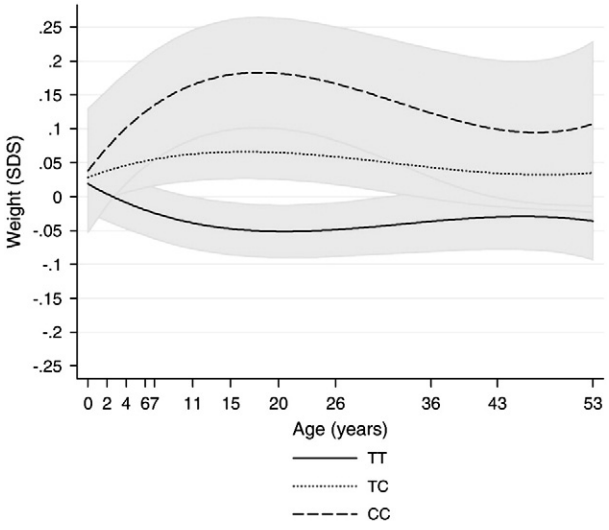

Fig. 4. Linear prediction of mean and 95% prediction interval from additive genetic models for weight SDS based on weight were measured or self-reported repeatedly at 11 time-points between ages 2 and 53 years by *MC4R* rs17782313 genotype in 1240 men and 1239 women born in 1946 (adapted from Hardy et al., 2010).

6. Conclusions

Mutations in *MC4R* have long been known to cause extreme and early onset obesity. Recent large-scale association studies have confirmed that also common variants in and near *MC4R* contribute to obesity susceptibility in the general population. While, two rather infrequent non-synonymous polymorphisms, V103I and I251L, reduce the risk of obesity by ~20% and ~50% respectively, common variants at 188 kb downstream of *MC4R* increase the risk of obesity by ~14%. It is currently not known how the variants downstream of *MC4R* confer increase obesity risk, but their association with adult height and with BMI in childhood is consistent with effects mediated through altered the MC_4 receptor function. While the associations for the near-*MC4R* locus have been firmly established, targeted analyses and experiments will be required to identify the causal variant and the biological pathways through which they increase obesity susceptibility.

References

Bauer, F., Elbers, C.C., Adan, R.A., Loos, R.J., Onland-Moret, N.C., Grobbee, D.E., van Vliet-Ostaptchouk, J.V., Wijmenga, C., van der Schouw, Y.T., 2009. Obesity genes identified in genome-wide association studies are associated with adiposity measures and potentially with nutrient-specific food preference. Am. J. Clin. Nutr. 90, 951–959.

Been, L.F., Nath, S.K., Ralhan, S.K., Wander, G.S., Mehra, N.K., Singh, J., Mulvihill, J.J., Sanghera, D.K., 2009. Replication of association between a common variant near melanocortin-4 receptor gene and obesity-related traits in Asian Sikhs. Obesity Res. 18, 425–429.

Cauchi, S., Stutzmann, F., Cavalcanti-Proena, C., Durand, E., Pouta, A., Hartikainen, A.L., Marre, M., Vol, S., Tammelin, T., Laitinen, J., Gonzalez-Izquierdo, A., Blakemore, A., Elliott, P., Meyre, D., Balkau, B., Jarvelin, M.R., Froguel, P., 2009. Combined effects of MC4R and FTO common genetic variants on obesity in European general populations. J. Mol. Med. 87, 537–546.

Chambers, J.C., Elliott, P., Zabaneh, D., Zhang, W., Li, Y., Froguel, P., Balding, D., Scott, J., Kooner, J.S., 2008. Common genetic variation near MC4R is associated with waist circumference and insulin resistance. Nat. Genet. 40, 716–718.

Cheung, C.Y.Y., Tso, A.W.K., Cheung, B.M.Y., Xu, A., Ong, K.L., Fong, C.H.Y., Wat, N.M.S., Janus, E.D., Sham, P.C., Lam, K.S.L., 2010. Obesity susceptibility genetic variants identified from recent genome-wide association studies: implications in a Chinese population. J. Clin. Endocrinol. Metab. 95, 1395–1403.

Cho, Y.S., Go, M.J., Kim, Y.J., Heo, J.Y., Oh, J.H., Ban, H.J., Yoon, D., Lee, M.H., Kim, D.J., Park, M., Cha, S.H., Kim, J.W., Han, B.G., Min, H., Ahn, Y., Park, M.S., Han, H.R., Jang, H.Y., Cho, E.Y., Lee, J.E., Cho, N.H., Shin, C., Park, T., Park, J.W., Lee, J.K., Cardon, L., Clarke, G., McCarthy, M.I., Lee, J.Y., Lee, J.K., Oh, B., Kim, H.L., 2009. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat. Genet. 41, 527–534.

Conrad, D.F., Andrews, T.D., Carter, N.P., Hurles, M.E., Pritchard, J.K., 2006. A high-resolution survey of deletion polymorphism in the human genome. Nat. Genet. 38, 75–81.

den Hoed, M., Ekelund, U., Brage, S., Grontved, A., Zhao, J.H., Sharp, S.J., Ong, K.K., Wareham, N.J., Loos, R.J., 2010. Genetic susceptibility to obesity and related traits in childhood and adolescence; influence of loci identified by genome-wide association studies. Diabetes 59, 2980–2988.

Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.j, Cone, R.D., 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385, 165–168.

Farooqi, I.S., Keogh, J.M., Yeo, G.S.H., Lank, E.J., Cheetham, T., O'Rahilly, S., 2003. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N. Engl. J. Med. 348, 1085–1095.

Frayling, T.M., Timpson, N.J., Weedon, M.N., Zeggini, E., Freathy, R.M., Lindgren, C.M., Perry, J.R.B., Elliott, K.S., Lango, H., Rayner, N.W., Shields, B., Harries, L.W., Barrett, J.C., Ellard, S., Groves, C.J., Knight, B., Patch, A.M., Ness, A.R., Ebrahim, S., Lawlor, D.A., Ring, S.M., Ben-Shlomo, Y., Jarvelin, M.R., Sovio, U., Bennett, A.J., Melzer, D., Ferrucci, L., Loos, R.J.F., Barroso, I., Wareham, N.J., Karpe, F., Owen, K.R., Cardon, L.R., Walker, M., Hitman, G.A., Palmer, C.N.A., Doney, A.S.F., Morris, A.D., Smith, G.D., The Wellcome Trust Case Control Consortium, Hattersley, A.T., McCarthy, M.I., 2007. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316, 889–894.

Geller, F., Reichwald, K., Dempfle, A., Illig, T., Vollmert, C., Herpertz, S., Siffert, W., Platzer, M., Hess, C., Gudermann, T., Biebermann, H., Wichmann, H.E., Schafer, H., Hinney, A., Hebebrand, J., 2004. Melanocortin 4 receptor gene variant I103 is negatively associated with obesity. Am. J. Hum. Genet. 74, 572–581.

Gotoda, T., Scott, J., Aitman, T.J., 1997. Molecular screening of the human melanocortin-4 receptor gene: identification of a missense variant showing no association with obesity, plasma glucose, or insulin. Diabetologia 40, 976–979.

Grant, S.F.A., Bradfield, J.P., Zhang, H., Wang, K., Kim, C.E., Annaiah, K., Santa, E., Glessner, J.T., Thomas, K., Garris, M., Frackelton, E.C., Otieno, F.G., Shaner, J.L., Smith, R.M., Imielinski, M., Chiavacci, R.M., Li, M., Berkowitz, R.I., Hakonarson, H., 2009. Investigation of the locus near MC4R with childhood obesity in Americans of European and African ancestry. Obesity Res. 17, 1461–1465.

Hardy, R., Wills, A.K., Wong, A., Elks, C.E., Wareham, N.J., Loos, R.J.F., Kuh, D., Ong, K.K., 2010. Life course variations in the associations between FTO and MC4R gene variants and body size. Hum. Mol. Genet. 19, 545–552.

Hart Sailors, M.L., Folsom, A.R., Ballantyne, C.M., Hoelscher, D.M., Jackson, A.S., Kao, W.H.L., Pankow, J.S., Bray, M.S., 2007. Genetic variation and decreased risk for obesity in the atherosclerosis risk in communities study. Diab. Obes. Metab. 9, 548–557.

Hasselbalch, A.L., Angquist, L., Christiansen, L., Heitmann, B.L., Kyvik, K.O., Sorensen, T.I.A., 2010. A variant in the fat mass and obesity-associated gene (FTO) and variants near the melanocortin-4 receptor gene (MC4R) do not influence dietary intake. J. Nutr. 140, 831–834.

Haupt, A., Thamer, C., Machann, J., Kirchhoff, K., Stefan, N., Tschritter, O., Machicao, F., Schick, F., Haring, H.U., Fritsche, A., 2008. Impact of variation in the FTO gene on whole body fat distribution, ectopic fat, and weight loss. Obesity Res. 16, 1969–1972.

Haupt, A., Thamer, C., Heni, M., Tschritter, O., Machann, J., Schick, F., Machicao, F., Haring, H.U., Staiger, H., Fritsche, A., 2009. Impact of variation near MC4R on whole-body fat distribution, liver fat, and weight loss. Obesity Res. 17, 1942–1945.

Heard-Costa, N.L., Zillikens, M.C., Monda, K.L., Johansson, A., Harris, T.B., Fu, M., Haritunians, T., Feitosa, M.F., Aspelund, T., Eiriksdottir, G., Garcia, M., Launer, L.J., Smith, A.V., Mitchell, B.D., McArdle, P.F., Shuldiner, A.R., Bielinski, S.J., Boerwinkle, E., Brancati, F., Demerath, E.W., Pankow, J.S., Arnold, A.M., Chen, Y.D.I., Glazer, N.L., McKnight, B., Psaty, B.M., Rotter, J.I., Amin, N., Campbell, H., Gyllensten, U., Pattaro, C., Pramstaller, P.P., Rudan, I., Struchalin, M., Vitart, V., Gao, X., Kraja, A., Province, M.A., Zhang, Q., Atwood, L.D., Dupuis, J.e., Hirschhorn, J.N., Jaquish, C.E., O'Donnell, C.J., Vasan, R.S., White, C.C., Aulchenko, Y.S., Estrada, K., Hofman, A., Rivadeneira, F., Uitterlinden, A.G., Witteman, J.C.M., Oostra, B.A., Kaplan, R.C., Gudnason, V., O'Connell, J.R., Borecki, I.B., van Duijn, C.M., Cupples, L.A., Fox, C.S., North, K.E., 2009. *NRXN3* is a novel locus for waist circumference: a genome-wide association study from the CHARGE Consortium. PLoS Genet. 5, e1000539.

Heid, I.M., Vollmert, C., Hinney, A., Doring, A., Geller, F., Lowel, H., Wichmann, H.E., Illig, T., Hebebrand, J., Kronenberg, F., the KORA Group, 2005. Association of the 103I MC4R allele with decreased body mass in 7937 participants of two population based surveys. J. Med. Genet. 42, e21.

Hindorff, L.A., Junkins, H.A., Manolio, Teri A., . NHGRI Catalog of published genome-wide association studiesat http://www.genome.gov/gwastudies Accessed October 2010.

Hinney, A., Schmidt, A., Nottebom, K., Heibult, O., Becker, I., Ziegler, A., Gerber, G., Sina, M., Gorg, T., Mayer, H., Siegfried, W., Fichter, M., Remschmidt, H., Hebebrand, J., 1999. Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. J. Clin. Endocrinol. Metab. 84, 1483–1486.

Holzapfel, C., Grallert, H., Huth, C., Wahl, S., Fischer, B., Doring, A., Ruckert, I.M., Hinney, A., Hebebrand, J., Wichmann, H.E., Hauner, H., Illig, T., Heid, I.M., 2010. Genes and lifestyle factors in obesity: results from 12,462 subjects from MONICA/KORA. Int. J. Obes. 34, 1538–1545.

Hotta, K., Nakamura, M., Nakamura, T., Matsuo, T., Nakata, Y., Kamohara, S., Miyatake, N., Kotani, K., Komatsu, R., Itoh, N., Mineo, I., Wada, J., Masuzaki, H., Yoneda, M., Nakajima, A., Funahashi, T., Miyazaki, S., Tokunaga, K., Kawamoto, M., Ueno, T., Hamaguchi, K., Tanaka, K., Yamada, K., Hanafusa, T., Oikawa, S., Yoshimatsu, H., Nakao, K., Sakata, T., Matsuzawa, Y., Kamatani, N., Nakamura, Y., 2009. Association between obesity and polymorphisms in SEC16B, TMEM18, GNPDA2, BDNF, FAIM2 and MC4R in a Japanese population. J. Hum. Genet. 54, 727–731.

Hotta, K., Nakamura, M., Nakamura, T., Matsuo, T., Nakata, Y., Kamohara, S., Miyatake, N., Kotani, K., Komatsu, R., Itoh, N., Mineo, I., Wada, J., Yoneda, M., Nakajima, A., Funahashi, T., Miyazaki, S., Tokunaga, K., Kawamoto, M., Masuzaki, H., Ueno, T., Hamaguchi, K., Tanaka, K., Yamada, K., Hanafusa, T., Oikawa, S., Yoshimatsu, H., Nakao, K., Sakata, T., Matsuzawa, Y., Nakamura, Y., Kamatani, N., 2010. Polymorphisms in NRXN3, TFAP2B, MSRA, LYPLAL1, FTO and MC4R and their effect on visceral fat area in the Japanese population. J. Hum. Genet. 54, 727–731.

Huszar, D., Lynch, C.A., Fairchild-Huntress, V., Dunmore, J.H., Fang, Q., Berkemeier, L.R., Gu, W., Kesterson, R.A., Boston, B.A., Cone, R.D., Smith, F.J., Campfield, L.A., Burn, P., Lee, F., 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88, 131–141.

Kang, S.J., Chiang, C.W.K., Palmer, C.D., Tayo, B.O., Lettre, G., Butler, J.L., Hackett, R., Adeyemo, A.A., Guiducci, C., Berzins, I., Nguyen, T.T., Feng, T., Luke, A., Shriner, D., Ardlie, K., Rotimi, C., Wilks, R., Forrester, T., McKenzie, C.A., Lyon, H.N., Cooper, R.S., Zhu, X., Hirschhorn, J.N., 2010. Genome-wide association of anthropometric traits in African- and African-derived populations. Hum. Mol. Genet. 19, 2725–2738.

Li, S., Zhao, J.H., Luan, J., Luben, R.N., Rodwell, S.A., Khaw, K.T., Ong, K.K., Wareham, N.J., Loos, R.J., 2010. Cumulative effects and predictive value of common obesity-susceptibility variants identified by genome-wide association studies. Am. J. Clin. Nutr. 91, 184–190.

Liem, E.T., Vonk, J.M., Sauer, P.J., van der Steege, G., Oosterom, E., Stolk, R.P., Snieder, H., 2010. Influence of common variants near INSIG2, in FTO, and near MC4R genes on overweight and the metabolic profile in adolescence: the TRAILS (TRacking Adolescents' Individual Lives Survey) Study. Am. J. Clin. Nutr. 91, 321–328.

Lindgren, C.M., Heid, I.M., Randall, J.C., Lamina, C., Steinthorsdottir, V., Qi, L., Speliotes, E.K., Thorleifsson, G., Willer, C.J., Herrera, B.M., Jackson, A.U., Lim, N., Scheet, P., Soranzo, N., Amin, N., Aulchenko, Y.S., Chambers, J.C., Drong, A., Luan, J., Lyon, H.N., Rivadeneira, F., Sanna, S., Timpson, N.J., Zillikens, M.C., Zhao, J.H., Almgren, P., Bandinelli, S., Bennett, A.J., Bergman, R.N., Bonnycastle, L.L., Bumpstead, S.J., Chanock, S.J., Cherkas, L., Chines, P., Coin, L., Cooper, C., Crawford, G., Doering, A., Dominiczak, A., Doney, A.S.F., Ebrahim, S., Elliott, P., Erdos, M.R., Estrada, K., Ferrucci, L., Fischer, G., Forouhi, N.G., Gieger, C., Grallert, H., Groves, C.J., Grundy, S., Guiducci, C., Hadley, D., Hamsten, A., Havulinna, A.S., Hofman, A., Holle, R., Holloway, J.W., Illig, T., Isomaa, B., Jacobs, L.C., Jameson, K., Jousilahti, P., Karpe, F., Kuusisto, J., Laitinen, J., Lathrop, G.M., Lawlor, D.A., Mangino, M., McArdle, W.L., Meitinger, T., Morken, M.A., Morris, A.P., Munroe, P., Narisu, N., Nordstrom, A., Nordstrom, P., Oostra, B.A., Palmer, C.N.A., Payne, F., Peden, J.F., Prokopenko, I., Renstrom, F., Ruokonen, A., Salomaa, V., Sandhu, M.S., Scott, L.J., Scuteri, A., Silander, K., Song, K., Yuan, X., Stringham, H.M., Swift, A.J., Tuomi, T., Uda, M., Vollenweider, P., Waeber, G., Wallace, C., Walters, G.B., Weedon, M.N., Witteman, J.C.M., Zhang, C., Zhang, W., Caulfield, M.J., Collins, F.S., vey Smith, G., Day, I.N.M., Franks, P.W., Hattersley, A.T., Hu, F.B., Jarvelin, M.R., Kong, A., Kooner, J.S., Laakso, M., Lakatta, E., Mooser, V., Morris, A.D., Peltonen, L., Samani, N.J., Spector, T.D., Strachan, D.P., Tanaka, T., Tuomilehto, J., Uitterlinden, A.G., van Duijn, C.M., Wareham, N.J., Watkins for the PROCARDIS consortia, H., Waterworth, D.M., Boehnke, M., Deloukas, P., Groop, L., Hunter, D.J., Thorsteinsdottir, U., Schlessinger, D., Wichmann, H.E., Frayling, T.M., Abecasis, G.R., Hirschhorn, J.N., Loos, R.J.F., Stefansson, K., Mohlke, K.L., Barroso, I., McCarthy for the GIANT consortium, M., The Wellcome Trust Case Control Consortium, 2009. Genome-wide association scan meta-analysis identifies three loci influencing adiposity and fat distribution. PLoS Genet. 5, e1000508.

Loos, R.J.F., 2009. Recent progress in the genetics of common obesity. Br. J. Clin. Pharmacol. 68, 811–829.

Loos, R.J., Lindgren, C.M., Li, S., Wheeler, E., Zhao, J.H., Prokopenko, I., Inouye, M., Freathy, R.M., Abecasis, G.R., Albai, G., Attwood, A.P., Beckmann, J.S., Berndt, S.I., Boehnke, M., Chanock, S.J., Hayes, R.B., Bergmann, S., Bennett, A.J., Bingham, S.A., Bochud, M., Brown, M., Cauchi, S., Chanock, S.J., Collins, F.S., Connell, J.M., Cooper, C., Smith, G.D., Day, I., Dina, C., De, S., Dermitzakis, E.T., Doney, A.S., Elliott, K.S., Elliott, P., Evans, D.M., Sadaf, F.I., Froguel, P., Ghori, J., Gieger, C., Groop, L., Groves, C.J., Gwilliam, R., Hadley, D., Hall, A.S., Hankinson, S.E., Hattersley, A.T., Hayes, R.B., Hebebrand, J., Heid, I.M., Illig, T., Lamina, C., Gieger, C., Illig, T., Kraft, P., Meitinger, T., Wichmann, H.E., Herrera, B., Hinney, A., Hu, F.B., Hunt, S.E., Hunter, D.J., Jackson, A.U., Jacobs, K.B., Jarvelin, M.R., Johnson, T., Jolley, J.D., Karpe, F., Keniry, A., Khaw, K.T., Lamina, C., Luben, R.N., Lyon, H.N., Mangino, M., Marchini, J., McArdle, W.L., McGinnis, R., Meitinger, T., Meyre, D., Munroe, P.B., Mohlke, K.L., Morris, A.D., Nagaraja, R., Ness, A.R., Neville, M.J., Nica, A.C., Ong, K.K., O'Rahilly, S., Owen, K.R., Palmer, C.N., Papadakis, K., Potter, S., Pouta, A., Qi, L., Randall, J.C., Rayner, N.W., Ridderstrale, M., Ring, S.M., Sandhu, M.S., Sanna, S., Scherag, A., Scheet, P., Sims, M.A., Schlessinger, D., Song, K., Soranzo, N., Speliotes, E.K., Syddall, H.E., Teichmann, S.A., Timpson, N.J., Tobias, J.H., Tuomilehto, J., Uda, M., Vogel, C.I., Voight, B.F., Wallace, C., Waterworth, D.M., Weedon, M.N., Wichmann, H.E., Willer, C.J., Wraight, Yuan, X., Zeggini, E., Hirschhorn, J.N., Strachan, D.P., Ouwehand, W.H., Caulfield, M.J., Samani, N.J., Frayling, T.M., Vollenweider, P., Waeber, G., Mooser, V., Deloukas, P., McCarthy, M.I., Wareham, N.J., Barroso, I., 2008. Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat. Genet. 40, 768–775.

Lubrano-Berthelier, C., Dubern, B., Lacorte, J.M., Picard, F., Shapiro, A., Zhang, S., Bertrais, S., Hercberg, S., Basdevant, A., Clement, K., Vaisse, C., 2006. Melanocortin 4 receptor mutations in a large cohort of severely obese adults: prevalence, functional classification, genotype–phenotype relationship, and lack of association with binge eating. J. Clin. Endocrinol. Metab. 91, 1811–1818.

MacKenzie, R.G., 2006. Obesity-associated mutations in the human melanocortin-4 receptor gene. Peptides 27, 395–403.

Meyre, D., Delplanque, J., Chevre, J.C., Lecoeur, C., Lobbens, S., Gallina, S., Durand, E., Vatin, V., Degraeve, F., Proenca, C., Gaget, S., Korner, A., Kovacs, P., Kiess, W., Tichet, J., Marre, M., Hartikainen, A.L., Horber, F., Potoczna, N., Hercberg, S., Levy-Marchal, C., Pattou, F., Heude, B., Tauber, M., McCarthy, M.I., Blakemore, A.I.F., Montpetit, A., Polychronakos, C., Weill, J., Coin, L.J.M., Asher, J., Elliott, P., Jarvelin, M.R., Visvikis-Siest, S., Balkau, B., Sladek, R., Balding, D., Walley, A., Dina, C., Froguel, P., 2009. Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. Nat. Genet. 41, 157–159.

Ng, M.C.Y., Tam, C.H.T., So, W.Y., Ho, J.S.K., Chan, A.W., Lee, H.M., Wang, Y., Lam, V.K.L., Chan, J.C.N., Ma, R.C.W., 2010. Implication of genetic variants near NEGR1, SEC16B, TMEM18, ETV5/DGKG, GNPDA2, LIN7C/BDNF, MTCH2, BCDIN3D/FAIM2, SH2B1, FTO, MC4R, and KCTD15 with obesity and type 2 diabetes in 7705 Chinese. J. Clin. Endocrinol. Metab. 95, 2418–2425.

North, K.E., Rose, K.M., Borecki, I.B., Oberman, A., Hunt, S.C., Miller, M.B., Blangero, J., Almasy, L., Pankow, J.S., 2004. Evidence for a gene on chromosome 13 influencing postural systolic blood pressure change and body mass index. Hypertension 43, 780–784.

Ohman, M., Oksanen, L., Kaprio, J., Koskenvuo, M., Mustajoki, P., Rissanen, A., Salmi, J., Kontula, K., Peltonen, L., 2000. Genome-wide scan of obesity in Finnish sibpairs reveals linkage to chromosome Xq24. J. Clin. Endocrinol. Metab. 85, 3183–3190.

Petry, C.J., Lopez-Bermejo, A., Diaz, M., Sebastiani, G., Ong, K.K.L., de Zegher, F., Dunger, D.B., Ibáñez, L., 2010. Association between a common variant near MC4R and change in body mass index develops by two weeks of age. Horm. Res. Paediatr. 73, 275–280.

Qi, L., Kraft, P., Hunter, D.J., Hu, F.B., 2008. The common obesity variant near MC4R gene is associated with higher intakes of total energy and dietary fat, weight change and diabetes risk in women. Hum. Mol. Genet. 17, 3502–3508.

Rankinen, T., Zuberi, A., Chagnon, Y.C., Weisnagel, S.J., Argyropoulos, G., Walts, B., Perusse, L., Bouchard, C., 2006. The human obesity gene map: the 2005 update. Obes. Res. 14, 529–644.

Redon, R., Ishikawa, S., Fitch, K.R., Feuk, L., Perry, G.H., Andrews, T.D., Fiegler, H., Shapero, M.H., Carson, A.R., Chen, W., Cho, E.K., Dallaire, S., Freeman, J.L., Gonzalez, J.R., Gratacos, M., Huang, J., Kalaitzopoulos, D., Komura, D., MacDonald, J.R., Marshall, C.R., Mei, R., Montgomery, L., Nishimura, K., Okamura, K., Shen, F., Somerville, M.J., Tchinda, J., Valsesia, A., Woodwark, C., Yang, F., Zhang, J., Zerjal, T., Zhang, J., Armengol, L., Conrad, D.F., Estivill, X., Tyler-Smith, C., Carter, N.P., Aburatani, H., Lee, C., Jones, K.W., Scherer, S.W., Hurles, M.E., 2006. Global variation in copy number in the human genome. Nature 444, 444–454.

Sarzynski, M.A., Jacobson, P., Rankinen, T., Carlsson, B., Sjostrom, L., Bouchard, C., Carlsson, L.M.S., 2010. Associations of markers in 11 obesity candidate genes with maximal weight loss and weight regain in the SOS bariatric surgery cases. Int. J. Obes. Online.

Saunders, C.L., Chiodini, B.D., Sham, P., Lewis, C.M., Abkevich, V., Adeyemo, A.A., de Andrade, M., Arya, R., Berenson, G.S., Blangero, J., Boehnke, M., Borecki, I.B., Chagnon, Y.C., Chen, W., Comuzzie, A.G., Deng, H.W., Duggirala, R., Feitosa, M.F., Froguel, P., Hanson, R.L., Hebebrand, J., Huezo-Dias, P., Kissebah, A.H., Li, W., Luke, A., Martin, L.J., Nash, M., Ohman, M., Palmer, L.J., Peltonen, L., Perola, M., Price, R.A., Redline, S., Srinivasan, S.R., Stern, M.P., Stone, S., Stringham, H., Turner, S., Wijmenga, C., Collier, D., 2007. Meta-analysis of genome-wide linkage studies in BMI and obesity. Obesity Res. 15, 2263–2275.

Scherag, A., Dina, C., Hinney, A., Vatin, V., Scherag, S., Vogel, C.I.G., Muller, T.D., Grallert, H., Wichmann, H.E., Balkau, B., Heude, B., Jarvelin, M.R., Hartikainen, A.L., Levy-Marchal, C., Weill, J., Delplanque, J., Korner, A., Kiess, W., Kovacs, P., Rayner, N.W., Prokopenko, I., McCarthy, M.I., Schafer, H., Jarick, I., Boeing, H., Fisher, E., Reinehr, T., Heinrich, J., Rzehak, P., Berdel, D., Borte, M., Biebermann, H., Krude, H., Rosskopf, D., Rimmbach, C., Rief, W., Fromme, T., Klingenspor, M., Schurmann, A., Schulz, N., Nothen, M.M., Muhleisen, T.W., Erbel, R., Jockel, K.H., Moebus, S., Boes, T., Illig, T., Froguel, P., Hebebrand, J., Meyre, D., 2010. Two new loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early-onset extreme obesity in French and German study groups. PLoS Genet. 6, e1000916.

Scuteri, A., Sanna, S., Chen, W.-M., 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, G.B., Fink, A.A., Weder, A.B., Cooper, R.S., Galan, P., Chakravarti, A., Schlessinger, D., Cao, A., Lakatta, E., Abecasis, G.R., 2007. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet. 3, e115.

Shi, J., Long, J., Gao, Y.T., Lu, W., Cai, Q., Wen, W., Zheng, Y., Yu, K., Xiang, Y.B., Hu, F.B., Zheng, W., Shu, X.O., 2010. Evaluation of genetic susceptibility loci for obesity in Chinese women. Am. J. Epidemiol. 172, 244–254.

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., 2007. Non-synonymous polymorphisms in melanocortin-4 receptor protect against obesity: the two facets of a Janus obesity gene. Hum. Mol. Genet. 16, 1837–1844.

Stutzmann, F., Tan, K., Vatin, V., Dina, C., Jouret, B.+., Tichet, J., Balkau, B., Potoczna, N., Horber, F., O'Rahilly, S., Farooqi, I.S., Froguel, P., Meyre, D., 2008. Prevalence of melanocortin-4 receptor deficiency in Europeans and their age-dependent penetrance in multigenerational pedigrees. Diabetes 57, 2511–2518.

Stutzmann, F., Cauchi, S., Durand, E., Calvacanti-Proenca, C., Pigeyre, M., Hartikainen, A.L., Sovio, U., Tichet, J., Marre, M., Weill, J., Balkau, B., Potoczna, N., Laitinen, J., Elliott, P., Jarvelin, M.R., Horber, F., Meyre, D., Froguel, P., 2009. Common genetic variation near MC4R is associated with eating behaviour patterns in European populations. Int. J. Obes. 33, 373–378.

Tabara, Y., Osawa, H., Guo, H., Kawamoto, R., Onuma, H., Shimizu, I., Takara, Y., Nishida, W., Yamamoto, M., Makino, H., Kohara, K., Miki, T., 2009. Prognostic significance of FTO genotype in the development of obesity in Japanese: the J-SHIPP study. Int. J. Obes. 33, 1243–1248.

Tenesa, A., Campbell, H., Theodoratou, E., Dunlop, L., Cetnarskyj, R., Farrington, S.M., Dunlop, M.G., 2008. Common genetic variants at the MC4R locus are associated with obesity, but not with dietary energy intake or colorectal cancer in the Scottish population. Int. J. Obes. 33, 284–288.

Thorleifsson, G., Walters, G.B., Gudbjartsson, D.F., Steinthorsdottir, V., Sulem, P., Helgadottir, A., Styrkarsdottir, U., Gretarsdottir, S., Thorlacius, S., Jonsdottir, I., Jonsdottir, T., Olafsdottir, E.J., Olafsdottir, G.H., Jonsson, T., Jonsson, F., Borch-Johnsen, K., Hansen, T., Andersen, G., Jorgensen, T., Lauritzen, T., Aben, K.K., Verbeek, A.L., Roeleveld, N., Kampman, E., Yanek, L.R., Becker, L.C., Tryggvadottir, L., Rafnar, T., Becker, D.M., Gulcher, J., Kiemeney, L.A., Pedersen, O., Kong, A., Thorsteinsdottir, U., Stefansson, K., 2009. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat. Genet. 41, 18–24.

Vaisse, C., Clement, K., Guy-Grand, B., Froguel, P., 1998. A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nat. Genet. 20, 113–114.

Vaisse, C., Clement, K., Durand, E., Hercberg, S., Guy-Grand, B., Froguel, P., 2000. Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. J. Clin. Invest. 106, 253–262.

Wang, C.L., Liang, L., Wang, H.J., Fen Fu, J., Hebebrand, J., Hinney, A., 2006. Several mutations in the melanocortin 4 receptor gene are associated with obesity in Chinese children and adolescents. J. Endocrinol. Invest. 29, 894–898.

Wang, D., Ma, J., Zhang, S., Hinney, A., Hebebrand, J., Wang, Y., Wang, H.J., 2010. Association of the MC4R V103I polymorphism with obesity: a Chinese case–control study and meta-analysis in 55,195 individuals. Obesity Res. 18, 573–579.

Willer, C.J., Speliotes, E.K., Loos, R.J.F., Li, S., Lindgren, C.M., Heid, I.M., Berndt, S.I., Elliot, A.L., Jackson, A.U., Lamina, C., Lettre, G., Lim, N., Lyon, H.N., McCarroll, S.A., Papadakis, K., Qi, L., Randall, J.C., Roccasecca, R.M., Sanna, S., Sheet, P., Weedon, M.N., Wheeler, E., Zhao, J.H., Jacobs, L.C., Prokopenko, I., Soranzo, N., Tanaka, T., Timpson, N.J., Almgren, P., Bennet, A., Bergman, R.N., Bingham, S.A., Bonnycastle, L.L., Brown, M., Burtt, N.P., Chines, P., Coin, L., Collins, F.S., Connell, J.M., Cooper, C., Smith, G.D., Dennison, E.M., Deodhar, P., Elliot, P., Erdos, M.R., Estrada, K., Evans, D.M., Gianniny, L., Gieger, C., Gillson, C.J., Guiducci, C., Hackett, R., Hadley, D., Hall, A.S., Havulinna, A.S., Hebebrand, J., Hofman, A., Isomaa, B., Jacobs, K.B., Johnson, T., Jousilahti, P., Jovanovic, Z., Khaw, K.T., Kraft, P., Kuokkanen, M., Kuusisto, J., Laitinen, J., Lakatta, E., Luan, J., Luben, R.N., Mangino, M., McArdle, W.L., Meitinger, T., Mulas, A., Munroe, P.B., Narisu, N., Ness, A.R., Northstone, K., O'Rahilly, S., Purmann, C., Rees, M.G., Ridderstrale, M., Ring, S.M., Rivadeneira, F., Ruokonen, A., Sandhu, M.S., Saramies, J., Scott, L.J., Scuteri, A., Silander, K., Sims, M.A., Song, K., Stephens, J., Stevens, S., Stringham, H.M., Tung, Y.C.L., Valle, T.T., van Duijn, C.M., Vimaleswaran, K.S., Vollenweider, P., Waeber, G., Wallace, C., Watanabe, R.M., Waterworth, D.M., Watkins, N., Witteman, J.C.M., Zeggini, E., Zhai, G., Zillikens, M.C., Altshuler, D.M., Caulfield, M.J., Chanock, S.J., Farooqi, I.S., Ferrucci, L., Guralnik, J.M., Hattersley, A.T., Hu, F.B., Jarvelin, M.R., Laakso, M., Mooser, V., Ong, K.K., Ouwehand, W.H., Salomaa, V., Samani, N.J., Spector, T.D., Tuomi, T., Tuomilehto, J., Uda, M., Uitterlinden, A.G., Wareham, N.J., Deloukas, P., Frayling, T.M., Groop, L.C., Hayes, R.B., Hunter, D.J., Mohlke, K.L., Peltonen, L., Schlessinger, D., Strachan, D.P., Wichmann, E.H., McCarthy, M.I., Boehnke, M., Barroso, I., Abecasis, G.R., Hirschhorn, J.N., 2009. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat. Genet. 41, 25–34.

Wu, L., Xi, B., Zhang, M., Shen, Y., Zhao, X., Cheng, H., Hou, D., Sun, D., Ott, J., Wang, X., Mi, J., 2010. Associations of six single nucleotide polymorphisms in obesity-related genes with body mass index and risk of obesity in the Chinese children. Diabetes 59, 3085–3089.

Xiang, Z., Litherland, S.A., Sorensen, N.B., Proneth, B., Wood, M.S., Shaw, A.M., Millard, W.J., Haskell-Luevano, C., 2006. Pharmacological characterization of 40 human melanocortin-4 receptor polymorphisms with the endogenous proopiomelanocortin-derived agonists and the agouti-related protein (AGRP) antagonist. Biochemistry 45, 7277–7288.

Yeo, G.S.H., Farooqi, I.S., Aminian, S., Halsall, D., Stanhope, R.G., O'Rahilly, S., 1998. A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nat. Genet. 20, 111–112.

Young, E.H., Wareham, N.J., Farooqi, S., Hinney, A., Hebebrand, J., Scherag, A., O'Rahilly, S., Barroso, I., Sandhu, M.S., 2007. The V103I polymorphism of the MC4R gene and obesity: population based studies and meta-analysis of 29,563 individuals. Int. J. Obes. 31, 1437–1441.

Yurtcu, E., Yilmaz, A., Ozkurt, Z., Kolukisa, E., Yilmaz, M., Keles, H., Ergun, M., Yetkin, I., Menevse, A., 2009. Melanocortin-4 receptor gene polymorphisms in obese patients. Biochem. Genet. 47, 295–300.

Zhao, J., Bradfield, J.P., Li, M., Wang, K., Zhang, H., Kim, C.E., Annaiah, K., Glessner, J.T., Thomas, K., Garris, M., Frackelton, E.C., Otieno, F.G., Shaner, J.L., Smith, R.M., Chiavacci, R.M., Berkowitz, R.I., Hakonarson, H., Grant, S.F.A., 2009. The role of obesity-associated loci identified in genome-wide association studies in the determination of pediatric BMI. Obesity Res. 17, 2254–2257.

Zobel, D.P., Andreasen, C.H., Grarup, N., Eiberg, H., Sorensen, T.I.A., Sandbaek, A., Lauritzen, T., Borch-Johnsen, K., Jorgensen, T., Pedersen, O., Hansen, T., 2009. 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 58, 757–764.