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Review

Association study of POMC variants with body composition measures and nutrient choice

Andrew Ternouth [a,1], Marek K. Brandys [b,c,1], Yvonne T. van der Schouw [d], Judith Hendriks [b], John-Olov Jansson [e], David Collier [a], Roger A. Adan [b,c,*]

[a] Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, United Kingdom
[b] Rudolf Magnus Institute of Neuroscience, Department of Neuroscience & Pharmacology, University Medical Center Utrecht, University of Utrecht, Utrecht, The Netherlands
[c] Rintveld Centre for Eating Disorders, Altrecht Mental Health Institute, Zeist, The Netherlands
[d] The Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands
[e] Department of Neuroscience and Physiology/Endocrinology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

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ABSTRACT

Genome linkage scans and candidate gene studies have implicated the pro-opiomelanocortin (POMC) locus in traits related to food intake, metabolic function, and body mass index. Here we investigate single nucleotide polymorphisms at the POMC locus in order to evaluate the influence of its genetic variance on body fat distribution and diet in a sample of middle-aged men from The Netherlands. 366 Dutch males from the Hamlet cohort were asked detailed questions about food choice, nutrient intake and exercise. Furthermore, their weight and body fat composition were measured. Each cohort member was genotyped for a set of single nucleotide polymorphisms (SNPs) at the POMC locus. Regression analysis, adjusted for several covariates, was used to test for the association between genetic variants and the phenotypes measured. POMC variation was associated with waist:hip ratio, visceral fat and abdominal fat (rs6713532, P = 0.020, 0.019, and 0.021, respectively), and nutrient choice (rs1042571, P = 0.034), but in light of limited power and multiple testing these results should be taken with caution. POMC is a strong candidate for involvement in appetite regulation as supported by animal, physiological, and genetic studies and variation at the POMC locus may affect an individual's energy intake which in turn leads to variation in body composition and body fat.

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* Corresponding author. The Rudolf Magnus Institute of Neuroscience, UMC Utrecht, STR 5.203, Heidelberglaan 100, P.O. Box 85500, 3508 GA Utrecht, The Netherlands. Tel.: +31 88 75 68517.
E-mail address: r.a.h.adan@umcutrecht.nl (R.A. Adan).
[1] These authors contributed equally.

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1. Introduction

The brain melanocortin system has an established role in the physiology of weight regulation and consists of the pro-opiomelanocortin (POMC) gene, which encodes the melanocortins (MCs) and β-endorphin, the melanocortin receptor genes and the Agouti-related protein (AgRP) gene. POMC, MC_3 and MC_4 receptor knockout mice are all obese. In addition to obesity, POMC deficient mice display strongly increased weight gain on a high fat diet, while eating only a modest amount more than the wild-type mice (Yaswen et al., 1999). Genetic as well as pharmacological studies in rodents indicate that reduction of MC receptor activity is associated with increased fat intake on choice diets (Hagan et al., 2001; Koegler et al., 1999). Activation of the MC system results in reduction of fat intake, an effect which is dependent on the MC_4 receptor (Samama et al., 2003). This implication in food intake and fat deposition, coming from rodent studies, makes genes belonging to the melanocortin system good candidates for human association studies with nutrient choice and anthropometric measures. Indeed, the melanocortin system is implicated in the development of obesity in humans, as mutations in several of its component genes are strongly associated with rare, penetrant, monogenic forms of obesity (Farooqi, 2006). For example heterozygous mutations in the melanocortin-4 receptor gene are the most common monogenic form of severe obesity in children, affecting about 2.6% of the population, and mis-sense mutations in POMC can result in monogenic sever early-onset obesity (Farooqi et al., 2006; Challis et al., 2002; Krude et al., 1998).

A common MC_4 receptor polymorphism has been associated with body mass index (BMI) (Heid et al., 2005) and a common variation in the AgRP gene has been associated with leanness and fat intake (Loos et al., 2005; Bonilla et al., 2006; Marks et al., 2004). For POMC itself, linkage studies have implicated its chromosomal locus at 2p23 in nutrient intake (Cai et al., 2004), fat mass (Comuzzie et al., 1997), and obesity (Hager et al., 1998). Other related phenotypes such as blood pressure (Rice et al., 2002), leptin levels (Suviolahti et al., 2003), physical activity (Simonen et al., 2003), and metabolic syndrome (Loos et al., 2003) have also been linked to the POMC region. Other studies found an association of the POMC gene with BMI, weight, and total fat (Chen et al., 2005) (in a female population), BMI, waist:hip ratio, subcutaneous fat, and visceral fat (Sutton et al., 2005) (in a Hispanic population), and waist:hip ratio (in a general UK population) (Baker et al., 2005). There have also been negative reports which do not show evidence for linkage or association with BMI or related phenotypes at the POMC locus (Suviolahti et al., 2003; Delplanque et al., 2000). There has been no attempt to associate macronutrient intake with genetic variation in the POMC gene. The strength of the current study is the detailed measurement of the body composition and macronutrient intake phenotypes available in the Hamlet cohort, which may help to overcome some of the difficulties potentially arising from the use of indirect phenotypes such as BMI.

The present study was designed to replicate associations between single nucleotide polymorphisms at the POMC locus, obesity phenotypes (BMI, total fat mass and waist:hip ratio) and measures of macronutrient intake.

2. Materials and methods

2.1. Subjects

We conducted a population-based, cross-sectional, single-centre study among 400 men aged between 40 and 80 years and living independently. The subjects and methods of recruitment have been described elsewhere (Muller et al., 2005). Data collection took place during two interviews in which the data were self reported or measured by a trained clinician. All participants gave written informed consent before enrolment in the study, and the study was approved by the institutional review board of the University Medical Center Utrecht. The data were collected between March 2001 and April 2002.

2.2. Phenotypes

2.2.1. BMI

Height and weight were measured in a standing position without shoes. BMI was calculated as the weight in kilograms divided by the square of the height in metres.

2.2.2. Waist:hip ratio

Waist circumference was measured midway between the lower rib margin and the iliac crest with the subject in a standing position. The hip circumference was measured in the same standing position at the level of the greater trochanter. Each reading was taken twice and the mean used for calculation of the ratio.

2.2.3. Visceral and subcutaneous fat

Visceral fat were measured using ultrasonography (Stolk et al., 2001) with an HDI 3000 (Philips Medical Systems, Eindhoven, The Netherlands) using a C 4-2 transducer. The distances between the posterior edge of the abdominal muscles and the lumbar spine or psoas muscles are measured using electronical callipers. For all images the transducer is placed on a straight line drawn between the left and right midpoint of the lower rib and the iliac crest. Distances are measured from three different angles: medial, left and right for intraabdominal fat mass and medial for subcutaneous fat mass in threefold. Measurements are made at the end of quiet expiration, applying minimal pressure without displacement of intraabdominal contents as observed by ultrasound image. Visceral fat was measured as the distance between the skin and the linea alba and intraabdominal fat as the distance between the peritoneum and the lumbar spine. Abdominal fat was calculated as the sum of the visceral and subcutaneous fat.

2.2.4. Total fat mass, and lean mass

Total and trunk lean body mass, and fat mass were measured using dual-energy x-ray absorptiometry (Hologic QDR 1000 densitometer, Hologic Inc., Waltham, MA, USA). Quality assurance for dual-energy x-ray absorptiometry, including calibration, was performed every morning, using the standard provided by the manufacturer.

2.2.5. Physical exercise

During the interview, the subjects completed the Voorrips questionnaire (Voorrips et al., 1991) which provides a validated reliable measure of physical activity.

2.2.6. Nutrient intake

A validated food frequency questionnaire (FFQ) was administered, designed to estimate the regular intake of 178 food items in the year before enrolment (Ocke et al., 1997a,b). From this questionnaire we calculated the daily total energy, protein, fat, carbohydrate, and alcohol intake. From these data we additionally calculated fat:protein, fat:carbohydrate, and carbohydrate:protein ratios.

2.3. Choice of genetic markers

Haplotype-tagging SNPs were selected in the coding sequence and up- and downstream of the POMC gene (UCSC browser coordinates chr2:25,292,212-25,303,950, May 2004 freeze), using HapMap (Haploview 3.32) with r^2 threshold set at >0.80, using the data available at the time of the study design (December 2006). Four SNPs were used to tag the POMC locus, although only three (rs6713532, rs6545975, and rs934778) were genotyped because a Taqman assay could not be designed for rs7565877. Secondly, a

literature search was conducted to investigate POMC SNPs which had already been associated with feeding or fat related phenotypes with a minor allele frequency >0.05 (Cai et al., 2004; Comuzzie et al., 1997; Hager et al., 1998; Baker et al., 2005). Three SNPs were chosen: rs1009388, rs1042571, and rs1866146 (Supplementary Fig. 1).

2.4. Genotyping

Genomic DNA had previously been isolated from peripheral lymphocytes using a high salt extraction procedure. DNA was not available for two men, and they were therefore excluded.

Genotyping was performed using the Taqman assay by design or assay on demand genotyping kits (http://www.appliedbiosystems.com) using standard protocol. Fluorescence intensities were quantified using an Applied Biosystems HT7900.

2.5. Statistical analysis

Phenotypes were tested for normality of distribution and, where necessary, logarithmic (natural) or square-root transformations were applied. SNPs were tested for violation of the Hardy–Weinberg equilibrium with the exact test (Wigginton et al., 2005). Association of SNPs with quantitative traits was tested via linear regression with adjustment for several covariates, assuming an additive model of genetic effect (allele dosage).

Analyses were conducted with Plink (Purcell et al., 2007) and SPSS 15.0 (SPSS, Chicago, Illinois).

3. Results

General characteristics of the subjects are presented in Table 1.

All 6 SNPs were in Hardy–Weinberg equilibrium (exact test, (Wigginton et al., 2005)) (Table 2). 32 out of 398 subjects were excluded due to low genotyping rate (more than 2 missing genotypes). Total genotyping rate in the remaining individuals was 0.89.

The successfully genotyped SNPs were re-evaluated using the Tagger program (http://www.broad.mit.edu/mpg/tagger/) (de Bakker et al., 2005) to estimate the amount of genetic variation captured. Applying a r^2 threshold of 0.8 and using the pairwise method, 4 HapMap SNPs genotyped in this study cover about 57% of the genetic variation on the POMC gene locus (43% when the region is extended by 5 kb at 5′ and 3′ ends). One Hapmap SNP had been excluded from analysis as no Taqman assay was available. Two genotyped SNPs (rs1042571 and rs1009388) were not in Hapmap. Their presence increases the extent of captured variance but it is difficult to quantify it.

Table 3 displays r^2 values between the SNPs. The highest r^2 value was 0.48 for rs1042571 and rs1009388. The values in this dataset are generally low or very low, meaning that each SNP contributes independent genetic information.

Results of the genetic association analysis are shown in Table 4, which displays P values for association, as determined by linear regression modelling with adjustment for age, education, physical activity, alcohol intake and BMI. The strongest associations were found for rs6713532 and the waist:hip ratio, visceral fat and abdominal fat (i.e. sum of visceral and subcutaneous fat). These associations became weaker when not controlled for BMI. SNP rs1042571 was associated with a ratio between fat and protein content in the diet. Furthermore, 2 other SNPs (rs6545975, and rs1009388) displayed suggestive association with investigated phenotypes (Table 4).

Interestingly, when, for exploratory purposes, an additional covariate – total lean mass – was controlled for, the three significant association signals of rs6713532 became slightly more pronounced and two association signals for rs6545975 became significant (with waist:hip ratio, P = 0.043, and visceral fat, P = 0.049; Supplementary Table 2).

Table 5 presents means and SDs of phenotypes per each genotype for SNPs significantly associated in a linear regression model, together with beta-coefficients. Furthermore, SNPs rs6713532 and rs1866146 were tested in a sample of 938 young men (mean age = 18.9) from the GOOD study (genotyped on Human610K Illumina array) (Andersson et al., 2009). Available phenotypes allowed testing for the association with BMI and total fat mass (both log-transformed), adjusted for age. There was no association with either of the phenotypes (Supplementary Table 1).

Table 1
Descriptive statistics detailing demographic, body composition and feeding phenotypes.

Phenotype	N[a]	Mean	Median	Standard deviation	Minimum	Maximum
Demographic						
Age (years)	366	60.53	61.00	11	40	80
Body fat and composition						
BMI (kg/m^2)	366	26.26	26.14	3.38	17.29	43.31
Waist:hip ratio	366	0.98	0.98	0.06	0.77	1.17
Visceral fat (cm)	366	7.53	7.10	2.18	3.3	14.75
Subcutaneous fat (cm)	366	2.65	2.60	0.86	0.3	6.5
Abdominal fat (cm)	366	10.18	9.78	2.43	4.95	20.45
Total fat mass (kg)	346	17.1	16.23	5.48	5.91	49.33
Total lean mass (kg)	346	61.52	61.34	7.17	44.81	88.77
Energy Balance						
Physical activity	363	18.08	17.67	7.47	1.1	43.95
Total energy intake (kcal)	364	2239.81	2216.28	533.36	735.79	4276.01
Protein intake (g/day)	364	83.63	81.98	20.73	38.06	162.46
Fat intake (g/day)	364	89.51	87.29	27.37	29.71	193.53
Carbohydrate intake (g/day)	364	241.39	232.38	66.91	69.75	605.24
Alcohol intake (g/day)	364	19.15	13.56	19.48	0	153.37
Nutrient choice						
Fat:protein ratio	364	1.07	1.07	0.19	0.61	1.75
Fat:carbohydrate ratio	364	0.38	0.37	0.1	0.13	0.79
Carbohydrate: protein ratio	364	2.94	2.88	0.66	1.38	5.94

[a] Sample consisted of males.

Table 2
Genotype distributions and Hardy–Weinberg equilibrium.

SNP	Polymorphism (minor allele first)	Genotype AA count (%)	Genotype AB count (%)	Genotype BB count (%)	Hardy–Weinberg P (exact test)
rs1866146	G/A	43 (12.1%)	167 (47.0%)	145 (40.8%)	0.73
rs1042571	T/C	9 (2.8%)	80 (24.7%)	234 (72.4%)	0.51
rs6713532	C/T	18 (5.1%)	129 (36.5%)	206 (58.3%)	0.77
rs6545975	C/T	58 (18.4%)	143 (45.5%)	113 (35.9%)	0.30
rs934778	G/A	4 (1.5%)	37 (13.7%)	229 (84.8%)	0.10
rs1009388	G/C	26 (7.7%)	122 (36.3%)	188 (55.9%)	0.32

Table 3
r^2 as a measure of linkage disequilibrium.

SNP	rs1866146	rs1042571	rs6713532	rs6545975	rs934778	rs1009388
rs1866146	–	0.10	0.38	0.00	0.04	0.17
rs1042571		–	0.03	0.06	0.10	0.48
rs6713532			–	0.11	0.00	0.09
rs6545975				–	0.00	0.15
rs934778					–	0.04
rs1009388						–

Table 4
P values<0.2 from the linear regression modelling of the association between POMC variants and quantitative phenotypes (additive model). Adjusted for age, education, physical activity, alcohol intake and BMI (BMI as a covariate was excluded when testing for the association with BMI).

Phenotype/SNP	rs1866146	rs1042571	rs6713532	rs6545975	rs934778	rs1009388
Body fat and composition						
BMI (kg/m2)[a]						0.104
Waist:hip ratio			**0.020**	*0.061*	0.141	
Visceral fat (cm)[a]			**0.019**	*0.090*	0.101	
Subcutaneous fat (cm)				*0.078*		
Abdominal fat (cm)[a]			**0.021**		0.133	
Total fat mass (kg)						
Total lean mass (kg)[a]	0.115				0.167	
Energy Balance						
Total energy intake (kcal)[a]			0.196			0.177
Protein intake (g/day)[b]			0.183	0.118		
Fat intake (g/day)[a]						*0.080*
Carbohydrate intake (g/day)[b]			*0.087*			
Nutrient Choice						
Fat:protein ratio[a]		**0.034**			0.147	*0.063*
Fat:carbohydrate ratio[a]		0.136				0.125
Carbohydrate:protein ratio[a]	0.166					

P<0.1 in italics; P<0.05 in bold.
[a] Natural log-transformed.
[b] Square-root transformed.

P values in this study are uncorrected for multiple testing. For a given sample size (n = 366), in order to have 80% statistical power to detect a true association, a SNP associated with a quantitative trait would have to explain about 2% of the total variance of the trait (assuming alfa at 0.05 and a minor allele frequency of 25%), testing for additive effects of the quantitative trait locus only.

4. Discussion

This study should be viewed in light of several limitations. P values are uncorrected for multiple testing, and it is clear that none would 'survive' such a correction (even though some of the tested phenotypes are highly correlated; Supplementary Table 1). Furthermore, our statistical power was not sufficient to detect minor genetic effects, if such exist. Also a genotyping rate of 90% and an incomplete coverage of genetic variation on the POMC locus are limitations. For the reasons above, and since case-only studies are particularly prone to spurious genetic associations (Sullivan, 2007), the current results should be taken with caution.

We have controlled for similar covariates as in Baker et al. (2005), except for smoking, for which no data were available. The association of rs1042571 and rs1009388 with the waist:hip ratio reported by Baker et al. (2005) was not confirmed in our study (SNP rs1009388 has also been tested in (Bienertova-Vasku et al., 2010) where no association with BMI or waist:hip ratio has been found). SNP rs1009388 had suggestive P values in tests with fat intake and fat:protein ratio and rs1042571 was associated with fat:protein ratio, indicating that effects of those variants may be stronger on food choice than on body composition. It should be noted that the sample in the current study was different from Baker et al. with respect to sex (males only), age and body composition measures.

We were able to test two SNPs from this study (rs6713532, and rs1866146) in an independent sample of 938 young men. There was no association with either the BMI or the total fat mass. It was not possible to test for association with abdominal or visceral fat or waist:hip ratio — phenotypes that displayed strongest signals in the current study. This shows that the association of rs6713532 with measures of fat and waist:hip ratio is either a false-positive or it is highly specific to those phenotypes or the studied population.

The strength of the Hamlet cohort lies in the availability of a number of potentially relevant phenotypes. Studies have suggested that the measurement of the waist circumference or waist:hip ratio, as indicators of abdominal obesity, may be better disease risk predictors than the BMI (Yusuf et al., 2005; Wang et al., 2005; Noble, 2001). Moreover, visceral fat levels are more strongly related to poor outcome than subcutaneous fat levels (Albu et al., 2000). The current study shows that the waist:hip ratio, abdominal fat and visceral fat may be better measures for genetic studies of obesity and body

Table 5
Means and SDs of the phenotype per each genotype, only for significant associations.

	Mean (SD) for the major homozygote	Mean (SD) for the heterozygote	Mean (SD) for the minor homozygote	Beta-coefficient		Variance explained	P
		rs6713532		Unstandardized	Standardized		
Phenotype							
Waist:hip ratio	0.98 (0.06)	0.97 (0.06)	0.94 (0.05)	−0.014	−0.09	0.82%	0.020
Visceral fat (cm)[a]	7.76 (2.29)	7.41 (2.06)	6.55 (1.63)	−0.065	−0.089	0.79%	0.019
Abdominal fat (cm)[a]	10.4 (2.59)	10.08 (2.24)	8.96 (1.82)	−0.060	−0.078	0.61%	0.021
		rs1042571					
Phenotype							
Fat:protein ratio[a]	1.06 (0.19)	1.08 (0.18)	1.23 (0.2)	0.071	0.119	1.41%	0.034

For log-transformed variables, one minor allele increase is associated with an average of (100 × unstandardized beta) percent increase in the phenotype. For the non-transformed variable (waist:hip ratio), unstandardized beta-coefficient represents an average increase in the phenotype with each additional minor allele (mean for the major homozygote represents the intercept).
[a] Log-transformed variables were used.

composition than the commonly used BMI, which is more likely influenced by factors other than the fat tissue, such as muscularity or bone structure. This is supported by our exploratory analysis with additional adjustment for total lean mass, which resulted in augmentation of the association signals.

Association of SNPs near to the MC_4 receptor gene is one of the most robust findings in genetic studies of body composition (Loos et al., 2008), (Chambers et al., 2008; Willer et al., 2009). POMC is located upstream of MC_4 in a genetic pathway and our results further support the involvement of the common variation in the melanocortin system in regulation of fat mass in humans, additionally suggesting that the effects on body composition may be mediated by the association of food choice and nutritional content of the diet. This study also shows that research could benefit from investigating phenotypes alternative to BMI (such as waist:hip ratio) and adjusting for confounders which are likely to obfuscate the relation between genetic variants and levels of fatness.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.ejphar.2010.10.112.

References

Albu, J.B., Kovera, A.J., Johnson, J.A., 2000. Fat distribution and health in obesity. Ann. NY Acad. Sci. 904, 491–501.

Andersson, N., Strandberg, L., Nilsson, S., Ljungren, O., Karlsson, M.K., Mellstrom, D., Lorentzon, M., Ohlsson, C., Jansson, J., 2009. Variants of the interleukin-1 receptor antagonist gene are associated with fat mass in men. Int. J. Obes. (Lond) 33, 525–533.

Baker, M., Gaukrodger, N., Mayosi, B.M., Imrie, H., Farrall, M., Watkins, H., Connell, J.M.C., Avery, P.J., Keavney, B., 2005. Association between common polymorphisms of the proopiomelanocortin gene and body fat distribution: a family study. Diabetes 54, 2492–2496.

Bienertova-Vasku, J., Bienert, P., Forejt, M., Tomandl, J., Brazdova, Z., Vasku, A., 2010. Genotype x nutrient association of common polymorphisms in obesity-related genes with food preferences and time structure of energy intake. Br. J. Nutr. 103, 352–359.

Bonilla, C., Panguluri, R.K., Taliaferro-Smith, L., Argyropoulos, G., Chen, G., Adeyemo, A.A., Amoah, A., Owusu, S., Acheampong, J., Agyenim-Boateng, K., Eghan, B.A., Oli, J., Okafor, G., Abbiyesuku, F., Johnson, T., Rufus, T., Fasanmade, O., Chen, Y., Collins, F.S., Dunston, G.M., Rotimi, C., Kittles, R.A., 2006. Agouti-related protein promoter variant associated with leanness and decreased risk for diabetes in West Africans. Int. J. Obes. (Lond) 30, 715–721.

Cai, G., Cole, S.A., Bastarrachea, R.A., Bastarrachea-Sosa, R.A., Maccluer, J.W., Blangero, J., Comuzzie, A.G., 2004. Quantitative trait locus determining dietary macronutrient intakes is located on human chromosome 2p22. Am. J. Clin. Nutr. 80, 1410–1414.

Challis, B.G., Pritchard, L.E., Creemers, J.W.M., Delplanque, J., Keogh, J.M., Luan, J., Wareham, N.J., Yeo, G.S.H., Bhattacharyya, S., Froguel, P., White, A., Farooqi, I.S., O'Rahilly, S., 2002. A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism. Hum. Mol. Genet. 11, 1997–2004.

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.

Chen, Y., Snieder, H., Wang, X., Kaviya, B., McCaffrey, C., Spector, T.D., Carter, N.D., O'Dell, S.D., 2005. Proopiomelanocortin gene variants are associated with serum leptin and body fat in a normal female population. Eur. J. Hum. Genet. 13, 772–780.

Comuzzie, A.G., Hixson, J.E., Almasy, L., Mitchell, B.D., Mahaney, M.C., Dyer, T.D., Stern, M.P., MacCluer, J.W., Blangero, J., 1997. A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. Nat. Genet. 15, 273–276.

de Bakker, P.I.W., Yelensky, R., Pe'er, I., Gabriel, S.B., Daly, M.J., Altshuler, D., 2005. Efficiency and power in genetic association studies. Nat. Genet. 37, 1217–1223.

Delplanque, J., Barat-Houari, M., Dina, C., Gallina, P., Clement, K., Guy-Grand, B., Vasseur, F., Boutin, P., Froguel, P., 2000. Linkage and association studies between the proopiomelanocortin (POMC) gene and obesity in Caucasian families. Diabetologia 43, 1554–1557.

Farooqi, I.S., 2006. Monogenic human obesity syndromes. Prog. Brain Res. 153, 119–125.

Farooqi, I.S., Drop, S., Clements, A., Keogh, J.M., Biernacka, J., Lowenbein, S., Challis, B.G., O'Rahilly, S., 2006. Heterozygosity for a POMC-null mutation and increased obesity risk in humans. Diabetes 55, 2549–2553.

Hagan, M.M., Rushing, P.A., Benoit, S.C., Woods, S.C., Seeley, R.J., 2001. Opioid receptor involvement in the effect of AgRP- (83–132) on food intake and food selection. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280, 814–821.

Hager, J., Dina, C., Francke, S., Dubois, S., Houari, M., Vatin, V., Vaillant, E., Lorentz, N., Basdevant, A., Clement, K., Guy-Grand, B., Froguel, P., 1998. A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. Nat. Genet. 20, 304–308.

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

Koegler, F.H., Schaffhauser, R.O., Mynatt, R.L., York, D.A., Bray, G.A., 1999. Macronutrient diet intake of the lethal yellow agouti (Ay/a) mouse. Physiol. Behav. 67, 809–812.

Krude, H., Biebermann, H., Luck, W., Horn, R., Brabant, G., Gruters, A., 1998. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat. Genet. 19, 155–157.

Loos, R.J.F., Katzmarzyk, P.T., Rao, D.C., Rice, T., Leon, A.S., Skinner, J.S., Wilmore, J.H., Rankinen, T., Bouchard, C., 2003. Genome-wide linkage scan for the metabolic syndrome in the HERITAGE family study. J. Clin. Endocrinol. Metab. 88, 5935–5943.

Loos, R.J.F., Rankinen, T., Rice, T., Rao, D.C., Leon, A.S., Skinner, J.S., Bouchard, C., Argyropoulos, G., 2005. Two ethnic-specific polymorphisms in the human Agouti-related protein gene are associated with macronutrient intake. Am. J. Clin. Nutr. 82, 1097–1101.

Loos, R.J.F., Lindgren, C.M., Li, S., Wheeler, E., Zhao, J.H., Prokopenko, I., Inouye, M., Freathy, R.M., Attwood, A.P., Beckmann, J.S., Berndt, S.I., Bergmann, S., Bennett, A.J., Bingham, S.A., Bochud, M., Brown, M., Cauchi, S., Connell, J.M., Cooper, C., Smith, G.D., Day, I., Dina, C., De, S., Dermitzakis, E.T., Doney, A.S.F., Elliott, K.S., Elliott, P., Evans, D.M., Sadaf Farooqi, I., Froguel, P., Ghori, J., Groves, C.J., Gwilliam, R., Hadley, D., Hall, A.S., Hattersley, A.T., Hebebrand, J., Heid, I.M., Herrera, B., Hinney, A., Hunt, S.E., Jarvelin, M., Johnson, T., Jolley, J.D.M., Karpe, F., Keniry, A., Khaw, K., Luben, R.N., Mangino, M., Marchini, J., McArdle, W.L., McGinnis, R., Meyre, D., Munroe, P.B., Morris, A.D., Ness, A.R., Neville, M.J., Nica, A.C., Ong, K.K., O'Rahilly, S., Owen, K.R., Palmer, C.N.A., Papadakis, K., Potter, S., Pouta, A., Qi, L., Randall, J.C., Rayner, N.W., Ring, S.M., Sandhu, M.S., Scherag, A., Sims, M.A., Song, K., Soranzo, N., Speliotes, E.K., Syddall, H.E., Teichmann, S.A., Timpson, N.J., Tobias, J.H., Uda, M., Ganz Vogel, C.I., Wallace, C., Waterworth, D.M., Weedon, M.N., Willer, C.J., Wraight, V.L., 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.

Marks, D.L., Boucher, N., Lanouette, C., Perusse, L., Brookhart, G., Comuzzie, A.G., Chagnon, Y.C., Cone, R.D., 2004. Ala67Thr polymorphism in the Agouti-related peptide gene is associated with inherited leanness in humans. Am. J. Med. Genet. A 126A, 267–271.

Muller, M., Grobbee, D.E., den Tonkelaar, I., Lamberts, S.W.J., van, d.S., 2005. Endogenous sex hormones and metabolic syndrome in aging men. J. Clin. Endocrinol. Metab. 90, 2618–2623.

Noble, R.E., 2001. Waist-to-hip ratio versus BMI as predictors of cardiac risk in obese adult women. West. J. Med. 174, 240–241.

Ocke, M.C., Bueno-de-Mesquita, H.B., Pols, M.A., Smit, H.A., van Staveren, W.A., Kromhout, D., 1997a. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int. J. Epidemiol. 26 (Suppl. 1), 49–58.

Ocke, M.C., Bueno-de-Mesquita, H.B., Goddijn, H.E., Jansen, A., Pols, M.A., van Staveren, W.A., Kromhout, D., 1997b. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int. J. Epidemiol. 26 (Suppl. 1), 37–48.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., Sham, P.C., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575. doi:10.1086/519795.

Rice, T., Rankinen, T., Chagnon, Y.C., Province, M.A., Perusse, L., Leon, A.S., Skinner, J.S., Wilmore, J.H., Bouchard, C., Rao, D.C., 2002. Genomewide linkage scan of resting blood pressure: HERITAGE family study. Health, risk factors, exercise training, and genetics. Hypertension 39, 1037–1043.

Samama, P., Rumennik, L., Grippo, J.F., 2003. The melanocortin receptor MCR4 controls fat consumption. Regul. Pept. 113, 85–88.

Simonen, R.L., Rankinen, T., Perusse, L., Rice, T., Rao, D.C., Chagnon, Y., Bouchard, C., 2003. Genome-wide linkage scan for physical activity levels in the Quebec family study. Med. Sci. Sports Exerc. 35, 1355–1359.

Stolk, R.P., Wink, O., Zelissen, P.M., Meijer, R., van Gils, A.P., Grobbee, D.E., 2001. Validity and reproducibility of ultrasonography for the measurement of intra-abdominal adipose tissue. Int. J. Obes. Relat. Metab. Disord. 25, 1346–1351.

Sullivan, P.F., 2007. Spurious genetic associations. Biol. Psychiatry 61, 1121–1126. doi:10.1016/j.biopsych.2006.11.010.

Sutton, B.S., Langefeld, C.D., Williams, A.H., Norris, J.M., Saad, M.F., Haffner, S.M., Bowden, D.W., 2005. Association of proopiomelanocortin gene polymorphisms with obesity in the IRAS family study. Obes. Res. 13, 1491–1498.

Suviolahti, E., Ridderstrale, M., Almgren, P., Klannemark, M., Melander, O., Carlsson, E., Carlsson, M., Hedenbro, J., Orho-Melander, M., 2003. Pro-opiomelanocortin gene is associated with serum leptin levels in lean but not in obese individuals. Int. J. Obes. Relat. Metab. Disord. 27, 1204–1211.

Voorrips, L.E., Ravelli, A.C., Dongelmans, P.C., Deurenberg, P., Van Staveren, W.A., 1991. A physical activity questionnaire for the elderly. Med. Sci. Sports Exerc. 23, 974–979.

Wang, Y., Rimm, E.B., Stampfer, M.J., Willett, W.C., Hu, F.B., 2005. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. Am. J. Clin. Nutr. 81, 555–563.

Wigginton, J.E., Cutler, D.J., Abecasis, G.R., 2005. A note on exact tests of Hardy–Weinberg equilibrium. Am. J. Hum. Genet. 76, 887–893.

Willer, C.J., Speliotes, E.K., Loos, R.J., Li, S., Lindgren, C.M., Heid, I.M., Berndt, S.I., Elliott, 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., Scheet, P., Weedon, M.N., Wheeler, E., Zhao, J.H., Jacobs, L.C., Prokopenko, I., Soranzo, N., Tanaka, T., Timpson, N.J., Almgren, P., Bennett, 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., Elliott, 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.G., 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., Valle, T.T., Van Duijn, C.M., Vimaleswaran, K.S., Vollenweider, P., Waeber, G., Wallace, C., Watanabe, R.M., Waterworth, D.M., Watkins, N., Wellcome Trust Case Control Consortium, Witteman, J.C., Zeggini, E., Zhai, G., Zillikens, M.C., Altshuler, D., 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, H.E., McCarthy, M.I., Boehnke, M., Barroso, I., Abecasis, G.R., Hirschhorn, J.N., Genetic Investigation of ANthropometric Traits Consortium, 2009. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat. Genet. 41, 25–34. doi:10.1038/ng.287.

Yaswen, L., Diehl, N., Brennan, M.B., Hochgeschwender, U., 1999. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. Nat. Med. 5, 1066–1070.

Yusuf, S., Hawken, S., Ounpuu, S., Bautista, L., Franzosi, M.G., Commerford, P., Lang, C.C., Rumboldt, Z., Onen, C.L., Lisheng, L., Tanomsup, S., Wangai, P., Razak, F., Sharma, A.M., Anand, S.S., 2005. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case–control study. Lancet 366, 1640–1649.