Brief Genetics Report

Heterozygosity for a *POMC*-Null Mutation and Increased Obesity Risk in Humans

I. Sadaf Farooqi,[1] Stenvert Drop,[2] Agnes Clements,[3] Julia M. Keogh,[1] Joanna Biernacka,[4] Sarah Lowenbein,[1] Benjamin G. Challis,[1] and Stephen O'Rahilly[1]

Congenital deficiency of proopiomelanocortin (POMC) results in a syndrome of hypoadrenalism, severe obesity, and altered skin and hair pigmentation. The concept that subtle variation in POMC expression and/or function might contribute to common obesity is suggested by studies reporting linkage of obesity-related traits to a locus on chromosome 2p22 encompassing the *POMC* gene. We identified a novel homozygous frameshift (C6906del) mutation in *POMC* in a child of Turkish origin with severe obesity and hypoadrenalism. This mutation would be predicted to lead to the loss of all POMC-derived peptides. The availability of a large extended pedigree provided the opportunity to address whether loss of one copy of the *POMC* gene was sufficient to alter obesity risk. Twelve relatives were heterozygous for the mutation and 7 were wild type. Of the heterozygotes, 11 of 12 heterozygotes were obese or overweight compared with only 1 of 7 of the wild-type relatives. The mean BMI SD score was 1.7 ± 0.5 in heterozygotes and 0.4 ± 0.4 in the wild-type relatives. Parametric linkage analysis of the trait "overweight" provided statistically significant evidence of linkage with this locus, with a maximum "location score" (comparable with multipoint logarithm of odds scores) of 3.191. We conclude that loss of one copy of the *POMC* gene predisposes to obesity in humans. Thus, genetic variants having relatively subtle effects on POMC expression and function could influence susceptibility to obesity. *Diabetes* 55:2549–2553, 2006

Proopiomelanocortin (POMC) is a complex propeptide encoding a range of melanocortin peptides that are released by tissue-specific proteolytic processing (1). These peptides have important roles in a range of functions such as skin pigmentation and the control of adrenal growth and function (2). In the central nervous system, POMC is most highly expressed in the arcuate nucleus of the hypothalamus, and POMC-expressing neurons are critically involved in the control of appetite and energy balance (3). Mice and humans lacking all POMC-derived peptides are severely hyperphagic and obese (4). A role for genetic variation in or near the *POMC* locus in determination of obesity or obesity-related quantitative traits in the general population has been suggested by a number of independent studies (5,6). If the *POMC* gene itself is the causative gene, this would imply that relatively subtle variation in POMC structure or function is sufficient to significantly alter energy balance. This notion is supported by the association of a number of point mutations in *POMC* with obesity (7,8), although some of these appear to have the capacity for dominant-negative interference with wild-type melanocortins (9). Limited data on existing human *POMC*-null heterozygotes show a trend toward increased adiposity (10).

In the present study, we report a patient homozygous for a novel frameshift mutation in *POMC* (C6906del) associated with severe obesity and ACTH deficiency. This mutation results in the complete loss of all POMC-derived peptides. A large number of family members were available for study, which gave us the opportunity to formally test the proposition that heterozygous loss of all the melanocortin components of POMC might be associated with a predisposition to overweight and obesity.

RESEARCH DESIGN AND METHODS

PCR and sequencing. All genetic studies were performed with the informed consent of subjects and the parents of children in this family. Genomic DNA was prepared from peripheral white blood cells using a commercial kit (blood amp kit; QIAGEN, Hilden, Germany). The human *POMC* gene on chromosome 2p22.3 is composed of three exons and two introns spanning ~8.6 kb. Exons 2 and 3 contribute to the coding region of the POMC protein. To amplify the entire coding region of the *POMC* gene from genomic DNA, PCR was performed using BioTaq (Bioline, London, U.K.) and carried out under standard conditions as described previously (9). Sequencing reactions were carried out using BigDye terminator chemistry (Perkin-Elmer, Foster City, CA) and analyzed on an ABI 377 automated DNA sequencer (Perkin-Elmer). Nucleotides and amino acids were numbered according to GenBank accession numbers V01510 and NP_000930 respectively.

Genotyping of control subjects. The nucleotide sequence of the *POMC* gene was analyzed in 100 control alleles from normal-weight Turkish control subjects.

Definitions of overweight and obesity. BMI was calculated as weight in kilograms divided by the square of height in meters. In adults, overweight was defined as BMI 25–29.9 kg/m^2 and obesity as >30 kg/m^2 according to World Health Organization criteria. In children (aged <18 years), there are currently no internationally recognized definitions for overweight and obesity. We used definitions proposed by the International Obesity Task Force and supported by a recent International Consensus on Childhood Obesity (11); overweight was defined as >91st and obesity as >99th percentile for age-adjusted BMI. Reference curves have been drawn for BMI in Turkish children; however, these are comparable with other European populations and different cutoffs have not been deemed necessary to date (12). We used U.K. population-

From the [1]University Department of Clinical Biochemistry, Cambridge Institute for Medical Research, Addenbrooke's Hospital, Cambridge, U.K.; [2]Sofia Children's Hospital, Erasmus University, Rotterdam, the Netherlands; the [3]MCH Westeinde, The Hague, the Netherlands; and the [4]Medical Genetics Department, Cambridge Institute for Medical Research, Addenbrooke's Hospital, Cambridge, U.K.

Address correspondence and reprint requests to Stephen O'Rahilly, University Department of Clinical Biochemistry, Cambridge Institute for Medical Research, Addenbrooke's Hospital, Cambridge, CB2 2XY, U.K. E-mail: so104@medschl.cam.ac.uk.

Received for publication 13 February 2006 and accepted in revised form 15 June 2006.

POMC, proopiomelanocortin.

DOI: 10.2337/db06-0214

© 2006 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

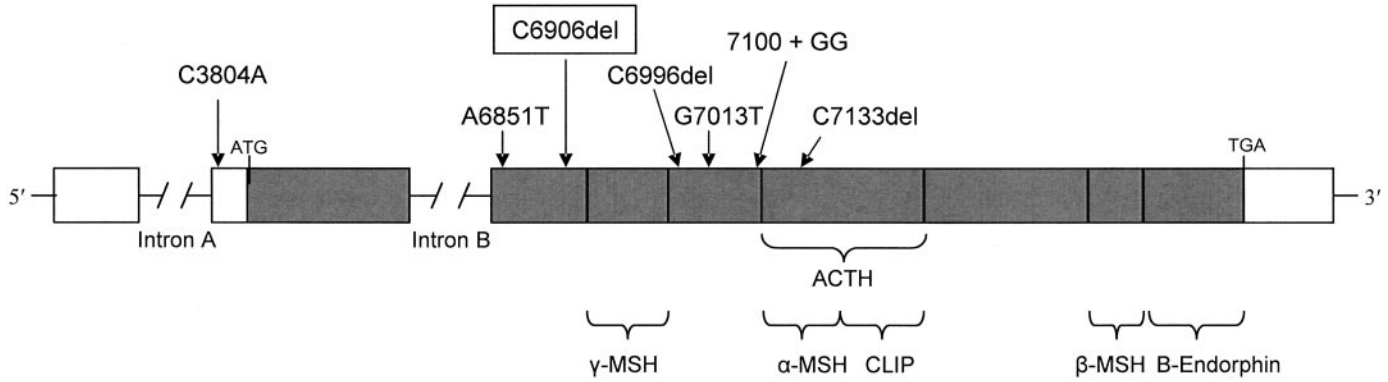

FIG. 1. Structure of the *POMC* gene and location of all homozygous and compound heterozygous mutations identified to date. Untranslated (white) and translated (filled) regions indicated. Proband 1: compound heterozygote for G7013T and C7133del. Proband 2: C3804A (homozygous). Proband 3: compound heterozygote for A6851T and 6996del. Proband 4: C3804A (homozygous). Proband 5: compound heterozygote for 7100 + GG and C3804A. Proband 6 (reported here and outlined)-C6906del (homozygous).

derived reference data for the calculation of BMI SD scores (SDS) in these Turkish subjects (13).

Statistical analyses. We undertook parametric linkage analysis with SIMWALK2 version 2.89 (14). We assumed a penetrance of 0.1, 0.9, and 1.0 for NN, NM, and MM subjects, respectively ("M" refers to mutant and "N" to wild-type allele).

RESULTS

In view of the combination of ACTH deficiency with a normal concentration of other pituitary hormones and severe obesity, a clinical diagnosis of probable POMC deficiency was made in the proband, a 2-year-old male. Direct sequencing of the *POMC* gene revealed the child to be homozygous for deletion of a single nucleotide in codon 69 of the open reading frame (Fig. 1). This deletion, which causes a proline-to-leucine change and a stop codon two amino acids downstream, occurs at the NH_2-terminal end of *POMC* and would lead to the loss of all POMC-derived peptides (Fig. 1). The deletion was present in the heterozygous state in both parents and also in other family members (Fig. 2). This family originates from Turkey; thus, we genotyped 100 control alleles from normal-weight Turkish control subjects and confirmed the absence of this mutation in control subjects.

We had an opportunity to perform a limited study of the 12 heterozygote members of this pedigree. We found that 11 of 12 heterozygotes for the null mutation in *POMC* were

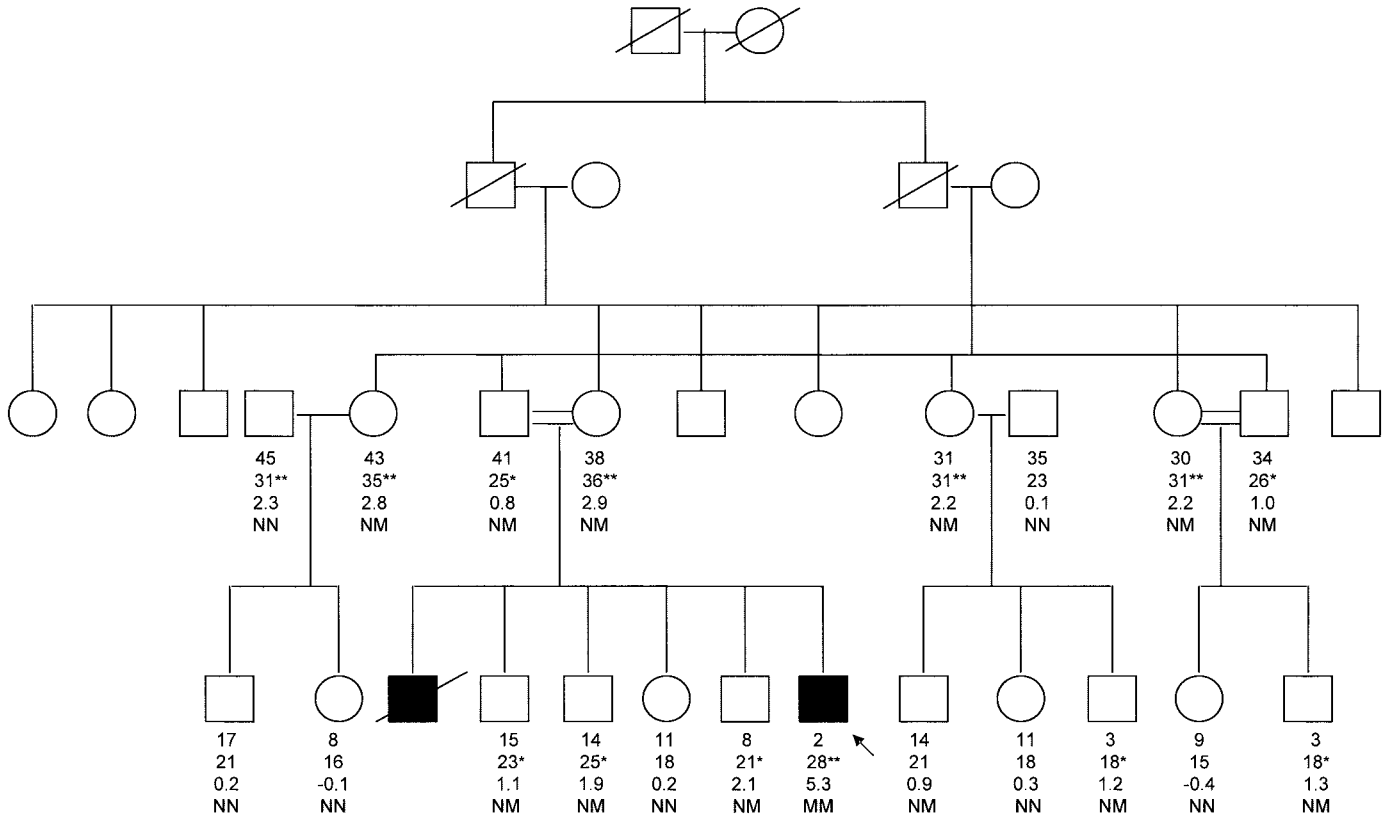

FIG. 2. C6906del *POMC* mutation in an extended Turkish pedigree. Filled symbols indicate patients with severe obesity. Arrow indicates the proband. Where available, age (years) followed by BMI (weight in kilograms divided by the square of height in meters) and BMI SDS (13) are indicated below each subject followed by the POMC genotype. Overweight (*) and obesity (**) using definitions in adults and children (see RESEARCH DESIGN AND METHODS). M, mutant allele; N, wild-type allele.

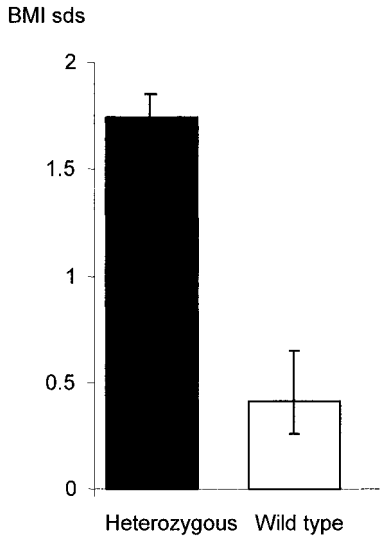

FIG. 3. BMI SD score (sds) in heterozygotes for the *POMC* mutation (●) and wild-type family members (○).

either overweight or obese compared with 1 of 7 wild-type family members. The mean BMI SDS of heterozygous relatives was 1.7 ± 0.5 compared with 0.4 ± 0.4 for wild-type relatives (Fig. 3). This pattern suggests that heterozygosity for this mutation may increase the probability of being overweight. We undertook parametric linkage analysis of the trait "overweight" with SIMWALK2 version 2.89 (14). This analysis gives a maximum "location score" (comparable with multipoint logarithm of odds scores) of 3.191 at position 0 cM from the variant investigated. Thus, analysis of this family using SIMWALK2 provides statistically significant evidence of linkage with this locus. None of the heterozygote subjects had clinical signs consistent with an increased susceptibility to the effects of infection or other features of hypoadrenalism. Regretably, dynamic testing of the hypothalamopituitary adrenal axis was not feasible.

The clinical phenotype of the proband was similar to the previously reported cases of complete POMC deficiency with plasma cortisol and ACTH below the limit of detection, severe hyperphagia reported in the first 6 months of life, and severe obesity (BMI SDS 5.3) (Fig. 4*A*). Notably, on initial examination, the patient did not have red hair (Fig. 4*B*), which has to date been considered a key clinical feature and a useful diagnostic clue to identify this rare monogenic obesity syndrome (15). On closer examination of the scalp, the patient was found to have brown hair with dark red roots. The patient had an obese sibling who had died at the age of 7 months, most likely from undiagnosed corticosteroid deficiency, and who likely harbored the same mutation. He was also reported to have had the same hair coloring.

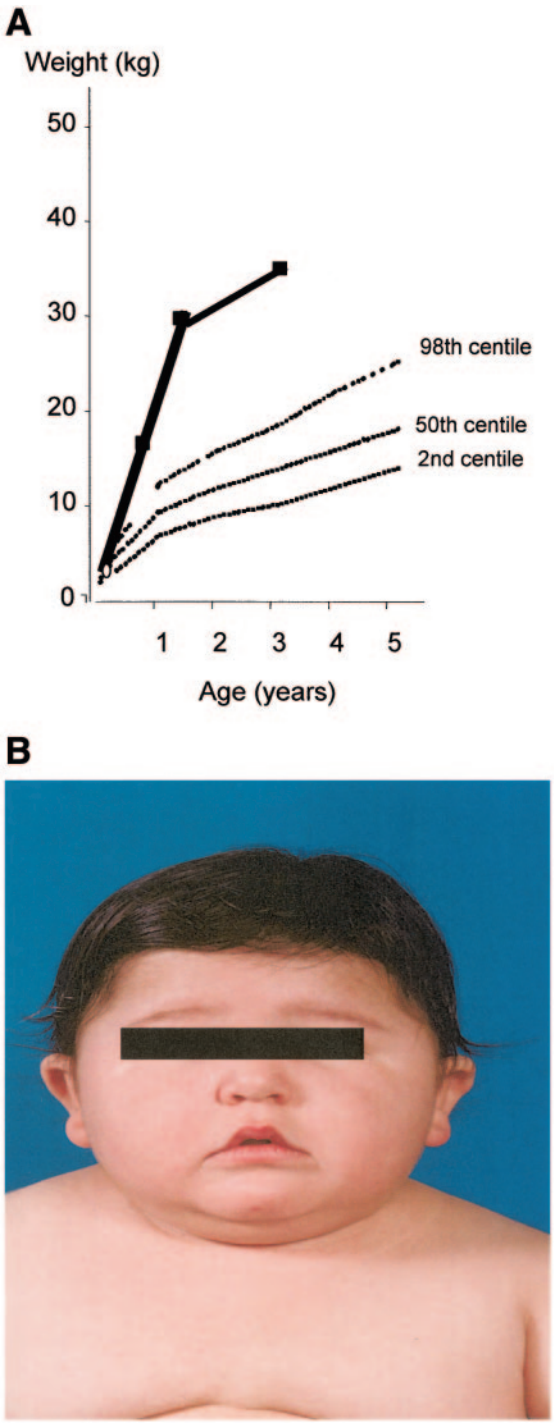

FIG. 4. Weight chart (*A*) and clinical photograph (*B*) of a Turkish patient homozygous for a *POMC*-null mutation, indicating severe obesity and lack of distinctive red hair phenotype.

DISCUSSION

In 1998, Krude et al. (15) provided the first description of humans congenitally lacking *POMC* gene products. One proband was a compound heterozygote for two nonsense mutations and a second patient was homozygous for a mutation in the 5′-untranslated region that introduced an additional out-of-frame start site, thus interfering with *POMC* translational initiation (Fig. 1). Subsequently, Krude et al. (10) have reported three additional unrelated European children with congenital POMC deficiency who were either homozygous or compound heterozygous for *POMC* mutations (Fig. 1). This child represents the sixth reported case of human POMC deficiency, being homozygous for a complete loss-of-function mutation (C6906del), which results in the loss of all POMC-derived peptides. Consistent with the previously reported children, this child also had severe hyperphagia and obesity. Birth weights have been unremarkable in all children reported to date, including our patient, indicating that the effects on growth and weight are exclusively postnatal. In this disorder, in both humans and murine models, obesity occurs

despite profound glucocorticoid deficiency, a condition normally associated with severe weight loss. In our patient, weight gain was documented before the commencement of hydrocortisone replacement therapy. Notably, in *pomc*-null mice, restoration of relatively normal glucocorticoid levels results in a marked worsening of obesity and insulin resistance (16), suggesting that the glucocorticoid deficiency modulates the severity of the metabolic phenotype.

Krude et al. (10) have previously attempted to assess the impact of loss of one *POMC* allele in the parents and heterozygous relatives of their probands. However, the 10 individuals they studied were from five different families, and no wild-type family members were available for comparison. Nevertheless, they estimated the maximum lifetime BMI SDS in adult *POMC* heterozygotes and suggested that most had a maximum lifetime BMI SDS of 1, which is at the upper end of the normal range (10). With this new kindred, we had the opportunity to study a large consanguineous pedigree with 12 heterozygote carriers and 7 wild-type subjects. The significantly higher prevalence of obesity/overweight in the carriers provides compelling support for the idea that loss of one copy of *POMC* is sufficient to markedly predispose to obesity. However, other modifier genes could also potentially influence body weight in members of this pedigree. Notably, we and others have described a variety of point mutations in *POMC*, including mutations in α- and β-melanocyte–stimulating hormone, which in the heterozygous state, significantly increase obesity risk (7,8) but are not invariably associated with obesity. It is possible, and in some cases likely, that some of these point mutations might result in peptides that somehow interfere with the function of wild-type melanocortins (7–9). It is only by studying the true heterozygous null subjects that a clear assessment of the role of *POMC* gene dosage in body weight control can be obtained.

It is notable that a number of genetic linkage studies have identified chromosome 2p22 (a region encompassing the *POMC* gene) as the site of a gene or genes influencing common obesity and obesity-related traits. The strongest evidence for a quantitative trait locus influencing obesity-related phenotypes comes from the San Antonio Family Heart Study undertaken in Mexican-American extended families, with a log odds ratio score of 7.5 for serum leptin levels on chromosome 2p22 (5). Strong evidence for linkage of plasma leptin levels, one of the most robust markers of fat mass, to this region of chromosome 2 was also seen in a genome-wide scan performed in French obese sibling pairs (6). Other studies have suggested a role for genes in this region in phenotypes such as serum triglycerides (17), blood pressure (18), physical inactivity (19), and type 2 diabetes (20). Association studies of the *POMC* gene and indexes of adiposity have been inconsistent (21,22), but most have been underpowered. The extent to which these effects are the consequence of variation in or around the *POMC* locus itself has yet to be determined, but the knowledge that the control of human energy balance is sensitive to *POMC* gene dose strengthens the candidacy of *POMC* as a site where variants affecting expression could influence body weight.

It is plausible that genetic variation around the *POMC* locus might confer a risk of obesity through a gene-environment interaction. We recently reported that 129 mice heterozygous for a null mutation in the *POMC* gene become significantly hyperphagic and obese on a high-fat diet but not on a normal chow diet (23). Interestingly, in a recent genome-wide scan analysis in Mexican-American families, suggestive evidence of linkage with saturated fat intake was found on chromosome 2p22 (24).

The proband of this pedigree is the first reported patient with POMC deficiency who does not have red hair. It is likely that this can be explained by the differing genetic background (Turkish), as opposed to the other reported cases who are all white Caucasian subjects of European ancestry. The retention of dark (and presumably eumelanin rich) hair in this child and his similarly affected deceased sibling indicates that the synthesis of eumelanin in humans is not absolutely dependent on the presence of melanocortin peptides. Clearly, epistatic genetic effects on skin and hair pigmentation are well described in animal models (25). It can be assumed, that in ethnic groups that are predominantly characterized by dark hair, other genetic variants act epistatically to maintain eumelanin synthesis in the absence of POMC-derived ligand, while in Northern European races, such eumelanin synthesis is more critically dependent on the presence of such ligands (26). Indeed, it has recently been shown that genetic variation in a putative cation exchanger in melanosomes (slc24a5) may contribute to variation in human pigmentation in different populations (27). This differential effect of POMC deficiency on hair and skin color has clinical relevance as well as biological interest. Thus, the cardinal features of congenital POMC deficiency are isolated ACTH deficiency, hyperphagia, and severe early-onset obesity. Although red hair may be an important diagnostic clue in patients of Caucasian origin, its absence in patients originating from other ethnic groups should not result in this diagnostic consideration being excluded.

In conclusion, we have reported the sixth case of human congenital POMC deficiency in a child of Turkish origin. In contrast to previously reported cases of Northern European origin, this child did not have red hair. The large number of family members available for study allowed us to examine the obesity risk conferred by haploinsufficiency for POMC, and this appeared to be substantial, a finding that further emphasizes the critical role of the central POMC system in the control of human energy balance.

ACKNOWLEDGMENTS

This work was supported by the Wellcome Trust (I.S.F. and S.O.R.) and the Medical Research Council (S.O.R.).

We thank Heather Cordell and John Todd for helpful discussions. We are indebted to the patients' families for their participation and to the physicians involved in the Genetics of Obesity Study.

REFERENCES

1. Bertagna X: Proopiomelanocortin-derived peptides (Review). *Endocrinol Metab Clin North Am* 23:467–485, 1994
2. Hadley ME, Haskell-Luevano C: The proopiomelanocortin system (Review). *Ann N Y Acad Sci* 885:1–21, 1999
3. Cone RD: Anatomy and regulation of the central melanocortin system (Review). *Nat Neurosci* 8:571–578, 2005
4. Coll AP, Farooqi IS, Challis BG, Yeo GS, O'Rahilly S: Proopiomelanocortin and energy balance: insights from human and murine genetics (Review). *J Clin Endocrinol Metab* 89:2557–2562, 2004
5. Comuzzie AG, Hixson JE, Almasy L, Mitchell BD, Mahaney MC, Dyer TD, Stern MP, MacCluer JW, Blangero J: A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nat Genet* 15:273–276, 1997
6. Delplanque J, Barat-Houari M, Dina C, Gallina P, Clement K, Guy-Grand B,

Vasseur F, Boutin P, Froguel P: Linkage and association studies between the proopiomelanocortin (POMC) gene and obesity in Caucasian families. *Diabetologia* 43:1554–1557, 2000
7. Lee YS, Challis BG, Thompson DA, Yeo GS, Keogh JM, Madonna ME, Wraight V, Sims M, Vatin V, Meyre D, Shield J, Burren C, Ibrahim Z, Cheetham T, Swift P, Blackwood A, Hung CC, Wareham NJ, Froguel P, Millhauser GL, O'Rahilly S, Farooqi IS: A POMC variant implicates beta-melanocyte-stimulating hormone in the control of human energy balance. *Cell Metab* 3:135–140, 2006
8. Biebermann H, Castaneda TR, van Landeghem F, von Deimling A, Escher F, Brabant G, Hebebrand J, Hinney A, Tschop MH, Gruters A, Krude H: A role for beta-melanocyte-stimulating hormone in human body-weight regulation. *Cell Metab* 3:141–146, 2006
9. Challis BG, Pritchard LE, Creemers JW, Delplanque J, Keogh JM, Luan J, Wareham NJ, Yeo GS, Bhattacharyya S, Froguel P, White A, Farooqi IS, O'Rahilly S: 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, 2002
10. Krude H, Biebermann H, Schnabel D, Tansek MZ, Theunissen P, Mullis PE, Gruters A: Obesity due to proopiomelanocortin deficiency: three new cases and treatment trials with thyroid hormone and ACTH4–10. *J Clin Endocrinol Metab* 88:4633–4640, 2003
11. Speiser PW, Rudolf MC, Anhalt H, Camacho-Hubner C, Chiarelli F, Eliakim A, Freemark M, Gruters A, Hershkovitz E, Iughetti L, Krude H, Latzer Y, Lustig RH, Pescovitz OH, Pinhas-Hamiel O, Rogol AD, Shalitin S, Sultan C, Stein D, Vardi P, Werther GA, Zadik Z, Zuckerman-Levin N, Hochberg Z: Childhood obesity. *J Clin Endocrinol Metab* 90:1871–1887, 2005
12. Bundak R, Furman A, Gunoz H, Darendeliler F, Bas F, Neyzi O: Body mass index references for Turkish children. *Acta Paediatr* 95:194–198, 2006
13. Cole TJ, Freeman JV, Preece MA: Body mass index reference curves for the UK, 1990. *Arch Dis Child* 73:25–29, 1995
14. Sobel E, Lange K: Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *Am J Hum Genet* 58:1323–1337, 1996
15. Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A: Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet* 19:155–157, 1998
16. Coll AP, Challis BG, Lopez M, Piper S, Yeo GS, O'Rahilly S: Proopiomelanocortin-deficient mice are hypersensitive to the adverse metabolic effects of glucocorticoids. *Diabetes* 54:2269–2276, 2005
17. Imperatore G, Knowler WC, Pettitt DJ, Kobes S, Fuller JH, Bennett PH, Hanson RL: A locus influencing total serum cholesterol on chromosome 19p: results from an autosomal genomic scan of serum lipid concentrations in Pima Indians. *Arterioscler Thromb Vasc Biol* 20:2651–2656, 2000
18. Rankinen T, An P, Rice T, Sun G, Chagnon YC, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C: Genomic scan for exercise blood pressure in the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study. *Hypertension* 38:30–37, 2001
19. Simonen RL, Rankinen T, Perusse L, Rice T, Rao DC, Chagnon Y, Bouchard C: Genome-wide linkage scan for physical activity levels in the Quebec Family study. *Med Sci Sports Exerc* 35:1355–1359, 2003
20. Demenais F, Kanninen T, Lindgren CM, Wiltshire S, Gaget S, Dandrieux C, Almgren P, Sjogren M, Hattersley A, Dina C, Tuomi T, McCarthy MI, Froguel P, Groop LC: A meta-analysis of four European genome screens (GIFT Consortium) shows evidence for a novel region on chromosome 17p11.2-q22 linked to type 2 diabetes. *Hum Mol Genet* 12:1865–1873, 2003
21. Hixson JE, Almasy L, Cole S, Birnbaum S, Mitchell BD, Mahaney MC, Stern MP, MacCluer JW, Blangero J, Comuzzie AG: Normal variation in leptin levels in associated with polymorphisms in the proopiomelanocortin gene, POMC. *J Clin Endocrinol Metab* 84:3187–3191, 1999
22. Baker M, Gaukrodger N, Mayosi BM, Imrie H, Farrall M, Watkins H, Connell JM, Avery PJ, Keavney B: Association between common polymorphisms of the proopiomelanocortin gene and body fat distribution: a family study. *Diabetes* 54:2492–2496, 2005
23. Challis BG, Coll AP, Yeo GS, Pinnock SB, Dickson SL, Thresher RR, Dixon J, Zahn D, Rochford JJ, White A, Oliver RL, Millington G, Aparicio SA, Colledge WH, Russ AP, Carlton MB, O'Rahilly S: Mice lacking proopiomelanocortin are sensitive to high-fat feeding but respond normally to the acute anorectic effects of peptide-YY(3–36). *Proc Natl Acad Sci U S A* 101:4695–4700, 2004
24. Cai G, Cole SA, Bastarrachea-Sosa RA, Maccluer JW, Blangero J, Comuzzie AG: Quantitative trait locus determining dietary macronutrient intakes is located on human chromosome 2p22. *Am J Clin Nutr* 80:1410–1414, 2004
25. Barsh GS: The genetics of pigmentation: from fancy genes to complex traits (Review). *Trends Genet* 12:299–305, 1996
26. Rees JL: Genetics of hair and skin color (Review). *Annu Rev Genet* 37:67–90, 2003
27. Lamason RL, Mohideen MA, Mest JR, Wong AC, Norton HL, Aros MC, Jurynec MJ, Mao X, Humphreville VR, Humbert JE, Sinha S, Moore JL, Jagadeeswaran P, Zhao W, Ning G, Makalowska I, McKeigue PM, O'Donnell D, Kittles R, Parra EJ, Mangini NJ, Grunwald DJ, Shriver MD, Canfield VA, Cheng KC: SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science* 310:1782–1786, 2005