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For debates

New insights into obesity genes

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Obesity is a major health problem in industrialized societies and the prevalence is increasing. Most human obesities appear to have a significant genetic component, but the multifactorial nature of the disease has confounded efforts to identify the genetic factors involved. In the last few years, however, major progress has been made toward the identification of the genes contributing to obesity.

Mouse models of obesity

Mouse models of polygenic obesity: Using a statistical approach known as quantitative trait locus analysis in two mouse models of polygenic obesity, seven separate chromosomal loci linked to body fat have been mapped [1–3]. To our knowledge, none of the genes present at these loci has yet been identified, but it is expected that this approach will yield interesting results in the near future.

Genetic analysis of mouse models of monogenic obesity has been more successful, leading to the identication of five "obesity genes": *agouti, fat, tub, ob* and *db*. This significant progress has been achieved by using the method of positional cloning.

Mutations at the agouti locus result in ectopic expression of the protein: At least two mutations at the mouse *agouti* locus (A^y and A^{vy}) are associated with adult-onset obesity. The mouse *agouti* gene was identified independently by two groups in 1992 [4, 5]. *Agouti* transcripts are normally expressed exclusively in the skin of neonatal mice, whereas they are detected in virtually all tissues in adult obese A^y mutants. This results from a large deletion which brings the coding region of the *agouti* gene under the control of a ubiquitous promoter [6]. Recent experiments with transgenic mice expressing the *agouti* cDNA under the control of the β-actin promoter confirmed that ubiquitous expression of the protein induces the obese phenotype [7, 8]. Agouti is a secreted protein, which shares homologies with toxins known to interact with ion channels. Indeed, it has been recently shown that intracellular free calcium concentrations are increased in cells incubated in the presence of the recombinant protein [9, 10]. Agouti is also known to antagonize the binding of the α-melanocyte stimulating hormone to its receptors, preventing the rise in cAMP normally induced by this hormone in melanocytes [11]. In humans, at variance with mice, the *agouti* gene is expressed in several tissues including adipose tissue [12, 13]. It is tempting to speculate that agouti has a direct effect on adipose cells. Excessive secretion could induce a decrease in cAMP levels, reducing lipolysis and favouring fat storage. Alternatively or concomitantly, by increasing calcium concentration, agouti has the potential to alter the signalling pathways of hormones involved in the regulation of fat storage. Such mechanisms could promote obesity if higher levels than normal of the agouti protein are secreted by the adipose cells.

The fat mutation results in the loss of carboxypeptidase-E activity: In 1995 a single base mutation was found to occur in the gene of the enzyme carboxypeptidase-E (CPE), in obese fat/fat mice [14]. This is associated with loss of CPE activity, leading to hyperproinsulinaemia which is one of the earliest phenotypic characteristics caused by the *fat* mutation. The relationship between this hormonal defect and the

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Abbreviations: CPE, Carboxypeptidase-E; PPAR, peroxisome proliferator activated receptor; PPRE, peroxisome proliferator response element; C/EBP, CCAAT enhancer binding protein; HMG, high mobility group.

later appearance of obesity remains unclear. To our knowledge, such a mutation in the CPE gene has not been reported in humans.

The tub mutation disrupts a gene of unknown function: The mutated gene responsible for the tubby obesity was identified in 1996 by two independent groups [15, 16]. A single base mutation in the gene abolishes a splice donor site, resulting in the replacement of 44-carboxy-terminal amino acids of the tub protein with 24 intron-encoded amino acids. Whether this induces a loss of function remains to be determined. The carboxy-half terminal of tub is similar to sequences previously cloned in various organisms, but nothing is known about their biochemical roles. Since *tub* mRNA is abundantly expressed in the hypothalamus, it is possible that the protein plays a role in the regulation of feeding behaviour and energy balance. A well-conserved human *tub* gene has been cloned [16], but it is not known whether mutations in this gene occur in obese humans.

The ob mutations result in the lack of leptin, whose receptor is encoded by the db gene: The mouse and human *ob* cDNA was cloned in 1994 and two mutations were discovered in ob/ob mice [17]. In the original strain, a single base mutation creates a premature stop codon resulting in the absence of the ob protein. The molecular basis for the second *ob* mutation is not known; however, it results in the absence of *ob* transcript, suggesting a promoter alteration. The *ob* gene, expressed exclusively in adipose tissue, encodes a new secreted protein, called leptin. Soon after the cloning of the gene it was demonstrated that daily injections of recombinant leptin induce a marked decrease in body weight and adipose tissue mass in ob/ob mice, diet-induced obese mice or wild type lean controls [18–20]. These observations conclusively demonstrate that leptin is an adipose-specific hormone, which plays a major role in the regulation of body weight.

The molecular mechanisms of leptin action are still poorly understood. Based on the data from previous parabiotic experiments [21], the effects of leptin were thought to be mediated by specific receptors encoded by the *db* gene. The cloning of the leptin receptor gene by virtue of leptin binding [22], followed by the demonstration that this gene is mutated in *db/db* mice [23, 24] brought clear evidence that *db* is the leptin receptor gene *(OB-R)*. The *db* mutation causes an abnormal splicing of *OB-R*, which results in a receptor lacking a cytoplasmic tail. Two recent reports show that this short form of OB-R is unable to activate the signal transducers and activators of transcription involved in leptin signalling through the full-length OB-R [25, 26]. Although the tissue sites at which leptin exerts its action remain to be determined, the presence of full-length OB-R in the hypothalamus [25] shows that this tissue is a target of the hormone. It is conceivable that leptin alters the expression of specific genes involved in energy homeostasis, like the hypothalamic neuropeptide-Y, which is a potent stimulator of food intake and whose synthesis is inhibited by leptin [27].

No evidence for a lack of leptin in obese people has been reported so far, although there are indications for linkage of the *ob* gene region with extreme obesity [28, 29]. In contrast, a positive correlation between body mass index and plasma leptin has been consistently observed, suggesting some kind of leptin resistance in obese humans. This potential syndrome does not appear to be due to defects in hypothalamic OB-R [30]. Thus, further detailed studies are needed to demonstrate the importance of leptin in the pathogenesis of human obesity.

Genes involved in adipose cell differentiation

In addition to the study of genes identified by the analysis of mouse models of obesity, significant progress has been made in research aimed at determining the factors involved in differentiation of adipose cells.

Transcription factors of the peroxisome proliferator activated receptor (PPAR) family: PPARs are members of the superfamily of nuclear hormone receptors which regulate the expression of genes possessing the specific response elements (peroxisome proliferator response element [PPREs]) in their regulatory sequences (reviewed in [31]). The pivotal role of PPARγ in adipose differentiation has been demonstrated by the induction of an adipocyte phenotype in fibroblasts and myoblasts infected with a retroviral vector overexpressing the gene [32, 33]. This effect is likely to be due to an activation of several key adipocyte genes containing functional PPREs in their regulatory sequence [31]. Finally, several natural or synthetic PPARγ activators, including fatty acids, prostaglandins and thiazolidinediones [34–37], have the potential to induce adipose differentiation through this transcription factor.

Transcription factors of the (C/EBP) family: C/EBP, which belong to the basic-leucine zipper superfamily of transcription factors, are also implicated in adipose differentiation. Arguments in support of a role of C/EBPα in adipose differentiation come from the temporal activation of C/EBPα expression before adipocyte specific genes [38], the capacity of antisense C/EBPα mRNA to inhibit differentiation [39] and the fact that premature induction or overexpression of C/EBPα triggers adipocyte differentiation [40, 41]. The important role of this factor in adipogenesis was confirmed in C/EBPα-null mice,

which failed to accumulate lipids in adipose tissue [42].

(HMG) DNA-binding proteins: HMGI-C is a member of the HMG family of DNA-binding proteins which are thought to play a role in chromatin conformation and hence, influence transcription. It was recently discovered that in benign tumours of adipose tissue, mutations in the HMGI-C gene result in the generation of fusion proteins with the HMGI-C DNA-binding domain linked to a transactivation domain, turning HMGI-C into a transcriptional activator [43, 44]. Furthermore, this gene is deleted in the mouse mutant *pygmy*, which is characterized by small size and disproportionately reduced body fat content [45]. Thus, the lack or reduced expression of HMGI-C could predispose to leaness, whereas the juxtaposition of the DNA-binding motif to transcriptional regulatory domains could promote adipogenesis.

Although major progress has been made into clarifying the basic role of transcription factors in adipocyte differentiation, major challenges lie ahead before this can be extrapolated to the human situation. Future research should determine whether altered function of certain of these factors might induce obesity, and if this is the case, find ways to overcome these potential defects.

References

1. Warden CH, Fisler JS, Shoemaker SM, et al. (1995) Identification of four chromosomal loci determining obesity in a multifactorial mouse model. J Clin Invest 95: 1545–1552
2. West DB, Waguespack J, York B, Goudey-Lefevre J, Price RA (1994) Genetics of dietary obesity in AKR/J × SWR/J mice: segregation of the trait and identification of a linked locus on chromosome 4. Mammalian Genome 5: 546–552
3. West DB, Goudey-Lefevre J, York B, Truett GE (1994) Dietary obesity linked to genetic loci on chromosomes 9 and 15 in a polygenic mouse model. J Clin Invest 94: 1410–1416
4. Bultman SJ, Michaud EJ, Woychik RP (1992) Molecular characterization of the mouse agouti locus. Cell 71: 1195–1204
5. Miller MW, Duhl DMJ, Vrieling H et al. (1993) Cloning of the mouse agouti gene predicts a secreted protein ubiquitously expressed in mice carrying the lethal yellow mutation. Genes Dev 7: 454–467
6. Michaud EJ, Bultman SJ, Klebig ML et al. (1994) A molecular model for the genetic and phenotypic characteristics of the mouse lethal yellow (A^y) mutation. Proc Natl Acad Sci USA 91: 2562–2566
7. Perry WL, Hustad CM, Swing DA, Jenkin NA, Copeland NG (1995) A transgenic mouse assay for agouti protein activity. Genetics 140: 267–274
8. Kleibig ML, Wilkinson JG, Geisler JG, Woychik RP (1995) Ectopic expression of the agouti gene in transgenic mice causes obesity, features of type II diabetes and yellow fur. Proc Natl Acad Sci USA 92: 4728–4732
9. Zemel MB, Han Kim J, Woychik RP, et al. (1995) Agouti regulation of intracellular calcium: role in the insulin resistance of viable yellow mice. Proc Natl Acad Sci USA 92: 4733–4737
10. Jones BH, Kim JH, Zemel MB, et al. (1996) Upregulation of adipocyte metabolism by agouti protein: possible paracrine actions in yellow mouse obesity. Am J Physiol 270: E192–E196
11. Lu D, Willard D, Patel IR, et al. (1994) Agouti protein is an antagonist of the melanocyte-stimulating hormone receptor. Nature 371: 799–802
12. Wilson BD, Ollmann MM, Kang L, Stoffel M, Bell GI, Barsh GS (1995) Structure and function of ASP, the human homolog of the mouse agouti gene. Hum Mol Genet 4: 223–230
13. Kwon HY, Bultman SJ, Löffler C, et al. (1994) Moecular structure and chromosomal mapping of the human homolog of the agouti gene. Proc Natl Acad Sci USA 91: 9760–9764
14. Naggert JK, Fricker LD, Varlamov O, et al. (1995) Hyperproinsulinemia in obese fat/fat mice associated with a carboxypeptidase E mutation which reduces enzyme activity. Nature Genet 10: 135–142
15. Noben-Trauth K, Naggert JK, North MA, Nishina PM (1996) A candidate gene for the mouse mutation *tubby*. Nature 380: 534–538
16. Kleyn PW, Fan W, Kovats SG, et al. Identification and characterization of the mouse obesity gene *tubby:* a member of a novel gene family. Cell 85: 281–290
17. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse *obese* gene and its human homologue. Nature 372: 425–432
18. Pelleymounter MA, Cullen MJ, Baker MB, et al. (1995) Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269: 540–543
19. Halaas JL, Gajiwala KS, Maffei M, et al. (1995) Weight-reducing effects of the plasma protein encoded by the *obese* gene. Science 269: 543–546
20. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269: 546–549
21. Coleman DL (1978) Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia 14: 141–148
22. Tartaglia LA, Dembski M, Weng X, et al. (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83: 1263–1271
23. Chen H, Charlat O, Tartaglia LA, et al. (1996) Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db/db* mice. Cell 84: 491–495
24. Lee G-H, Proenca R, Montez JM, et al. (1996) Abnormal splicing of the leptin receptor in *diabetic* mice. Nature 379: 632–635
25. Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC (1996) Defective STAT signaling by the leptin receptor in *diabetic* mice. Proc Natl Acad Sci USA 93: 6231–6235
26. Baumann H, Morella KK, White DW, et al. (1996) The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. Proc Natl Acad Sci USA 93: 8374–8378
27. Stephens TW, Basinski M, Bristow PK, et al. (1995) The role of neuropeptide Y in the antiobesity action of the *obese* gene product. Nature 377: 530–532
28. Clement K, Garner C, Hager J, et al. (1996) Indication for linkage of the human *OB* gene region with extreme obesity. Diabetes 45: 687–690

29. Reed DR, Ding Y, Xu W, Cather C, Green ED, Price RA (1996) Extreme obesity may be linked to markers flanking the human *OB* gene. Diabetes 45: 691–694
30. Considine RV, Considine EL, Williams CJ, Hyde TM, Caro JF (1996) The hypothalamic leptin receptor in humans. Identification of incidental sequence polymorphisms and absence of the *db/db* mouse and *fa/fa* rat mutation. Diabetes 19: 992–994
31. Schoonjans K, Staels B, Auwerx J (1996) Role of the peroxisomal proliferator activated receptors (PPAR) in mediating effects of fibrates and fatty acids on gene expression. J Lipid Res 37: 907–925
32. Tontonoz P, Hu E, Spiegelman BM (1994) Stimulation of adipogenesis in fibroblasts by PPAR γ 2, a lipid-activated transcription factor. Cell 79: 1147–1156
33. Hu EP, Tontonoz P, Spiegelman BM (1995) Transdifferentiation of myoblasts by the adipogenic transcription factors PPARγ and C/EBPα. Proc Natl Acad Sci USA 92: 9856–9860
34. Yu K, Bayona CB, Kallen HP, et al. (1995) Differential activation of peroxisome proliferator-activated receptors by eicosanoids. J Biol Chem 270: 23975–23983
35. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkinson WO, Willson TM, Kliewer SA (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). J Biol Chem 270: 12953–12956
36. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM (1995) 15-Deoxy-Δ 12,14 prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ. Cell 83: 803–812
37. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehman JM (1995) A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. Cell 83: 813–819
38. Christy RJ, Yang VW, Ntambi JM, et al. (1989) Differentiation-induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoters of two adipocyte-specific genes. Genes Dev 3: 1323–1335
39. Lin FT, Lane MD (1992) Antisense CCAAT/enhancer binding protein RNA supresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 adipocytes. Genes Dev 6: 533–544
40. Freytag SO, Paielli DL, Gilbert DJ (1994) Ectopic expression of the CCAAT/enhancer-binding protein α promotes the adipogenic program in a variety of mouse fibroblastic cells. Genes Dev 8: 1654–1663
41. Lin FT, Lane MD (1994) CCAAT/enhancer binding protein is sufficient to initiate the 3T3-L1 adipocyte differentiation program. Proc Natl Acad Sci USA 91: 8757–8761
42. Wang N, Finegold MJ, Bradley A et al. (1995) Impaired energy homeostasis in C/EBPα knockout mice. Science 269: 1108–1112
43. Ashar HR, Schoenberg Fejzo M, Tkachenko A, et al. (1995) Disruption of the architectural factor HMGI-C: DNA-binding AT hook motif fused in lipomas to distinct transcriptional regulatory domains. Cell 82: 57–65
44. Schoenmakers EFPM, Wanschura S, Mols R, Bullerdiek J, Van den Berghe H, Van de Ven WJW (1995) Recurrent rearrangements in the high mobility group protein gene, HMGI-C, in benign mesenchymal tumours. Nature Genet 10: 436–443
45. Zhou X, Benson KF, Ashar HR, Chada K (1995) Mutation responsible for the mouse *pygmy* phenotype in the developmentally regulated factor HMGI-C. Nature 376: 771–774