Genetics of Pathways Regulating Body Weight in the Development of Obesity in Humans

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Although rapid globalization of the Westernized way of life is responsible for the large rise in the number of obesity cases (about 1 billion individuals are now overweight or frankly obese), obesity is a typical common multifactorial disease in that environmental and genetic factors interact, resulting in a disease state (1). There is strong evidence for a genetic component to human obesity: e.g., the familial clustering (the relative risk among siblings being 3–7) (2) and the high concordance of body composition in monozygotic twins (3). However, the role of genetic factors in many human obesities (referred to as "common obesity" in this review) is complex, being determined by interaction of several genes (polygenic), each of which may have relatively small effects (i.e., they are "susceptibility" genes and work in combination with each other as well as with environmental factors such as nutrients, physical activity, and smoking). [Exp Biol Med Vol. 226(11):991–996, 2001]

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Monogenic Human Obesity

Most of the syndromic forms of obesity like Prader-Willi, Cohen, Alstrom, and Bardet-Biedl have been genetically mapped, but causative genes have not yet been isolated (4). The extreme rarity of these mutations has made the search for the causative genes difficult. In contrast, the strategy used to identify nonsyndromic forms of human obesity has been relatively successful. This strategy has focused on screening large numbers of subjects for mutations in candidate genes that are homologous to murine genes known to be involved in energy homeostasis and when mutated, cause obesity in rodents. In only 2 years, five different human obesity genes have been identified: leptin, leptin receptor, pro-opiomelanocortin (POMC), melanocortin 4 receptor (MC4R), and proconvertase (PC1) (5–9). Significantly, all of the proteins encoded by these five genes are part of the same pathway regulating food intake (Fig. 1). The leptin (or *ob* gene) codes for a hormone synthesized and is secreted by adipocytes in proportion to their fat content. In the hypothalamus, leptin binds the long form of its receptor (coded for by the *db* gene) and among its effects is enhanced expression of the POMC gene. The enzyme PC1 cleaves POMC to yield ACTH and α-melanocyte-stimulating hormone (α-MSH), which reduces food intake when it binds to the brain-specific MC4R. The fact that no mutations in genes involved in other pathways potentially regulating energy intake have been found in human monogenic obesity suggests that the leptin pathway may be the primary regulator of energy balance in humans.

The five human genes causing monofactorial obesity fall into two categories based on prevalence. The first includes the genes coding for leptin, the leptin receptor, POMC, and very rare, recessive forms of obesity associated with pituitary endocrine dysfunction. For example, loss-of-function mutations in leptin have been found in only two individuals (cousins). Their phenotype of morbid obesity with onset in the first weeks of life included increased appetite, constant hyperphagia, and hypogonadotropic hypogonadism. Treatment with recombinant leptin was fully successful, resulting in reduced food intake, recovery of satiety, and a dramatic decrease of fat mass with no change in lean mass (10). Similarly, only one family to date has been identified with leptin receptor mutations (6). In the individuals with homozygous mutations, a truncation of the receptor before the transmembrane domain completely abolishes leptin signaling, leading to a massive obesity similar to that of individuals with leptin deficiency. There is also significant growth retardation and central hypothyroidism. Although these mutations are rare, they emphasize the central role that leptin can play in regulating energy balance in humans as well as in rodents. Along similar lines, the key role of the melanocortin system in the control of body weight in humans is evidenced by the discovery of mutations in POMC and MC4R genes that result in massive obesity. Two children with homozygous or compound heterozygous loss-of-function mutations in POMC exhibited a complex phenotype reflecting the lack of neuropeptides derived from the POMC gene. This led to impaired signaling via several

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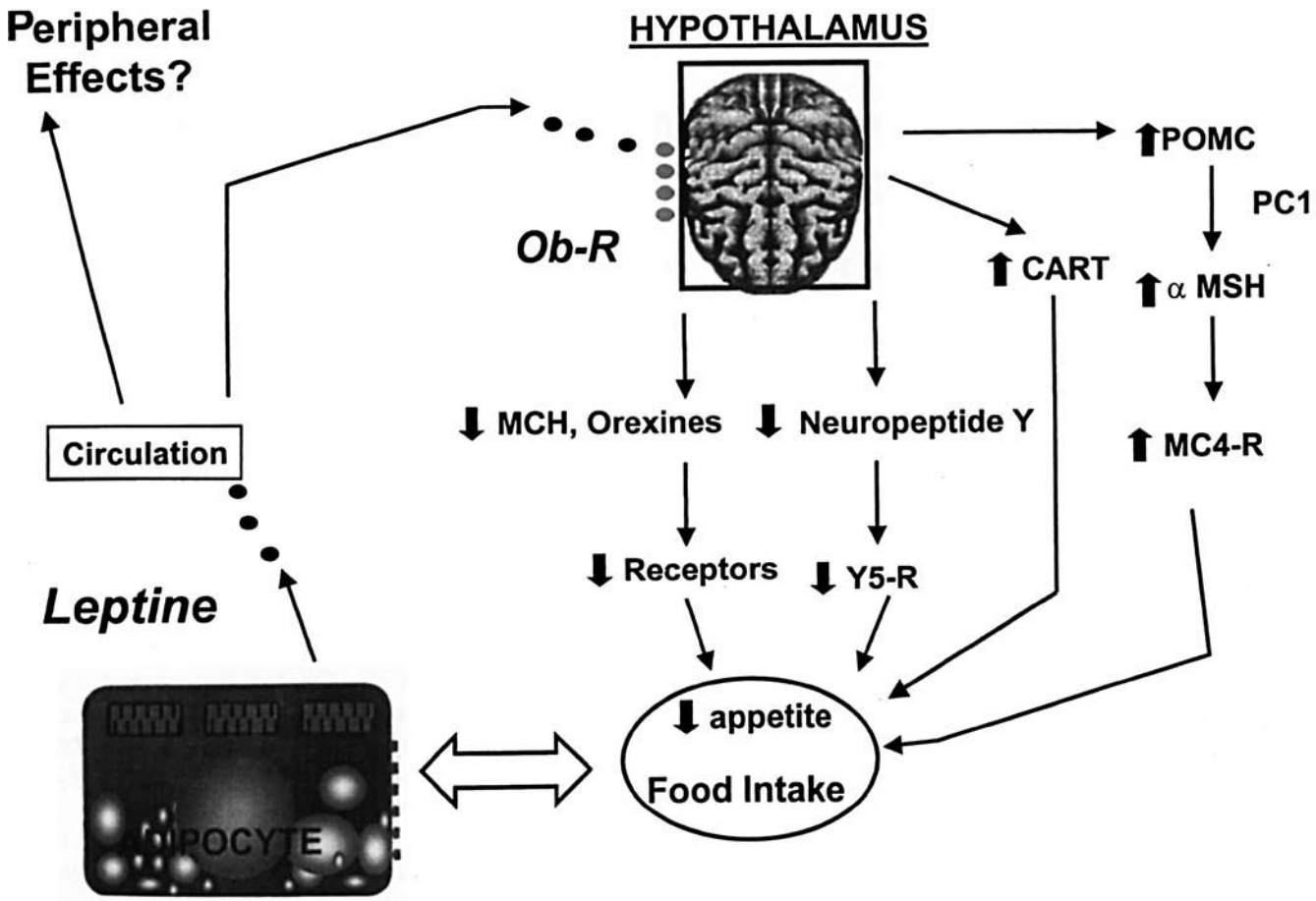

Figure 1. Leptin pathway for weight control. Reprinted from *Best Practice and Research Clinical Endocrinology and Metabolism,* Vol. 15, No. 3, pp391–404, 2001.

melanocortin receptors (7). The absence of α-MSH was responsible for the ensuing obesity (a result of the absence of the melanocortin ligand for MC4R) as well as for altered pigmentation and red hair (from the absence of the ligand for the MC1 receptor). Additionally, the lack of ACTH interacting with the MC3 receptor led to adrenal deficiency.

The second group of monogenic forms of nonsyndromic obesity thus far consists of the numerous mutations in the MC4R gene (8). The MC4R gene is the most prevalent obesity gene to date, being involved in 1%–4% of very obese individuals (11). MC4R mutations generally segregate in families via an autosomal dominant mode of inheritance with variable penetrance. However, in some consanguineous pedigrees, MC4R mutations with relatively modest loss of function appear to be codominantly or even recessively associated with obesity. In this regard, obesity caused by MC4R mutations is similar to more common forms of obesity, with an earlier age of onset and with a trend for hyperphagia in infancy, a trait that seems to disappear with age. Recent data obtained in mice as well as in humans suggest that impaired MC4R signaling could be involved in hyperinsulinemia through impaired negative neuronal control of insulin secretion. In this respect, MC4R might be considered as a "thrifty gene" (i.e., a gene whose product promotes energy efficiency) and could serve as a primary target for small anti-obesity molecules, regardless of the proximate cause of the obesity, and possibly as a target for drugs treating the metabolic syndrome (12).

Common Human Obesity

Two general approaches have been conducted to date in the search for genes underlying common polygenic obesity in humans. The first approach focuses on "candidate genes"; that is, genes selected as having some plausible role in obesity based on their known or presumed biological role in energy homeostasis. Efforts to identify candidate genes for obesity have concentrated on adipose tissue. In brown adipose tissue, regulation of thermogenesis by the sympathetic nervous system is mediated by β-adrenergic receptors. A Trp64Arg mutation located in the first transmembrane domain of these receptors was first identified as being correlated with obesity in different populations (13, 14). However, discordant data were also published (15) indicating that the role of this candidate gene in human obesity, if any, is modest, or should be considered in relation with others in the same pathway. Importantly, in mature brown adipocyte cells, stimulation of β_3-adrenergic receptors by norepinephrine activates uncoupling protein 1 (UCP-1) via the cAMP metabolic pathway. Uncoupling proteins (UCPs) are inner

mitochondrial membrane proton transporters that dissipate the proton gradient, releasing stored energy in the form of heat. An A-to-G variation in UCP-1 was associated with a gain of fat mass in a Quebec family study (16). Additional effects on weight gain of the G allele of the −3826 variant of UCP-1 with the Trp64Arg mutation of the β_3-adrenergic receptor gene occurred in the French morbid obese population (17). Moreover, polymorphisms in other members of the uncoupling gene family, UCP-2 and UCP-3, are associated with body mass index (BMI, a general measure of adiposity) in Pima Indians (18). However, variations in β_3-adrenergic receptors and UCP genes are probably not sufficient to induce obesity alone.

Several other candidate genes have been studied, and among them is leptin. The role of the leptin gene in common polygenic obesity was first suggested by linkage studies (19, 20). Polymorphisms in the 5′-untranslated region of the human leptin gene were associated with low leptin levels and with resistance to a low calorie diet (20). The peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor that plays a key role in adipogenesis and in some respect may control the thrifty gene response to environmental signals like nutrients (e.g., fatty acids are likely to bind PPARγ), leading to efficient energy storage. A Pro12Ala variation in the PPARγ gene is associated with improved insulin sensitivity, reduced obesity (21), and a modest decrease in the risk of type 2 diabetes (odds ratio = 0.85) (22).

The second approach used for identifying genes underlying common polygenic obesity utilizes genome-wide scans in order to detect chromosomal regions showing linkage with obesity in large collections of nuclear families. This strategy requires no presumptions on the function of genes at the susceptibility loci, since it attempts to map genes purely by position. Genotyping of 400 multiallelic markers (short tandem repeats with a density of 1 marker/10 cM) enables identification of polymorphic markers showing strong allele identity by descent in obese family members (i.e., allele sharing in sibships is significantly higher than 50%). Identification of such susceptibility gene(s) for obesity may then be positionally cloned in the intervals of linkage.

Five genome-wide scans for obesity genes have been published to date. These were carried out in Mexican American families (24), French pedigrees (25), Pima Indians (26, 27), and in White Americans (28, 29). Both Comuzzie *et al.* (24) and Hager *et al.* (25) provided a candidate region on chromosome 2p21 that could explain a significant part of the variance of leptin levels in humans. This linkage was replicated in a cohort of African-American families (30). Mapping within this region is a strong candidate gene for obesity, the POMC gene. However, no strong association was shown between POMC polymorphisms and obesity in Mexican American, French, or Danish populations (31).

Other major gene loci for obesity and leptin levels (Fig. 2) have been identified on chromosome 10p11 and on 5cenq in French families. The involvement of this chromosomal region in obesity was recently confirmed in a cohort of young obese Germans (32) as well as in White Caucasians and in African Americans (33). In addition to this 10p locus, a genome scan performed in White Americans showed evidence for linkage on chromosome 20q 13 and on 10q (28). In Pima Indians, the most interesting region was shown to be on chromosome 11q. Recently, Comuzzie (29) described a new locus at 3q27 that was linked to various quantitative traits characterizing the metabolic/insulin resistance syndrome. Interestingly, this 3q27 locus was previously identified as a type 2 diabetes (NIDDM) locus in the French population (34). Several candidate genes map to this region, including the APM1 gene encoding the differentiated adipocyte secreted protein ACRP30/adiponectin, which is abundantly present in plasma. The purified C-terminal domain of adiponectin has been reported to protect mice on a high fat diet from obesity, and to rescue obese or lipoatropic murine models from severe insulin resistance by decreasing levels of plasma free fatty acids and enhancing lipid oxidization in muscle (35). Moreover, plasma levels of adiponectin have been shown to be decreased in obese diabetic subjects (36), which makes ACRP30 an attractive candidate gene for fat-induced metabolic syndrome and type 2 diabetes. Recent data suggest a role for variations in the ACRP30 gene in obesity-associated type 2 diabetes in different ethnic groups (Froguel P, Kadowaki T, unpublished data).

Although some concerns have been raised about the heterogeneity and reliability of genetic data in multifactorial diseases in general (e.g., the lack of replication), the results from genome scans in obesity studies are surprisingly reproducible, despite differences in ethnicity and in environmental factors. Indeed, loci at chromosome 2 and 10 are largely confirmed, as well as to a lesser extent, loci on chromosome 5. These data show that among complex traits, adiposity is one of the most inheritable, and that a few major loci may contribute to the genetic risk for obesity in humans. A working hypothesis based on available data is that obesity is an oligogenic disease whose development can be modulated by various polygenic (modifier) genes and by environmental influences.

The current epidemic of obesity represents a major public health concern given the strong association of adiposity with cardiovascular, metabolic, and other morbidities. Preventative and therapeutic approaches are hampered by a lack of fundamental understanding of the control of human body fat mass and disturbance of this control in obese states. Human geneticists have pioneered the understanding of the genetic basis of obesity through their discovery of the first monogenic defects leading to extreme childhood obesity. The more challenging problem is identification of the genetic variants that underlie susceptibility to the common forms of human obesity. Based on high-quality family material and recent widely confirmed genome-wide scans, it is likely that putative etiological variants in candidate genes will emerge. The success of these studies will require a

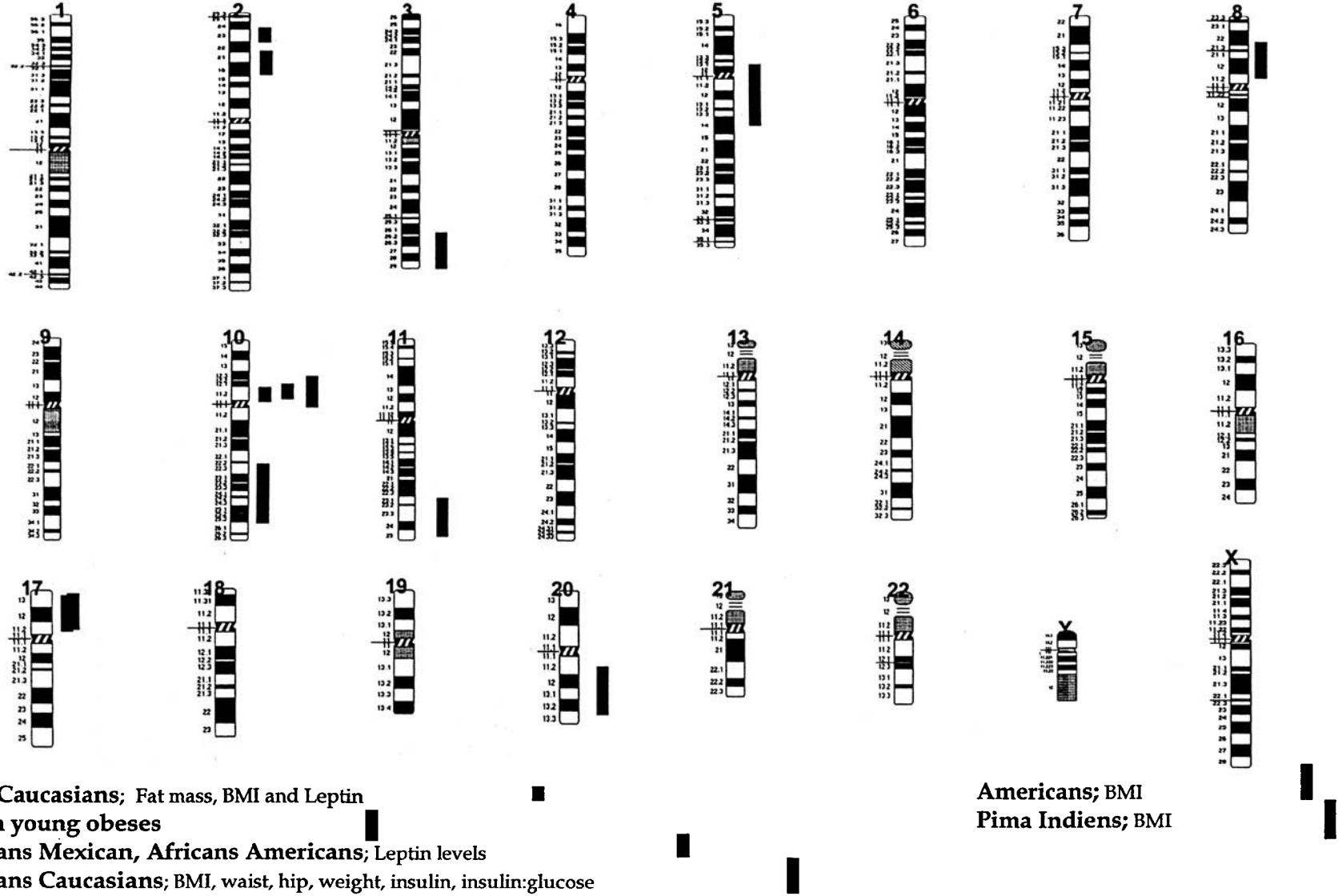

Figure 2. Chromosomal location of obesity loci identified in genome-wide scans studies for different populations as indicated by the various symbols. Impossible to discriminate linkage results in different populations (all bars in black). Reprinted from *Best Practice and Research Clinical Endocrinology and Metabolism*, Vol. 15, No. 3, pp391–404, 2001.

multidisciplinary approach combining genomics, bioinformatics, expression profiling, biochemistry, human physiology, and molecular epidemiology. Although the task is considerable, the breadth and depth of expertise now available in human genetics of complex traits provide unique scientific opportunities for significant advances.

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