Pathophysiology and genetics of obesity

Neena Srivastava1, Ram Lakhan2 & Balraj Mittal2
1Department of Physiology, K G Medical University, Lucknow 226 003, India
2Department of Medical Genetics, SGPGIMS, Lucknow 226 014, India

Obesity, a global problem, is a multifactorial disorder. The factors are environmental, metabolic and genetic and their interaction with each other regulates the body weight. Imbalance in either of the factors may be responsible for weight gain. With advancement of research techniques in the last decade, genetic studies have been undertaken for several different causative mutations involving obesity loci on different chromosomes. Monogenic and polygenic obesity has been observed however, polygenic forms are more common. So far more than 200 genes in mouse and more than 100 genes in humans have been identified which result in phenotypes that affect body weight regulation. In spite of this knowledge, the field of obesity has still not been explored extensively. There remain a lot of lacuna regarding causes and treatment of obesity. Challenges are still there to identify the exact cause of weight gain and the use of current knowledge for development of anti-obesity drugs targeted for body weight regulation. In this review, we have explained neuropathophysiologic regulation of feeding behaviour and some aspects of obesity-genetics especially with single nucleotide polymorphism of selected candidate genes and their functional aspects mainly in monogenic obesity.

Keywords: Body mass index, Genetics, Obesity, Polymorphism, Single nucleotide polymorphism

Obesity, a chronic, relapsing, stigmatized, neurochemical disease that is more prevalent in developing/developed countries and leading to much comorbidity. Multiple factors are involved that contribute to the development of obesity. These may be social, behavioural, environmental and genetic. It is a global health problem in the present era. During the early 20th century, the prevalence of obesity rose slowly, but it began to rise more rapidly in 1980. A continuously rising trend in the prevalence of obesity in childhood and adolescence has been noted in several studies, associated with this rise in obesity rates presage a dire future for these children as complications of blindness, heart disease, renal failure, amputation and compromised quality of life1,2. Understanding of the pathophysiology of obesity has increased markedly over the last decade, but this knowledge is insufficient for the management of obesity. A better understanding of molecular mechanism of pathogenesis and role of environmental and genetic factors will provide hope for planning the treatment strategies of weight reduction.

The regulation of appetite relies on complex hypothalamic neurocircuitry in which the arcuate nucleus and the hormone leptin, ghrelin play important roles. Excess body fat is stored in adipose tissue which forms over 10% of total body weight, but it is now clear that adipocytes have functions other than simple storage cells. The most significant of these appears to be the secretory3. The control of appetite and body composition has been explained by the occurrence of physiological set point for body weight4.

The commonly used clinical methods of fat assessment and classification are body mass index (BMI; weight in kg/height in m²) and waist to hip ratio (WHR), respectively. The range of BMI varies significantly according to the stage of economic transition and associated industrialization of a country, the shift from dietary deficit to one of dietary excess. Obesity, defined as a BMI of more than 30, is a common condition in Europe and the United States. A BMI cut-off of >23 has been suggested by WHO as indicative of overweight for Asia-Pacific inhabitants due to their greater fat deposits5. BMI may not be necessarily best measure for obesity and BMI data should be taken with bit of caution as there is potential misclassification bias. (Putting individuals in to wrong category: such as muscled athlete in the ‘overweight’ category). Evidences show that individuals with a body mass index (BMI; within the “normal” range) of 23 to 25 are at increased risk of diabetes, compared with individuals with a lower BMI.
Prevalence of obesity

Prevalence of obesity is increasing rapidly in developed and developing countries. There is a growing body of evidence that prevalence of overweight and obesity increases in areas where tremendous socioeconomic changes have taken place such as in East Germany. Prevalence of obesity in Indian population is 20% in adults and 10% in children. In Punjab state of India, frequency of overweight and obesity has found to be more among urban females than in their rural female counterparts. Another similar study reported that the prevalence of overweight (BMI ≥ 25) was high among urban southern Indian children. (17.8% in boys, 15.8% in girls) obesity (BMI ≥ 30 kg/m² in this study) was seen in 3.6% of boys and 2.9% of girls. Level of overall and central adiposity, as well as body fat, was found to be high among Marwari’s, as compared with other ethnic populations of India. The prevalence of overweight and obesity among the affluent children in Amritsar was as high or higher as in some industrialized countries due to lifestyle changes and change in eating behaviour. The prevalence of overweight among affluent Bengali children in Kolkata was higher than those reported from other Asian countries.

Pathophysiology of obesity

Modulation of the amount of energy that we take in as food involves several mechanisms and networks that connect the brain with the gut, this process being key to the regulation of body weight over time, as well as to the modification of long-term eating behaviours. Obesity is characterized by an increase in subcutaneous adipose tissue. Its metabolic consequences, such as insulin resistance, are primarily attributable to increased fat deposition at sites such as the omentum, liver, and skeletal muscles. Recently, a virus has also been found to be associated with obesity. Human adenovirus Ad-36 causes adiposity in animal models and enhances differentiation and lipid accumulation in human and 3T3-L1 pre-adipocytes, which may, in part, explain the adipogenic effect of Ad-36.

Body weight regulation is at both, short term (day to day) and long term basis. Obesity occurs as a result of imbalance in energy input and expenditure. For weight regulation there is a set point in every individual. A lot of information is available regarding genes, peptides, neurotransmitters, and receptors in the hypothalamus and neighbouring areas which regulate appetite and body weight. Neuropeptide which increases appetite is neuropeptide Y.

Other appetite increasing neuropeptides are orexin A and B, agouti related peptides (AGRP) and melanin concentrating hormones (MCH). Neuropeptides which decrease food intake are pro-opiomelanocortin (POMC) derivatives which act on MC4 receptor, cocaine and amphetamine related transcripts (CART), corticotropin releasing hormone (CRH), prolactin releasing peptide (PrRP), α-melanocyte stimulating hormone (α-MSH), 5-hydroxy triptamine (5HT), serotonin and leptin receptor (LEPR).

There are four hypotheses regarding afferent mechanisms involved in appetite regulation. (Fig. 1) According to lipostatic hypothesis adipose tissue produces a hormonal signal that is proportionate to the amount of fat. Obesity is also said to be an inflammatory condition of the body. A growing list of adipocytokines involved in inflammation (IL-1beta, IL-6, IL-8, IL-10, IL-18, TNF-alpha, TGF-beta,) and the acute-phase response (serum amyloid A, PAI-1) have been found to be increased in the metabolic syndrome. From white adipose tissue, there is release of leptin and resistin which decrease appetite. There is also release of adiponectin and adipocytokines like tumor necrosis factor-α and interleukin-6 (IL-6) which increase appetite. Brown adipose tissue also releases PPAR-γ and uncoupling protein (UCP-1) which are responsible for high metabolic rate and thus, for weight reduction.

Gut peptide hypothesis determines release of peptides like GRP from the stomach. Glucagon and somatostatin from pancreas which decrease appetite and control weight. Some more peptides like CCK and PYY released from intestine and colon also are responsible for appetite and body weight regulation. Polypeptide ghrelin is released from stomach. It exerts orexigenic effect through NPY/AGRP pathway in the arcuate nucleus.

Glucostatic hypothesis holds that reduced blood glucose level increases appetite, frequent fasts lead to reduction in basal metabolic rate and increase in adiposity. Thermostatic hypothesis postulates that fall in body temperature below set point stimulates appetite and above set point inhibits.

Role of genetics in obesity

Genetics has shown tremendous effect on the process of weight gain. Overall, data from twin and adoption studies are consistent with a genetic
contribution for body mass index (BMI) of between 40 and 70%. Comprehensive profiling technologies coupled with creative statistical analyses have revealed that interactions between genetic and environmental factors are responsible for the common obesity which is currently challenging many developed and developing countries.

Recent obesity related genetic studies have produced a large repertoire of predisposing alleles of diverse importance. Current obesity appears to be an interaction of paramount genetic factors with an abundance of calorically dense food and decline in physical activity. In the genetic perspectives identification and characterization of monogenic and polygenic obesity syndromes have led to an improved understanding of the precise nature of inherited component of severe obesity. Genome wide scans in different ethnic populations have identified major obesity loci on chromosomes 2, 5, 10, 11 and 20.

Recent genetic studies have identified several different causative mutations underlying such syndromes. There are a large number of genes in humans which are believed to affect body weight and adiposity. The obesity gene map shows putative loci on all chromosomes except Y. Around 176 human obesity cases due to single-gene mutations in 11 different genes have been reported, 50 loci related to mendelian syndromes relevant to human obesity have been mapped to a genomic region, and causal genes or strong candidates have been identified for most of these syndromes.

Inherited forms of obesity are syndromic and are result of abnormal functioning of single genes leading to weight gain i.e., obesity as salient clinical feature. About 30 mendelian disorders with obesity as a prominent clinical feature, often are in association with mental retardation, dysmorphic features and organ-specific developmental abnormalities (i.e. pleiotropic syndromes) have been identified which include mainly— Prader-willi, Bardet–Biedl syndrome, Albright’s hereditary osteodystrophy, Fragile X syndrome, Borjeson–Forssman–Lehmann syndrome, Binge eating syndrome, Cohen syndrome, WAGR syndrome (Wilms tumour, anorexia, ambiguous genitalia and mental retardation) and Alström syndrome etc.

The more common forms of obesity are however polygenic. For most overweight people, obesity is a product of gene environment interaction. The assimilation, storage, and utilization of nutrient energy include a number of metabolic pathways that control body weight and body fat content by a set-point mechanism. This system involves a pool of genes, several of which have been recently identified on the basis of their known roles in energy homeostasis in animals combined with the finding of gene mutations that appear to be associated with obesity phenotypes in humans; mainly leptin,
leptin receptor pro-opiomelanocortin, pro-hormone convertase -1, insulin gene, peroxisome proliferator-activator receptor-γ, uncoupling protein, melanocortin-3 and melanocortin-4 receptor genes. Mutations in mitochondrial genome also have been associated with severe forms of obesity.

**Selected candidate genes associated with obesity**

**Role of POMC gene**—In cases of very severe obesity that starts in infancy, a single gene might be playing a permissive role allowing environmental factors to have major impact. Mutations in leptin gene and its receptor, pro-opiomelanocortin (POMC), or more frequently, melanocortin receptor 4 mutations, are evidence of the existence of an obesity gene. It has been observed that inactivity of either of these genes would be sufficient to produce early onset anomalous eating habits. Adipocytokine, leptin released from adipocytes acts on hypothalamic neurons to release pro-opiomelanocortin (POMC), leading to a cascade of neuronal and hormonal events that inhibit feeding behaviour\(^{18}\). Some null mutations of the pro-opiomelanocortin gene (POMC) have been found to cause obesity in humans and rodents.

Genetic findings have proven that the loss of only one copy of the POMC gene is sufficient to render mice susceptible to the effects of high fat feeding to emphasize the potential importance of this locus as a site for gene-environment interactions predisposing to obesity\(^{19}\). Rare mutations in POMC gene cause severe early-onset childhood obesity. A recent study has demonstrated that central nervous system POMC peptides play a critical role in energy homeostasis that is not substituted by peripheral POMC\(^{20}\).

**Melanocortin-4-receptor**—Increasing number of human disorders resulting from genetic disruption of the leptin-melanocortin pathways have been identified\(^{21}\). However, effects of mutations in melanocortin-4 receptor (MC4R) gene, for which the obese phenotype varies in the degree of severity among individuals, are also thought to be influenced by environmental surroundings\(^{16}\).

Polymorphisms of the human melanocortin-4-receptor have been found in severely obese individuals, suggesting that melanocortin-4 receptor malfunction might be involved in human obesity and obesity-associated diabetes. It has been seen that deletion of MC4-R and POMC gene increases feeding and weight in mouse models. A recent meta-analysis of 29563 individuals confirms that the V103I polymorphism protects against human obesity at a population level. It suggests that genetic variants, at least in part, explain susceptibility and resistance to common forms of human obesity\(^{22}\). Since it is known that MC4-R activation generates intracellular cAMP, knowledge of molecules that can affect cAMP generation or otherwise mimic MC4-R-induced signaling specifically in adipocytes, these cells could provide targets for novel anti-obesity drugs. Stimulation of cAMP production, the human melanocortin type 4 (hMC-4) receptor recently has been shown to mediate p44/42 MAPK activation.

**Uncoupling proteins**—Uncoupling proteins are considered as candidate genes for association with energy metabolism and obesity, constitute a subgroup of mitochondrial transporter-superfamily that uncouples protein entry in mitochondrial matrix from ATP synthesis. Four homologous UCP isoforms have been identified. UCP-1, the first UCP to be described, is found exclusively in brown adipose tissue, UCP-2 in several tissues, UCP-3 in human skeletal muscle and rat brown adipose tissue and skeletal muscle, whereas UCP-4 is expressed in the brain. The promoter polymorphism of UCP-2, -866G/A, has been associated with increased gene expression and also contribute to the biological variation of insulin secretion in humans\(^{23}\). A study has evaluated the prevalence of -866G>A change of UCP-2 gene in Spanish pediatric population to study its influence on the phenotype of obese children and found that subjects carrying the ‘A’ nucleotide present higher values of tricepital and sub-scapular skin-folds as compared to non-mutant subjects, which may indicate a relationship between the presence of ‘A’ allele in obese children and higher amounts of subcutaneous fat. The homozygote of UCP-2 gene, Ala55Val mutation, increases the risk of obesity in Chinese population. UCP2 gene mutation or ADR beta gene mutation alone is not associated with obesity, the possible additive effects of these two micro-genes increase the occurrence of obesity. Emerging data indicate that primary physiological role of UCP3 may be the mitochondrial handling of fatty acids rather than the regulation of energy expenditure through thermo-genesis. Increased expression of UCP-2 and UCP-3 under conditions of increased fatty acid metabolism implies as yet undefined role in lipid metabolism\(^{24}\) and body weight regulation. Various studies to-date suggest that uncoupling proteins do not cause obesity but can act as modifier genes.
**Insulin and insulin receptor gene** —Insulin substrate-1 gene occupies key position in insulin signalling pathway. After insulin binding to alpha subunit of insulin receptor, the beta subunit undergoes auto-phosphorylation and in turn phosphorylates other endogenous substrates in the cascade insulin action. Several polymorphisms have been identified in IRS-I gene, but Gly > Arg substitution at codon 972 is quite prevalent in patients in Type II diabetes than in healthy controls. The polymorphism has also been associated with impaired glucose tolerance, this association has been more marked in obese subjects (BMI > 25 kg/m²).

**β2-adrenergic and glucocorticoid receptor** — Catecholamines stimulate lipolysis in fat cells through β-AR (Adrenergic receptor) and inhibited through α-ARs. An association between codon 27 (Gln > Glu) of β2-AR has been associated with obesity. It has been found that in the regulatory region of β2-AR, there is polymorphism, which is in linkage disequilibrium with codon 27 polymorphism. The 5′ leader region of β2-AR mRNAs includes a short open reading frame encoding a 19 amino acid leader peptide. A short synthetic peptide corresponding to the peptide encoded by β2-AR short open reading frame potently inhibits translation in vitro, suggesting that leader cistron may play a role in the regulation of β2-AR expression. Therefore, there may be association of 5′ leader polymorphism with obesity and obesity related metabolic disorders. A polymorphism in the glucocorticoid receptor gene at codon 363 results in amino acid from aspargine to serine which leads to increased sensitivity to glucocorticoids and results in higher sensitivity to glucocorticoids and leads to energy imbalance.

**Adiponectin** — An adipocytokine encoded by APMI gene localized on chromosome 3q27 is one of the adipocyte-expressed proteins which regulate the homeostatic control of glucose, lipid, and energy metabolism. Evidences suggest its role in the genetic predisposition to metabolic X syndrome, such as insulin resistance, obesity, type 2 diabetes, and coronary artery disease. Adiponectin also enhances the transcription of other genes involved in fatty acid metabolism, most notably peroxisome proliferator-activated receptor-α (PPAR-α). It also contains response elements for peroxisome proliferator-activator receptor γ (PPAR γ), a key regulator of glucose and lipid metabolism. Mackeivics et al. have investigated the association of 2 SNPs of ACDC (Adipocyte, C1q, and Collagen Domain Containing) gene, 45T-G and 276G-T, and their haplotypes with serum adiponectin concentrations. Adiponectin downregulates its own production and the expression of its AdipoR2 receptor in transgenic mice. Evidences also suggest that adiponectin secretion is modulated by interleukins such as IL-15 which indicates that interleukins may modulate fat, lean body composition and insulin sensitivity. Combined deficiencies of IL-6 and IL-1 have been shown to cause obesity in young mice.

**Leptin** — It is a 16 kDa adipocyte derived hormone that circulates in the serum in the free and bound form. Serum levels of leptin reflect the amount of energy stored in adipose tissue. Studies have confirmed that leptin plays not only a crucial role in the control of body weight in human, but also in several endocrine functions. Leptin is the paradigm of adipose tissue endocrine function. It is almost exclusively produced by adipocyte and it has a central role in energy storage regulation and fertility. When leptin signalling is defective, through a defect in either receptor or in peptide itself (ob/ob mouse), the NPY system is up-regulated (mRNA over-expression) and leads to increased peptide release, whereas the content and/or release of some inhibitory peptides (neurotensin, cholecystokinin) are diminished.

Genetic factors related to the leptin gene are important in defining the set point of obese individuals (i.e., the circulating leptin level for a given degree of body fatness). Le Stunff et al. have shown that girls of comparable adiposity have different circulating leptin levels, depending on their genotype at promoter region of the leptin gene. Girls with -/- Lep-2549 genotype have 25% lower mean leptin levels than the girls with other genotypes.

**Peptide YY (PYY)** — It is secreted as a 36 amino acid, straight chain polypeptide, and is found in maximum concentration at the terminal ileum, colon and rectum. PYY participates in regulation of appetite and weight balance through hypothalamic-based mechanisms. PYY (1-36) stimulates appetite and weight gain through Y1 and Y5 receptors. Variations in peptide YY and Y2 receptor genes are associated with severe obesity in Pima Indian men. Some studies have suggested that peripheral administration of peptide YY (3-36) and glucagon-like peptide-17-36 inhibit food intake additively. In a study, three rare non-synonymous variants have been identified, only one of which, PYY Q62P, exhibited familial segregation with body mass. A common and
conserved variant of PYY and NPY receptor Y2R variant is also protective for obesity.\textsuperscript{38}

Resistin— It is a cysteine-rich 12.5 kDa polypeptide, adipocytokine, with a controversial history regarding its role in pathogenesis of obesity-mediated insulin resistance and type 2 diabetes mellitus. The serum resistin concentration significantly correlates with the degree of obesity and distribution of fat.\textsuperscript{39} Variability in the serum resistin levels might be related to polymorphic variants of the promoter region of the gene. Chung \textit{et al.}\textsuperscript{40} have shown that stimulatory protein 1 (Sp1) interacts with resistin, a common polymorphism of human resistin promoter, \textendash \textendash 420C >G, is critical for binding of Sp1 and modulates the transcriptional activity of the resistin gene by changing the binding ability of Sp1. A recent study concerning 123 middle-aged women and 120 healthy young subjects has found that serum resistin levels do not correlate with markers of adiposity (including BMI, waist to-hip ratio, insulin resistance, lipid profile, and serum leptin levels\textsuperscript{41} while increased serum resistin in adults with Prader-Willi syndrome is related to obesity and not to insulin resistance. Resistin expression is significantly decreased in the white adipose tissue of several different models of obesity including ob/ob, db/db, tub/tub, and KKA(y) mice compared with their lean counterparts.

Ghrelin — Predominantly secreted from the stomach, is the natural ligand for the growth hormone secretagogue receptor in the pituitary gland thus, fulfilling criteria of a brain-gut peptide. It has profound orexigenic, adipogenic, and somatotrophic properties, increasing food intake and body weight. Ghrelin has ability to stimulate appetite by its activation of neuropeptide Y neurons and inhibition of pro-opiomelanocortin neurons. A negative feedback regulation may exist between adipocytokines and ghrelin production. Ukkola \textit{et al.}\textsuperscript{42} have identified a mutation at amino acid position 51 (Arg51Gln) of the pre-proghrelin sequence in obese subjects, and found that a mutation at codon 72 of pre-proghrelin gene (Leu72Met) is associated with lower age of onset of obesity. Circulating pre-prandial ghrelin to obestatin ratio is increased in the visceral adipose tissue of both humans and rodents. Interleukin-1 receptor antagonist gene polymorphism has been found to be highly enriched in the visceral adipose tissue of both humans and rodents. Interleukin-1 receptor antagonist gene polymorphism has been found to be associated with higher BMI in north Indian populaton\textsuperscript{50}. Obese people are more prone to gall stone diseases, but one study has shown that LRPAP insertion deletion polymorphism respective of BMI in gall stone patients\textsuperscript{51}.

Several studies have been conducted and reviewed the interaction of environmental and behavioural factors responsible for obesity. Overall existing knowledge regarding contributing factors in development of obesity suggests involvement of environmental cognitive and genetic factors in the progress of disease. A better understanding of their interactions in the process of weight gain will provide avenues for prevention and management.

Deletion of CART gene in mice resulted in diet induced obesity.\textsuperscript{48} Mutational screening of CART gene have shown that (Leu34Phe), mutation cosegregates with the severe obesity phenotype over three generations and has not been found in the control population. While in other study\textsuperscript{49} no clear association with obesity was found. A recently identified adipocytokine ‘visfatin’, found to be highly enriched in the visceral adipose tissue of both humans and rodents. Interleukin-1 receptor antagonist gene polymorphism has been found to be associated with higher BMI in north Indian populaton\textsuperscript{50}.

The data in this review article is based on MEDLINE and Pub Med searches using term “obesity” and “genetics” in combination with other forms of eating disorders. Neuromedin beta is found to be very strong candidate gene of eating behaviours and predisposition to obesity.\textsuperscript{44} A novel missense substitution (Val1483Ile) in the fatty acid synthase gene (FAS) is associated with percentage of body fat and substrate oxidation rates in non-diabetics. A study performed on Pima Indians indicated that Val1483Ile substitution in FAS is protective against obesity. The SLC6A14 gene is an interesting novel candidate for obesity. It encodes an amino acid transporter, which potentially regulates tryptophan availability for serotonin synthesis that possibly affects appetite control.\textsuperscript{45} In Zucker rats, continuous stimulation of beta3-adrenoceptors by KTO-7924 (a chemical compound) causes brown adipose tissue-like adipocytes to appear in retroperitoneal white adipose tissue, and improves lipid metabolism.\textsuperscript{46} Analysis of lineages of diabetic individuals indicates that SHP [orphan nuclear receptor small heterodimer partner (SHP, NR0B2)] mutation is associated with obesity rather than with diabetes\textsuperscript{47}.

Other candidate genes— Genetic studies in humans have shown that mutations in BDNF or TrkB genes may account for certain types of obesity or other forms of eating disorders. Neuromedin beta is found to be very strong candidate gene of eating behaviours and predisposition to obesity. A novel missense substitution (Val1483Ile) in the fatty acid synthase gene (FAS) is associated with percentage of body fat and substrate oxidation rates in non-diabetics. A study performed on Pima Indians indicated that Val1483Ile substitution in FAS is protective against obesity. The SLC6A14 gene is an interesting novel candidate for obesity. It encodes an amino acid transporter, which potentially regulates tryptophan availability for serotonin synthesis that possibly affects appetite control. In Zucker rats, continuous stimulation of beta3-adrenoceptors by KTO-7924 (a chemical compound) causes brown adipose tissue-like adipocytes to appear in retroperitoneal white adipose tissue, and improves lipid metabolism. Analysis of lineages of diabetic individuals indicates that SHP [orphan nuclear receptor small heterodimer partner (SHP, NR0B2)] mutation is associated with obesity rather than with diabetes.
key words “prevalence”, “genes”, “mutation”, “Asian” and “India”

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