Genetic factors associated with susceptibility to obesity

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Abstrak

Tinjauan pustaka ini membicarakan berbagai faktor genetik dan protein yang berhubungan dengan obesitas. Tiga mekanisme yang mendasari obesitas adalah: peningkatan relatif masukan energi, penurunan relatif penggunaan energi, dan kecenderungan penyimpanan kalori dalam bentuk lemak. Kelainan genetik dapat mendasari salah satu dari ketiga mekanisme tadi, baik sendiri ataupun bersamaan. Banyak faktor genetik yang berhubungan dengan kecenderungan mengalami obesitas, yaitu: peningkatan ekspresi protein 'agouti' atau protein 'serupa agouti'; defisiensi leptin, defisiensi atau mutasi reseptor leptin, gangguan pada jalur karboksipeptidase, mutasi 'tubby', peningkatan ekspresi GLUT4 transporter glukosa, mutasi reseptor serotonin 5-HT2C, dan gangguan pada CCK atau reseptor CCK-A.

Abstract

This review discusses the various genetic factors and gene products (proteins) related to obesity. The three fundamental mechanisms underlying obesity are: relative increase in energy intake; relative decrease in energy expenditure; and preferential partitioning of ingested calories to fat storage. Gene defects can be related to any one of these mechanisms, either alone or together. Many genetic factors are associated with susceptibility to obesity, i.e. over expression in agouti signaling protein or agouti related proteins; leptin deficiency, leptin receptor mutation or deficiency, abnormality in carboxypeptidase pathway, tubby mutation, over-expression of GLUT4 glucose transporter, serotonin 5-HT2C receptor mutation, and defect in CCK or CCK-A receptor.

Keywords: *agouti, leptin, leptin receptor, carboxypeptidase, tubby, GLUT4 glucose transporter, serotonin 5-HT2C receptor, CCK, CCK-A receptor*

The three fundamental mechanisms underlying obesity are: 1) relative increase in energy intake; 2) relative decrease in energy expenditure; and 3) preferential partitioning of ingested calories to fat storage.[1] Gene defects can be related to any one of these mechanisms, either alone or together. Experiments using transgenic animals showed that obesity was related to various genetic factors, either single or multiple. This review discusses the various genetic factors and gene products (proteins) related to obesity i.e. the agouti signaling protein (ASP), OB protein/leptin (lep), OB receptor/leptin receptor (*Lepr*), and many other factors.

Agouti signaling protein (ASP)

In mouse, ASP is coded by the agouti locus. ASP is a 131-amino acid peptide with a 22-amino acid signal peptide, a central basic region and a cysteine-rich C-terminus. It is normally produced only in the hair folicle and testes.

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A mouse with autosomal dominant agouti gene defect is characterized by obesity, hyperphagia, hyperinsulinemia, and hypercorticosteronism. The defect in the gene encoding ASP is located in the noncoding region that control the promoter, resulting in overexpression of a structurally unaltered ASP. Overexpression of ASP occurs in usual and ectopic (e.g. brain) sites.[1] In brain, ASP competes with high affinity against melanocyte-stimulating hormon (MSH) at one type of MSH receptor (melanocortin receptor, MC4R).[1,2] Fan et al (1977) showed that melanocortic neurons exert a tonic inhibition of feeding behaviour.[3] Thus, it appears likely that some of the obesity-producing efeects of ASP expression in the brain may be due to its interference with signal generation by MSH at MC4R, a signal which normally act to suppress food intake. Furthermore, ASP also appears to induce lipogenesis by enhancing insulin sensitivity,[1] due to the increasing intracellular Ca2+ [Ca+2]i.[4-6] In addition, recombinant agouti exerts a potent antilipolytic effect in human adipocytes via a [Ca2+]i dependent mechanism.[4]

Kesterson et al (1997) showed that expression of neuropeptide Y (NPY), a potent stimulator of food

intake, in the dorsal medial hypothalamic nucleus (DMH) was seen in MC4R-knock out (MC4-RKO, -/-) mice, but not in heterozygous (+/-) or wild type (+/+) mice.[7] This identifies the DMH as a brain region that is functionally altered by the disruption of melanocortic signaling and suggests that this nucleus induced feeding via elevated NPY expression.[7,8]

In humans, ASIP (gene symbol for the human homolog of agouti) maps to 20q11.2, and is primarily expressed in adipocytes.[4,6] In addition it is also express at low level in the skin, testes, ovary, heart, liver and kidney, suggesting that it may have roles in humans which are different from the mouse.[1] Whether ASIP is normally expressed in human brain is not known. ASIP has not yet been directly linked to human obesity.[1] However, as humans express agouti in adipose tissue, it may exert paracrine effects on [Ca2+]i, and thereby stimulate de novo lipogenesis.[6]

Agouti related protein

Agouti related protein (AGRP) is also known as agouti related transcript (ART).[1] The gene maps to 16q22 in human. The protein has 132 amino-acids and is 25% identical to human agouti.[9] ART is expressed primarily in the adrenal gland, subthalamic nucleus and hypothalamus, with a lower level of expression occuring in testes, lung and kidney.[9] As agouti, AGRP is an antagonist of the melanocortin receptors, MC3R and MC4R.[2]

In mouse, ART showed localized expression in the arcuate nucleus of the hypothalamus, the median eminence, and the adrenal medula. In addition, the hypothalamic expression of ART was elevated approximately 10 fold in obese mice.[9]

OB protein/leptin (lep)

In rodent, leptin is coded by the leptin gene and synthesized as a 167-amino acid protein and secreted from adipose tissue after axcision of a 21-aa signal peptide. Leptin has a cytokine-like predicted tertiary structure, which includes 4 α-helices, 2 β-sheets, and a single disulfide bond between cysteine 96 and 146.[1]

In the *ob/ob* mouse, a mutation of the leptin gene causes leptin deficiency,[10,11] and results in hyperphagia, decreased energy expenditure, severe obesity, and insulin-resistant diabetes.[11] Central or peripheral administration of leptin to these mice leads to acute decrease in food intake; and chronic administration of leptin results in marked reduction in body fat.[11]

The human leptin gene (LEP) is 85% identical to the murine gene at the amino acid level and maps to 7q31.3. Extreme obesity in humans has been linked to genetic markers near the leptin gene.[1] Until 1996, direct coding sequence analysis of a large number of human subjects has failed to identify any functionally significant coding variation in this gene.[10] However, in 1997 Montague et al confirmed the definite role for leptin in human energy balance, by the demonstration of homozygozity for a frameshift mutation, resulting from a single nucleotide deletion in the protein coding region of leptin gene in two cousins with massive and early onset obesity.[12]

Regulation of leptin secretion

Numerous studies in humans have reported that circulating leptin concentrations are highly correlated with adiposity.[10,13] However, fasting or energy restriction decreases leptin concentrations[1] acutely and disproportionately to the relative modest changes in adiposity.[12] The response of the body will be to increase appetite and decrease energy expenditure to restore adipose tissue mass. On the other hand, one day of massive overfeeding, which does not change body weight, results in a 40% increase in serum leptin. Thus, factors other than adipose tissue mass are likely to influence leptin secretion. One of these factors may be caloric intake.[10] In addition, there is rhythmicity of adipose tissue leptin-mRNA expression which is entirely linked to changes in food intake.[10]

Infusion of small amounts of glucose is sufficient to prevent a decrease in glycemia and insulinemia during fasting in humans, also to prevent a decrease in plasma leptin. The decrease in circulating leptin concentrations during energy restriction in humans is related closely to the decrease in plasma glucose. Thus, adipocyte glucose metabolism may be involved in the regulation of leptin secretion.[11]

Adipocytes from obese subjects maintained in culture up to 5 days continued producing severalfold more leptin than those from lean patients. Thus it is important to understand how the microenvironment of adipocytes, in the absence of extracellular messengers, provides the correct information to the leptin promoter. It seems that cell size is an important determinant of leptin mRNA expression. Small fat cells expressed less leptin mRNA than large fat cells even when obtained from the same individual. This suggests the possibility that cell wall stretching itself may initiate signal, since exogenously applied tension to the plasma membrane has been demonstrated to lead to the induction of gene

expression in other cell system. It is also possible that products of intracellular adipocyte metabolism, free fatty acids, diacylglycerol, lysophosphatidic acid, etc., could act as modifiers to regulate leptin gene expression.[10]

Agouti antagonism of central nervous system MCR binding inhibits the anorexic effects of leptin, whereas agouti up-regulates adipocyte leptin expression, serving to limit the magnitude of agouti-induced obesity.[14] Finally, hormones, such as insulin, glucocorticoid and adrenergic agents, regulate leptin secretion.[10]

Leptin-insulin relationship

Whereas systemic administration of leptin to leptin-deficient mice restores insulin-sensitive glucose disposal, insulin resistance to glucose disposal in obese humans occurs in the presence of high ambient leptin. Thus both total leptin deficiency and extreme leptin excess are associated with resistance to insulin action.[1]

Acute (hours) and chronic (days) administration of insulin in vivo and in vitro increases adipose tissue leptin mRNA in rodents. Short-term incubation of freshly isolated human adipocytes in either the presence or absence of insulin did not result in an insulin stimulated leptin release into the medium. Therefore it is clear that acute insulin does not stimulate leptin secretion in humans. However, chronically, insulin appears to play an important role in leptin mRNA expression and leptin secretion.[10]

Leptin action

It is not clear whether leptin must enter the cerebrospinal fluid to affect the brain, which receive its putative signal regarding fat mass. One of the circumventricular organs (vascular elements lacking the blood brain barrier) is located in the median eminence, just below the arcuate nucleus of the hypothalamus. The arcuate, which mediates aspects of ingestive behaviour, projects axons to the median eminence, and it is possible that the arcuate cell bodies are exposed directly to the circulation. In non-obese humans and animals, cerebrospinal fluid leptin concentration is about 5% of the plasma concentration, whereas in obese individuals, the ratio of cerebrospinal fluid to plasma leptin concentration is diminished. This is apparently due to saturation of the transport system at a plasma leptin concentration of approximately 25 ng/ml, well below the circulating concentration of leptin in most obese individual.[1]

Leptin's effect on body weight appears to be mediated primarily via effects on the hypothalamus. Some of leptin effects on energy homeostasis are apparently conveyed by the suppression of the arcuate nucleus in the expression of neuropeptide Y (NPY). NPY stimulated food intake, decreases thermogenesis, and increases plasma insulin and corticosteroid level. Therefore, NPY appears to be a logical transducer system for leptin action.[10]

Leptin resistance

Some leptin effects on energy homeostasis are apparently conveyed by suppression of the expression of neuropeptide Y (NPY) a potent stimulator of food intake.[10] Therefore, higher concentration of leptin in obese individuals should reduce food intake. However, this is not occurred. Furthermore, leptin concentration are higher per unit adiposity in obese than in normal-weight subjects.[11] These facts can be interpreted to indicate resistance to leptin action in obese individuals. This is supposed to be due to central nervous system insensitivity to leptin.

OB receptor/leptin receptor (*lepr*)

In human, *Lepr* is encoded by *Lepr* gene, and the gene maps to 1p31.[1] *Lepr* belong to the class I cytokine receptor family. They usually have an extracellular ligand binding domain of 840 amino acids, a transmembrane domain of 34 amino acids, and a variable intracellular domain.[10]

Lepr isoforms

To date, several *lepr* isoforms have been identified. A short leptin receptor, named *ob* Ra, has a 34-amino acid intracellular domain and is believed to function as a transporter. A long leptin receptor, named *ob* Rb, has a 304-amino acid intracellular domain and is believed to function as the first leptin-signaling step. Another leptin receptor, *ob* Rc, with intracellular domain of 32 amino acids, may also function as a transporter. The final leptin receptor, *ob* Re, is the shortest and lacks transmembrane domain; therefore, it may be a soluble receptor.[10]

Lepr expression

Lepr is expressed in multiple tissues.[1] Therefore, *lepr* isoforms are present in the hypothalamus, brain, choroid plexus, liver, lung, heart, kidney, testes, adipose tissue, spleen, etc.[10]

Lepr mutation

In diabetic (*db/db*) mouse and Zucker fatty (*fa/fa*) rat, obesity is due to a mutation in the *Lepr* gene. In *db/db* mouse, *Lepr* mutation is due to a point mutation that generates a novel splice donor site in the 3'-untranslated region of the penultimate exon of the gene. The resulting frameshift affects only the longest isoform, the *ob* Rb, resulting in the deficiency of just this isoform, which is sufficient to produce the obesity/diabetes syndrome. In *fa/fa* rat, *Lepr* mutation is due to a point mutation of codon 269 (CAG → CCG, Q269P), which is within the cytokine motif of the receptor.[1]

In humans, several polymorphisms within the *Lepr* gene have been reported in both obese and normal weight subjects. However, the functional effects of these polymorphisms have yet to be determined. No human subject has yet been described with an unambigous loss-of-function mutation in the *Lepr* gene.[1]

Lepr function

The long leptin receptor (*ob* Rb) is believed to function as the first leptin-signaling step. The long intracellular domain contains putative motifs for Janus protein-tyrosine kinase (JAK) and signal transducers and activators of transcription (STAT) binding. JAK and STAT binding are key steps for cytokine class I receptor signaling.[10]

In addition, *Lepr* is suggested to function in receptor-mediated transport, which may be necessary for leptin tissue uptake and catabolism. *Lepr* mRNA is highly expressed in the kidney, which is confirmed to be the significant site of leptin clearance in humans.[15]

Other factors

Carboxypeptidase E (Cpe)

CPE is required for the excision of paired dibasic residues remaining at the C terminus of peptide prohormone intermediates such as proinsulin, which is previously cleaved by prohormone convertase 1.[1]

In mouse, homozygous CPE mutation leads to abolish virtually all of the activity of the enzyme and causes obesity, hyperproinsulinemia, and transient hyperglycemia. However, transgenic replacement of CPE activity in the pancreatic islets does not alter the obesity. Therefore, the mechanism of obesity in CPE mutation is not caused by the defect in insulin processing *per se*.[1] CPE plays a role in processing neurohormones such as cholecystokinine (CCK),[16] and gastrin.[17] In addition, CPE has putative roles in processing other prohormones and proneuropeptides, such as neurotensin, MSH, etc. This suggests possible mechanisms for CPE in causing obesity via effects on these neurohormones that regulate food intake and energy expenditure.[1]

In humans, no instance of obesity can be related to sequence variations in the gene encoding CPE (4q32). However, obesity due to compound heterozygosity for mutations in prohormone convertase 1 has been found in a 47-year old woman with moderate obesity and hyperproinsulinemia.[18] Thus, the CPE pathway has been implicated in the control of body weight in humans.[1]

Tubby gene product (tub)

In mouse, *tub* shows 62% amino acid similarity to a putative phosphodiesterase, but may actually be a member of a novel protein family. *Tub* is expressed in high level in the hypothalamus, a region of the brain that plays a critical role in control of food intake and energy expenditure.[1]

A mouse with homologous tubby gene defect (autosomal recessive mutation)[19] does not develop obesity until at least 12 weeks of age, and the obesity is much milder in degree than any of the other rodent single gene mutation.[1] In addition, the mouse with tubby gene defect develops retinal degeneration, degeneration of the organ of Corti and the ganglion cells of the cochlea at 3 weeks, but has no other neurological effects.[20] However, in adult mice, *tub* is transcribed in several brain areas, including cerebral cortex, hippocampus, several nuclei of the hypothalamus controlling feeding behavior, in the spiral ganglion of the inner ear and in the photoreceptor cells of the retina. These structures contain potential cellular targets of the tubby mutation-induced pathogenesis.[21] Therefore it has been hypothesized that lack of *tub* may result in apoptosis of neural cells of these organs.[1] Apoptosis of specific cells within the hypothalamic ventromedial nucleus, which is believed to be the satiety center,[22] prevent the inhibition of the feeding center and lead to obesity, such as observed when these nuclei are ablated.[23]

In comparison with the wild-type controls, a significant reduction (20%) of pro-opiomelanocortin (POMC) mRNA expression was observed in the arcuate nucleus (ARC) of the mature obese, but not in the juvenile non-obese tubby mice. Similarly, an age and body mass dependent induction (about 30-fold) of

NPY mRNA was observed in the dorsomedial (DMH) and ventromedial (VMH) hypothalamic nuclei of the tubby mice. However, NPY mRNA in the ARC was decreased by approximately 30-40% in both juvenile and mature tubby mice. These results suggest that the altered hypothalamic POMC and/or NPY functions may be important contributing factors for the development of obesity in this animal model.[19]

The human tubby gene homolog maps to 11p15, and is 94% identical to the mouse gene at the amino acid level with particularly high conservation within the final 260 amino acids. To date, no mutations in the human homolog have been reported.

Glucose transporter (GLUT4)

The GLUT4 glucose transporter plays a pivotal role in insulin-stimulated glucose transport in fat and muscle. In transgenic mice that overexpress GLUT4 selectively in fat, *in vivo* glucose disposal is enhanced and massively increased glucose transport into isolated adipocytes occurs. These mice remarkably develop increased adiposity due to adipocyte hyperplasia. These data demonstrated that increased expression of the GLUT4 gene in fat may alter nutrient partitioning so as to increase adipose mass, and that increase glucose transport into fat may set into motion events that foster the replication of immature adipocytes and/or the differentiation of precursor cells into adipocytes.[24]

Serotonin 5-HT2C receptor

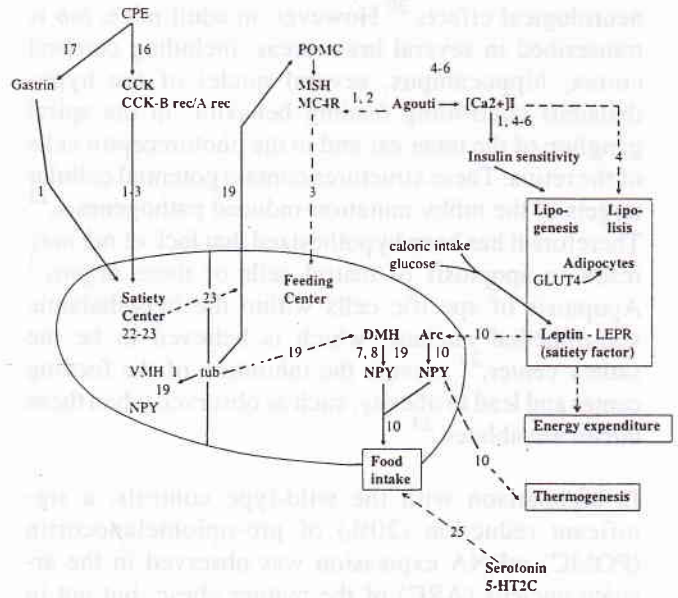
Young adult mice with a targeted mutation of the serotonin 5-HT receptor gene consume more food despite normal responses to exogenous leptin administration. Chronic hyperphagia leads to a 'middle-aged'-onset obesity associated with a partial leptin resistance of late onset. In addition, older mice develop insulin resistance and impaired glucose tolerance. Mutant mice also responded more to high-fat feeding, leading to hyperglycemia without hyperlipidemia. These data establish a role for 5-HT2C receptors in the serotonergic regulation of body weight and food intake.[25]

In human, anorexigenic agents, that induce serotonin (5-hydroxytryptamine, 5-HT) release from nerve terminals and reduce its re-uptake, are used to decrease appetite and body weight in obese subjects. In addition, some of these agents may have effects on thermogenesis.

Neurohormones and its receptors

Cholecystokinin (CCK) is a 33-amino acid peptide with multiple functions in both the central nervous system (via CCK-B receptors) and the periphery (via CCK-A receptors). CCK mediation of satiety via the A-receptor subtype suggests a role for CCK in the management of obesity.

Inter-relationship between various factors influencing food intake, energy expenditure, and fat storage is summarized in Figure 1.



--- > = inhibition, → = induction, numbers = reference number, CPE = carboxypeptidase, POMC = pro-opiomelanocortin, CCK = cholecystokinin, CCK-B rec = CCK-b receptor, MSH = melanocyte stimulating hormone, MC4R = melanocortine receptor, LEPR = leptin receptor, VMH = ventromedial nuclei of hypothalamus, DMH = dorsomedial nuclei of hypothalamus, Arc = arcuate nuclei of hypothalamus, NPY = neuropeptide Y, 5-HT2C = serotonin receptor

Figure 1. Various factors influencing food intake, energy expenditure and fat storage

In conclusion: many genetic factors are associated with susceptibility to obesity, i.e. over expression of agouti signaling protein or agouti related proteins; leptin deficiency; leptin receptor mutation or deficiency; abnormality in carboxypeptidase pathway; tubby mutation; over-expression of GLUT4 glucose transporter; serotonin 5-HT2C receptor mutation; and defect in CCK or CCK-A receptor.

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