

## Obesity – a genetic disease of adipose tissue?

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Although the rapid increase in the prevalence of obesity in many countries suggests that environmental factors (mainly overeating and physical inactivity) play the most important role in the development of overweight, it is very likely that genetic factors also contribute. It appears that one major gene in combination with one or several minor genes constitute the genetic components behind excess accumulation of body fat in most obese individuals. However, monogenic obesity has been described in a few families due to changes in leptin, leptin receptor, prohormone convertase, pro-opiomelanocortin or melanocortin-4 receptor. None of the monogenic variants is of great importance for common human obesity; the latter genes are unknown so far. Results from genomic scans suggest that major obesity genes are located on chromosomes 2, 10, 11 and 20. Studies of candidate genes indicate that the minor obesity genes control important functions of adipose tissue, and that structural variance in these genes may alter adipose tissue function in a way that promotes obesity. Such genes are  $\beta_2$ - and  $\beta_3$ -adrenoceptors, hormone-sensitive lipase, tumour necrosis factor alpha, uncoupling protein-1, low-density lipoprotein receptor, and peroxisome proliferator activator receptor gamma-2. Some of these genes may promote obesity by gene–gene interactions (for example  $\beta_3$ -adrenoceptors and uncoupling protein-1) or gene–environment interactions (for example  $\beta_2$ -adrenoceptors and physical activity). Some are important for obesity only among women (for example  $\beta_2$ - and  $\beta_3$ -adrenoceptors, low-density lipoprotein receptor and tumour necrosis factor alpha). Few ‘non-adipose’ genes have so far shown a firm association to common human obesity, which could suggest that the important genes for the development of excess body fat also control adipose tissue function.

**Fat cells: Body mass index: Lipolysis: Thermogenesis: Adipocyte differentiation**

### Introduction

The prevalence of obesity increases rapidly in most countries, reaching almost epidemic proportions in areas where there has been a recent change in habits towards a more westernized lifestyle. As discussed (Hill & Peters, 1998) the environmental contribution to the obesity epidemic is apparent. Nevertheless, the strong genetic component of obesity is established in numerous studies on twins and relatives (Bouchard, 1993). A recent analysis of some of these studies suggests that as much as 50–70 % of variation in body mass index is attributable to genetic difference (Allison *et al.* 1996). In this review the recent development in genetic research on human obesity is summarized, in particular focusing on the notion that several important obesity genes may be involved in the regulation of human adipose tissue function and might cause excess body fat by a direct interaction with the fat cell. For comprehensive overviews of earlier work in the field of human obesity

genetics, see Bouchard (1993); Bouchard & Pérusse, (1996); Pérusse *et al.* (1996); Chagnon *et al.* (1998). In the interest of space, review articles rather than original papers are cited whenever possible. Studies only showing a lack of association between a particular gene and obesity have not been incorporated in this review.

### Evidence for a genetic cause of rare forms of obesity

Using recent developments in DNA technology, most of the monogenic obesities in rodent models have been identified within a few years (Rosenbaum *et al.* 1997). Some rare forms of monogenic obesity in humans have also been described (Table 1). Families with juvenile-onset morbid obesity have been screened for mutations in the genes causing monogenic obesity in the mouse. So far, mutations in the human genes coding for leptin (Montague *et al.* 1997; Strobel *et al.* 1998), leptin receptor (Clément *et al.* 1998),

**Table 1.** Monogenic forms of obesity

Human gene	Reference*	Corresponding mouse model
Leptin	Montague <i>et al.</i> 1997	ob/ob
Leptin receptor	Clément <i>et al.</i> 1998	db/db
Proopiomelanocortin	Krude <i>et al.</i> 1998	Agouti
Prohormone convertase 1	Jackson <i>et al.</i> 1997	fat/fat
Melanocortin 4 receptor	Vaisse <i>et al.</i> 1998; Yeo <i>et al.</i> 1999	Mc4r-deficient mice

\* Relevant first published references.

pro-opiomelanocortin (Krude *et al.* 1998), prohormone convertase-1 (Jackson *et al.* 1997) and melanocortin-4 receptor (Vaisse *et al.* 1998; Yeo *et al.* 1999) have been described, all of which associate with juvenile-onset morbid obesity. The mechanisms responsible for excess fat accumulation in the rare forms of monogenic human obesity are not known. However, it is likely that they share features of the pathophysiology behind the equivalent genetic forms of obesity among rodents (Table 1) which have been discussed extensively (Chua & Leibel, 1997). As mentioned, these mutations are rare and they can only explain obesity among a few families. Several of the genes have been screened for an association to obesity among large populations but no important associations have been found (Echwald *et al.* 1999; Norman *et al.* 1999; Pérusse *et al.* 1999).

#### Number of genes involved in common forms of human obesity

The genes of importance for common forms of human obesity remain to be identified. The results of segregation analysis of several different pedigree samples suggest that a major gene is in control of the fat mass (see Ginsburg *et al.* 1998). On the other hand, a number of chromosomes have been identified using a genome-wide scan approach in family studies; at least one major obesity gene is located in each of these genes (Table 2). Thus chromosome numbers 2 (Comuzzie *et al.* 1997; Rotimi *et al.* 1999), 10 (Häger *et al.* 1998), 11 (Norman *et al.* 1998) and 20 (Lembertas *et al.* 1997; Lee *et al.* 1999) appear to contain one or several genes which are in control of body weight. The quantitatively most important locus in each of these genes is partly identified, but we do not know yet exactly which genes and which mutation(s) in the genes associate with obesity. It is of interest to note that some candidate genes believed to be involved in the etiology of obesity are located near the chromosomal loci described in Table 2. This includes leptin and pro-opiomelanocortin on chromosome 2, as well as agouti-signalling protein, CEBPB (an adipocyte differentiation gene) and adenosine deaminase on chromosome 20.

**Table 2.** Location of major human obesity genes

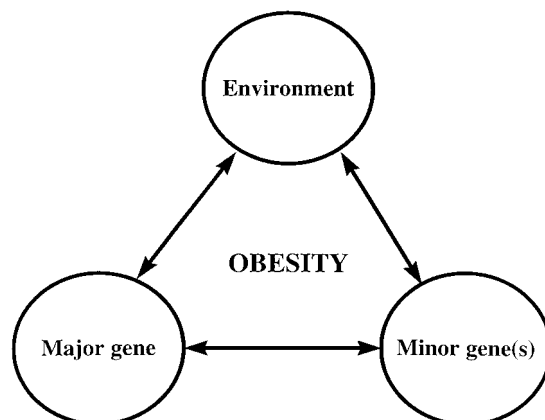
Chromosome	Reference*	Locus
2	Comuzzie <i>et al.</i> 1997	p21
10	Häger <i>et al.</i> 1998	5 cM from p-terminal
11	Norman <i>et al.</i> 1998	q21–22
20	Lembertas <i>et al.</i> 1997	q13

\* Relevant first published references.

It is evident from the discussion above that segregation analysis and genome-wide scanning give different answers to the question whether one or several genes are involved in the etiology of common forms of human obesity. It is thought that several genes (in combination with environmental factors) cause obesity in a certain individual (Comuzzie & Allison, 1998). A schematic picture of gene–environment interactions is given in Fig. 1. One of these genes may be a major one and the others could be minor obesity genes. The particular major and minor obesity genes may differ between families or ethnic groups. In the rest of this paper I discuss the relative importance of genes with a known function for human obesity. Through the so-called candidate gene approach, a number of polymorphisms in different genes have recently been found to be linked to human obesity. It is apparent that all of these are in control of major functions of adipose tissue.

#### $\beta_3$ -adrenergic receptor

In 1995 it was discovered in three independent populations that a coding mutation in the  $\beta_3$ -adrenergic receptor (Trp64Arg) associated with obesity and certain features of the so-called metabolic (insulin resistance) syndrome (Strosberg, 1997a; Arner & Hoffstedt, 1999). This polymorphism has given rise to enormous interest and almost 100 articles on the mutation have been published so far according to *Medline*. About half of the obesity studies show an association to overweight whereas the other half fail to demonstrate a relationship with obesity. Even meta-analyses show divergent results as regarding the link of Trp64Arg to body weight and obesity (Allison *et al.* 1998;

**Fig. 1.** Gene–environment interactions leading to obesity.

Fujisawa *et al.* 1998). Although the reasons for the discrepant findings are not yet known, it is quite possible that the  $\beta_3$ -adrenoceptor is an important obesity gene.

The  $\beta_3$ -adrenoceptor is above all expressed and functional in white and brown fat cells (Lowell & Fliers, 1997; Strosberg, 1997b). Although the brown adipose tissue mass is small in adult humans, the thermogenic effect in brown human fat cells can be activated both *in vivo* and *in vitro* by  $\beta_3$ -adrenergic agonists. Furthermore,  $\beta_3$ -receptors can stimulate lipolysis in white human adipose tissue although the receptor is less efficient in this respect in comparison with the co-existing  $\beta_1$  and  $\beta_2$  subtypes. Recent studies have shown that the Trp64Arg substitution in the  $\beta_3$ -adrenoceptor molecule markedly impairs the receptor function in human fat cells (Hoffstedt *et al.* 1999b; Umekawa *et al.* 1999) and decreases resting metabolic rate (Sipiläinen *et al.* 1997).

When all available information is considered together, it is possible that the adipocyte  $\beta_3$ -adrenoceptor is an obesity gene. The Trp64Arg mutation impairs receptor function and is associated with decreased thermogenesis. This may explain its association with obesity in several populations.

#### *$\beta_2$ -adrenergic receptor*

The  $\beta_2$ -adrenoceptor plays several roles in the regulation of energy homeostasis, such as stimulation of glycogen breakdown and stimulation of lipid mobilization. It co-exists with  $\beta_1$ - and  $\beta_3$ -adrenoceptors in human white adipose tissue (see Carpené *et al.* 1998; Large & Arner, 1998 for thorough reviews on its normal and pathophysiological function). The  $\beta_2$ -adrenoceptor is the dominating lipolytic adrenoceptor subtype under normal conditions and is downregulated in subcutaneous adipose tissue of obese subjects. The latter is an important underlying mechanism for the frequently observed lipolytic catecholamine resistance in human obesity.

Several coding mutations in the  $\beta_2$ -adrenoceptor gene are described that markedly impair receptor function in transfected cell lines (Büscher *et al.* 1999). At least one of these polymorphisms (Arg16Gly) associates with altered receptor function in human white fat cells (Large *et al.* 1997). Another  $\beta_2$ -receptor polymorphism, Gln27Glu, was strongly associated with obesity among Swedes (Large *et al.* 1997). It was confirmed that this polymorphism is linked to obesity in Japanese subjects (Ishiyama-Shigemoto *et al.* 1999; Mori *et al.* 1999). On the other hand, it is not associated with early onset obesity in Danes (Echwald *et al.* 1998). Gene–environment interactions for the Gln27Glu polymorphism are described (Meirhaeghe *et al.* 1999). An interaction between this polymorphism and body mass index is observed in sedentary but not in physically active subjects.

It could be speculated that the Gln27Glu polymorphism influences body fat accumulation (maybe in combination with physical inactivity) by changing the lipolytic response of adipose tissue to catecholamines, which are the major lipolysis-stimulating hormones in man. Bearing in mind the broad action of  $\beta_2$ -adrenoceptors, there are a number of other possibilities by which this receptor may promote obesity.

#### *Hormone-sensitive lipase*

Another important lipolysis-regulating protein is hormone-sensitive lipase (Langin *et al.* 1996). Hormone-sensitive lipase is the final rate-limiting step for triglyceride breakdown in fat cells during lipolysis. It is stimulated by catecholamines and inhibited by insulin. As well as in adipose tissue, the enzyme is also functionally expressed in skeletal muscle and testis. The physiological importance in the latter two organs is not well understood. A number of studies have demonstrated decreased ability of catecholamines to stimulate lipolysis in subcutaneous adipose tissue (Large & Arner, 1998). It was recently shown that this lipolysis defect could be explained in part by a decreased expression of hormone-sensitive lipase in fat cells (Large *et al.* 1999). The same type of lipolysis defect in hormone-sensitive lipase also is found in non-obese subjects with heredity for obesity (Hellström *et al.* 1996).

Association between the hormone-sensitive lipase gene and obesity has been presented in two studies. A non-coding dinucleotide repeat was shown to associate with obesity in a French population (Magré *et al.* 1998). Genetic variability, detected by restriction fragment length polymorphism, associated with abdominal obesity in a Scandinavian population (Klannemark *et al.* 1998). It is not known if structural variants in hormone sensitive lipase gene are linked to altered enzyme function. It is possible that the polymorphisms only reflect more important genetic variance on chromosome 19 near the hormone-sensitive lipase locus, although no such evidence was found in a linkage study on French sib-pairs (Clément *et al.* 1999). When genetic and functional data are combined it is tempting to speculate that gene mutations directly or indirectly impair hormone-sensitive lipase so that lipolysis in fat cells is decreased which, in turn, may promote obesity.

#### *Uncoupling proteins*

Uncoupling proteins belong to a family of proteins which can uncouple ATP production from mitochondrial respiration, thereby dissipating energy as heat and affecting energy expenditure (Schrauwen *et al.* 1999). Three different proteins are discovered. The first, UCP-1, is exclusively expressed in brown adipose tissue where it regulates the combustion of fatty acids (Cannon *et al.* 1998). Brown adipose tissue is present in considerable amounts in human newborns and plays an important role for thermogenesis in the intra-uterine and early phase of extra-uterine life. In adults, little brown adipose tissue is present but brown fat cells are found in several human tissues (Krief *et al.* 1993), and decreased gene expression of UCP-1 is detected in white adipose tissue of obese as compared to lean adult subjects (Oberkofler *et al.* 1997). It is possible to activate UCP-1 in human adipose tissue by  $\beta_3$ -adrenergic agonists through stimulation *in vivo* or *in vitro*. UCP-2 is expressed in several tissues (including adipose tissue) whereas UCP-3 is predominantly expressed in skeletal muscle. Also, the tissue expression of mRNA for both UCP-2 and UCP-3 is influenced by the obese state in man.

Polymorphisms in the genes for UCP-2 and UCP-3 show some association to resting metabolic rate, but published

work suggests that none of these genes is likely to play an important role in human obesity (Otabe *et al.* 1999; Ricquier, 1999; Warden, 1999). On the other hand, a polymorphism (A to G) at position -3826 in the 5' flanking region of the UCP-1 gene influences weight loss and weight gain in obese subjects from France or Quebec (Cassard-Doulcier *et al.* 1996; Fumeron *et al.* 1996; Oppert *et al.* 1999). Interestingly, there are also important interactions between the genes for UCP-1 and  $\beta_3$ -adrenergic receptor. Thus synergistic effects of A(-3826)G in UCP-1 and Trp64Arg in  $\beta_3$ -adrenoceptor on basal metabolic rate in obese Finns (Valve *et al.* 1998) and on weight gain or weight loss in obese French or Finnish subjects (Clément *et al.* 1996; Fogelholm *et al.* 1998) are reported. It should be noted, though, that no relationship between the described UCP-1 polymorphism and obesity variables was found in Swedish subjects (Cagnon *et al.* 1998). Based on the findings discussed above it is possible that UCP-1 is an obesity gene in humans. Mutations in or near this gene could influence metabolic rate and thereby cause changes in body fat stores. Such an effect on thermogenesis may be of particular importance when genetic variance in UCP-1 and  $\beta_3$ -adrenoceptor genes co-exists.

#### *Peroxisome proliferator-activated receptor gamma-2 (PPAR $\gamma$ -2)*

Peroxisome proliferator-activated receptors, especially PPAR $\gamma$ -2, are key regulators of adipocyte differentiation and energy storage (Schoonjans *et al.* 1997). The expression of mRNA coding for PPAR $\gamma$ -2 is increased in adipose tissue of obese subjects. Two coding mutations, which are functional, have been described in PPAR $\gamma$ -2. A Pro115Gln mutation accelerates the differentiation of adipocytes and is associated with morbid obesity in Germans (Ristow *et al.* 1998). A Pro12Ala substitution associated with decreased receptor activity and lower body mass index in Finns (Deeb *et al.* 1998), and in lean but not in obese Danes (Ek *et al.* 1999).

The PPAR $\gamma$ -2 mutations are very rare. So far only a handful of subjects have been described who are homozygous for either of the two mutations; heterozygotes seem to have a normal phenotype (Mori *et al.* 1998; Ek *et al.* 1999). More research, including investigations of additional and large populations, is needed to ascertain whether PPAR $\gamma$ -2 is an important obesity gene. It could be speculated, though, that genetic variance in PPAR $\gamma$ -2 affects adipocyte differentiation and subsequently alters the normal development of the fat mass.

#### *Tumor necrosis factor alpha (TNF- $\alpha$ )*

The cytokine TNF- $\alpha$  modulates the expression of several genes in adipose tissue and is implicated in the development of obesity (Hotamisligil & Spiegelman, 1994). The most important TNF- $\alpha$  effect for obesity is the ability to modulate insulin sensitivity, mainly by inhibiting insulin signalling in fat cells (Hotamisligil, 1999). TNF- $\alpha$  has also other actions in adipose tissue that could play a role in obesity: it can induce apoptosis of fat cells (Prins *et al.* 1997) and stimulate lipolysis; the latter is achieved by decreasing the expression of the lipolysis-inhibiting G<sub>i</sub> protein in adipocytes (Gasic *et al.* 1999).

Genetic studies using sibling-pair analysis demonstrated linkage between a marker near the TNF- $\alpha$  and body fat content in Pima Indians (Norman *et al.* 1995). A polymorphism upstream of the transcription start site of TNF- $\alpha$  (G to A at position -308) associated with obesity in several European populations (Férendez-Real *et al.* 1997; Herrmann *et al.* 1998). It was recently found in Swedes that the latter polymorphism influences body fat content only in homozygous subjects (Hoffstedt *et al.* 2000). A/A (-308) subjects had excessive fat accumulation in comparison to heterozygous and G/G (-308) subjects. The latter may suggest that TNF- $\alpha$  is a recessive obesity gene. It is unclear how the -308 polymorphism alters the function of TNF- $\alpha$ , although *in vitro* studies have documented that this genetic variance influences the transcription of the TNF- $\alpha$  gene in lymphocytes (Wilson *et al.* 1997).

TNF- $\alpha$  is widely expressed and has many divergent actions which could influence storage of body fat. However, it is possible that structural variance in the gene alters the expression of TNF- $\alpha$  in fat cells so that lipolysis, insulin sensitivity and apoptosis in adipose tissue are changed in a way which causes excess body fat accumulation.

#### *Low-density lipoprotein receptor (LDL-R)*

LDL-R is a widely expressed receptor which has multiple roles in lipid metabolism (Willnow, 1999). Of particular interest for adipose tissue is that it mediates fat cell uptake of lipids from remnant lipoproteins (Descamps *et al.* 1993). Several studies have shown that a polymorphic marker near the locus for LDL-R has relatively strong linkage to obesity (see Pérusse *et al.* 1999 for references). The exact nature of this polymorphism remains to be established. Although several obesity-promoting mechanisms not directly involving adipose tissue are possible for LDL-R, it could be speculated that structural variance in the corresponding gene leads to increased lipid uptake by fat cells so that excess storage of fat in adipose tissue is developed.

#### *Other genes*

Single nucleotide substitution and small base deletions and insertions are the most common forms of DNA polymorphism and disease-causing mutations in the human genome. Using recent advances in DNA sequencing and computerized bioinformatics, it is possible to rapidly screen several mutations simultaneously in large population samples (Gu *et al.* 1998; Wacey & Tuddenham, 1998). Using similar or simpler approaches, a number of polymorphisms in several genes other than those mentioned above have been investigated regarding an association with obesity (see Pérusse *et al.* 1999 for references). Most of these genes have no known important function in adipose tissue. So far either no or a weak association between these 'non-adipose tissue' genes and obesity has been demonstrated; usually only a single report for each of the genes is published.

#### **Gender and obesity genes**

It is well known that body fat distribution usually differs between men and women. Men often have upper-body

obesity, whereas peripheral obesity is the most common form among women. Family studies strongly suggest that body fat distribution, in particular visceral fat level, is governed by genetic factors (Katzmarzyk *et al.* 1999). Several of the polymorphisms discussed in this review have a different impact on body fat in men than in women.

Many studies suggest that the Trp64Arg polymorphism in the  $\beta_3$ -adrenoceptor is associated with obesity only in women (Arner & Hoffstedt, 1999). The Gln27Glu polymorphism in the  $\beta_2$ -adrenoceptor is linked to obesity in women but not in men in a Swedish population (Hellström *et al.* 1999). The A/G(-3081) polymorphism in the TNF- $\alpha$  gene associated with excess body fat in women but not in men in a study on Swedes (Hoffstedt *et al.* 2000). Association of an LDL-R microsatellite variant with obesity was found in females, but not in males (Rutherford *et al.* 1997). These data indicate that men and women have different obesity genes. Clearly more studies are needed on the interactions between gender, genes and obesity.

### Conclusions

In the past few years knowledge of the genetics behind obesity has increased dramatically. Most of the genes causing monogenic obesity in the mouse have been discovered, and the mechanisms behind their control of body fat accumulation have been elucidated in part. Some of these genes do cause excess fat accumulation in a few human subjects. However, genetic control of common forms of human obesity is poorly understood, although family studies suggest that major genes in the control of body fat stores are located in chromosomes 2, 10, 11 and 20. Through the candidate gene approach, several genes have been identified which might be considered as minor obesity genes because polymorphism in these genes is associated with obesity in some but not all subjects. It is apparent that

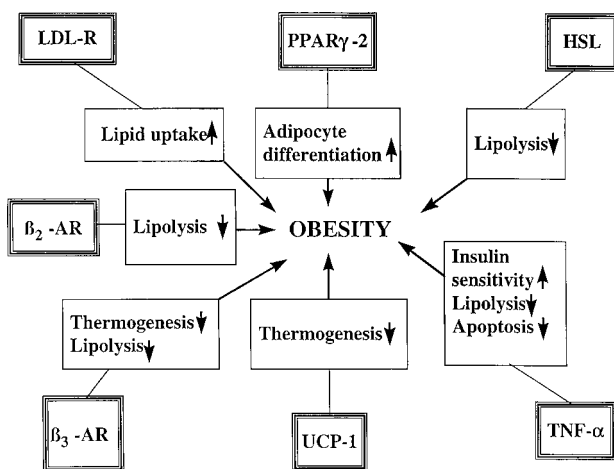
most of the minor candidate genes control important functions in adipose tissue. Therefore genetic variations in the latter genes may alter adipose tissue function in a way that promotes the development of obesity (Fig. 2). Thus changes in the genes encoding for  $\beta_2$ -adrenoceptors,  $\beta_3$ -adrenoceptors, hormone-sensitive lipase and TNF- $\alpha$  may inhibit lipolysis. TNF- $\alpha$  polymorphism could also improve insulin sensitivity and reduce adipocyte apoptosis. A genetic variation in PPAR $\gamma$ -2 may increase adipocyte differentiation. Genetic variation in UCP-1 (and  $\beta_3$ -adrenoceptors) could decrease thermogenesis. LDL-R polymorphisms might increase lipid storage in adipose tissue. All these variations in the genetic code could promote excess fat accumulation. Some genes may interact in the development of an obese phenotype, such as UCP-1 and  $\beta_3$ -receptor. Gene-environment interactions may also play a pathophysiological role in the cause of obesity, for example between physical activity and the  $\beta_2$ -adrenoceptor gene. Finally, there might be different obesity genes (at least minor ones) among women and men: for example, the genes encoding for  $\beta_2$ -adrenoceptors,  $\beta_3$ -adrenoceptors, TNF- $\alpha$  and LDL-R may be more important for obesity development in women than in men. I do want to emphasize that genes which are widely expressed such as the  $\beta_2$ -adrenoceptor, TNF- $\alpha$  and LDL-R may cause obesity in a number of ways not directly involving adipose tissue. On the other hand, it is difficult to understand how the genes for hormone sensitive lipase,  $\beta_3$ -adrenoceptor, UCP-1 and PPAR $\gamma$ -2 could promote obesity without directly involving the fat cells. It is therefore an attractive speculation that structural variability in genes controlling the function of adipose tissue is important for common forms of human obesity.

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**Fig. 2.** A tentative model showing how changes in the structure of genes controlling important actions in human adipose tissue may cause obesity by altering fat cell function.  $\beta_2$ -AR= $\beta_2$ -adrenoceptor;  $\beta_3$ -AR= $\beta_3$ -adrenoceptor; HSL=hormone-sensitive lipase; PPAR $\gamma$ -2=peroxisome proliferator-activated receptor gamma-2; UCP-1=uncoupling protein-1; TNF- $\alpha$ =tumour necrosis factor alpha; LDL-R=low-density lipoprotein receptor.

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