Genetics of the metabolic syndrome

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The clustering of cardiovascular risk factors such as abdominal obesity, hypertension, dyslipidaemia and glucose intolerance in the same persons has been called the metabolic or insulin-resistance syndrome. In 1998 WHO proposed a unifying definition for the syndrome and chose to call it the metabolic syndrome rather than the insulin-resistance syndrome. Although insulin resistance has been considered as a common denominator for the different components of the syndrome, there is still debate as to whether it is pathogenically involved in all of the different components of the syndrome. Clustering of the syndrome in families suggests a genetic component. It is plausible that so-called thrifty genes, which have ensured optimal storage of energy during periods of fasting, could contribute to the phenotype of the metabolic syndrome. Common variants in a number of candidate genes influencing fat and glucose metabolism can probably, together with environmental triggers, increase susceptibility to the syndrome. Among these, the genes for β_3-adrenergic receptor, hormone-sensitive lipase, lipoprotein lipase, IRS-1, PC-1, skeletal muscle glycogen synthase, etc. appear to increase the risk of the metabolic syndrome. In addition, novel genes may be identified by genome-wide searches.

Abdominal obesity: Hypertension: Dyslipidaemia: Glucose intolerance: Metabolic syndrome: Insulin-resistance syndrome

About 10 years ago Gerald Reaven re-introduced the concept of syndrome X for the clustering of cardiovascular risk factors such as hypertension, obesity, high triglyceride and low HDL cholesterol concentrations (Reaven, 1988). The syndrome is, however, much older: as early as 1923 Kylin described the clustering of hypertension, obesity and gout (Kylin, 1923). Since then the syndrome has been given several names, including the insulin-resistance syndrome, the cardiovascular metabolic syndrome, the deadly quartet, etc. (Modan *et al*. 1985; DeFronzo & Ferrannini, 1991; Haffner *et al*. 1992; Meigs *et al*. 1997). The name insulin-resistance syndrome has been widely used and refers to insulin resistance as a common denominator of the syndrome (Balkau *et al*. 1999). The prevalence of the metabolic syndrome has varied markedly between different studies, most probably because of a lack of accepted criteria for the definition of the syndrome (Haffner *et al*. 1997; Bonora *et al*. 1998; Rantala *et al*. 1999). In 1998 WHO proposed a unifying definition for the syndrome and chose to call it the metabolic syndrome rather than the insulin-resistance syndrome (Alberti & Zimmet, 1998). The reason was mainly that it was not considered to be established that insulin resistance is the cause of all the components of the syndrome.

Definition of the metabolic syndrome

According to the WHO proposal, a person with type 2 diabetes or impaired glucose tolerance has the metabolic syndrome if two of the criteria listed below are fulfilled. A person with normal glucose tolerance has the metabolic syndrome if he/she fulfils two of the criteria in addition to being insulin resistant. Insulin resistance is defined as lowest quartile of measures of insulin sensitivity (e.g. insulin-stimulated glucose uptake during euglycaemic clamp) or highest quartile of fasting insulin or HOMA insulin-resistance index (Alberti & Zimmet, 1998). It should be kept in mind that fasting insulin concentrations or the HOMA index represent only surrogate measures of insulin sensitivity, and that changes in insulin sensitivity explain only about 30 % of the variance in fasting insulin concentrations. The components of the metabolic syndrome are:

(1) hypertension defined as antihypertensive treatment and/or blood pressure $>$ 160/90 mm Hg;
(2) dyslipidaemia defined as elevated plasma triglyceride ($\geqslant$ 1·7 mmol/l) and/or low HDL cholesterol concentrations ($<$ 0·9 mmol/l in men, $<$ 1·0 mmol/l in women);
(3) obesity defined as high BMI ($\geqslant$ 30) and/or high waist–hip ratio, WHR ($>$ 0·90 in males, $>$ 0·85 in women);
(4) microalbuminuria (overnight urinary albumin excretion rate $\geqslant$ 20 μg/min; Fig. 1).

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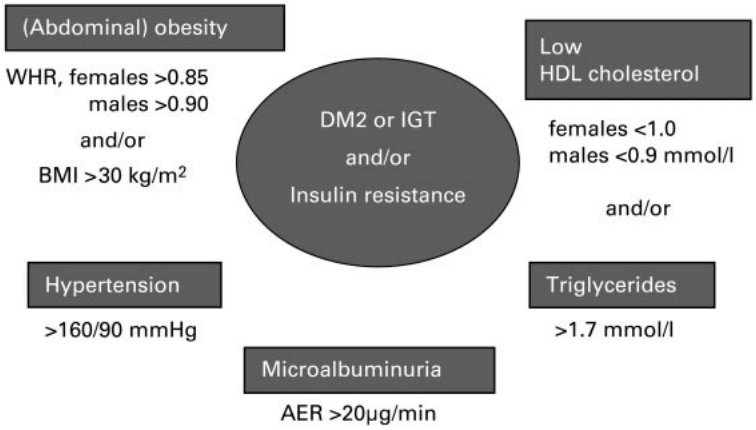

Fig. 1. Components of the metabolic syndrome (Alberti *et al.* 1998).

The cut-off for WHR would make 80–90 % of the population obese, and it may therefore be wise to increase the cut-off levels for WHR to $>1{\cdot}00$ in males and $>0{\cdot}90$ in females.

Prevalence and risk associated with the metabolic syndrome

Applying this definition to the population from the Botnia study in Finland and Sweden (Groop *et al*. 1996), about 10 % of persons with normal glucose tolerance, 40 % of persons with IGT and 70 % of patients with type 2 diabetes would have the metabolic syndrome (B. Isomaa, unpublished results). Importantly, the presence of the metabolic syndrome was associated with a threefold increased risk of coronary heart disease, myocardial infarction and stroke.

Inheritance of the metabolic syndrome

Insulin resistance clusters in families: 45 % of first-degree relatives of patients with type 2 diabetes are insulin resistant, compared with 20 % of people without a family history of diabetes (Beck-Nielsen & Groop, 1994; Groop *et al*. 1996; Groop & Tuomi, 1997). Despite this, the heritability for insulin resistance is lower than the heritability for insulin secretion in twin studies (Lehtovirta *et al*. 2000). This may simply reflect the fact that insulin resistance is also strongly influenced by environmental factors.

The heritability of blood pressure is about 40–50 %, and hypertension is associated with insulin resistance (Ferrannini *et al*. 1987). The heritability of HDL cholesterol is stronger than the heritability of triglycerides (Hong *et al*. 1998; Lehtovirta *et al*. 2000); the triglyceride levels are also dependent on the duration of fasting and blood glucose levels. Both high triglycerides and low HDL cholesterol concentrations are associated with insulin resistance (Widèn *et al*. 1992). Microalbuminuria also seems to be under genetic control; it clusters in families and the heritability of albumin excretion is about 30 % (Forsblom *et al*. 1999). It is still debated whether microalbuminuria *per se* is associated with insulin resistance or whether the observed association between microalbuminuria and insulin resistance is due to concomitant hypertension (Forsblom *et al*. 1995; Mykkänen *et al*. 1998).

About 40 % of the variation in body fat is being attributed to genetic factors (Bouchard *et al*. 1988, 1996). The genetic factor is even more impressive for abdominal obesity, and is considered to explain 60 % of the variance in abdominal fat of post-menopausal women (Carey *et al*. 1996; Samaras *et al*. 1997). First-degree relatives of patients with type 2 diabetes have an increased WHR compared with their spouses without a family history of non-insulin-dependent diabetes mellitus (Groop *et al*. 1996), and this increase in abdominal fat is seen without a significant increase in total body fat. Importantly, the redistribution of fat to the abdominal region is seen at completely normal glucose tolerance. The inheritance of type 2 diabetes thus seems to favour fat accumulation in the intra-abdominal region. Intra-abdominal fat is metabolically very active, with a high rate of free fatty acid (FFA) turnover. Intra-abdominal FFA metabolism is relatively resistant to the effect of insulin in persons with abdominal obesity (Lönnqvist *et al*. 1995). Instead, the β_3-adrenergic receptor of visceral fat is sensitive to stimulation by catecholamines (Krief *et al*. 1993). This, in turn, will ensure a large supply of FFA to the portal vein for further transport to liver and other tissues such as muscle. In contrast, lipolysis in subcutaneous fat is more sensitive to the inhibitory effect of insulin, which will favour re-esterification of FFA to triglycerides (Reynisdotter *et al*. 1994).

Abdominal obesity and insulin resistance

There is a strong correlation between the amount of intra-abdominal fat measured by CT scan (Banerji *et al*. 1995) or waist circumference and insulin sensitivity. Although abdominal obesity and insulin resistance could be coincidental expressions of a third unknown factor, the possibility that they are causally related must be considered. Abdominal fat tissue could provide a signal for the chain of events leading to skeletal muscle insulin resistance. One such candidate could be leptin (Zhang *et al*. 1994); another could be TNF-α (Hotamisigil *et al*. 1995). A change in the affinity of intra-abdominal and subcutaneous fat tissue lipolysis for

catecholamines or insulin could result in increased lipolysis in abdominal fat and increased re-esterification in subcutaneous fat. An increased supply of FFA could, in turn, result in increased FFA uptake and re-esterification in muscle. In fact, increased intramuscular triglyceride concentrations have been reported in insulin-resistant obese individuals, and correlate with the rate of insulin-stimulated glucose metabolism (Storlien *et al*. 1991). An increased FFA turnover within the muscle could, through activation of the FFA–glucose cycle (Randle cycle), lead to impaired insulin-stimulated glycogen synthesis (Groop & Ferrannini, 1993).

Thrifty genotype

Why does the metabolic syndrome develop in individuals switching from a rural to an urban lifestyle? The thrifty gene hypothesis was put forward by Neel (1962), who proposed that individuals living in a harsh environment with an unstable food supply would maximize their probability of survival if they could maximize storage of surplus energy. Genetic selection would thus favour energy-conserving genotypes in such environments. Storage of energy as fat rather than as glycogen would ensure energy during periods of starvation. Support for this hypothesis comes from a study in the *ob* and *db* mice (Coleman, 1979). Heterozygous animals (only homozygous animals will develop obesity or diabetes) with the same body weight as the wild type survived longer during total fasting than the insulin-sensitive wild-type mice. The sand rat is another example of such an insulin-resistant thrifty genotype, with a metabolism aimed at storing energy to ensure survival during long periods of fasting in the desert. When this energy-storing genotype is exposed to the abundance of food typical for Western society it becomes detrimental, causing glucose intolerance. It can therefore be assumed that thrifty genes predispose to the metabolic syndrome. Such putative genes could be expected to influence lipolysis, fuel oxidation and skeletal muscle glucose metabolism.

Thrifty phenotype

An alternative explanation has also been proposed by which most of the metabolic syndrome is programmed *in utero*, the so called thrifty phenotype hypothesis (Hales & Barker, 1992). According to this theory, intra-uterine malnutrition would lead to a low birth weight and increased risk of the metabolic syndrome later in life. Although these findings have been replicated in several studies, it has also been shown that the risk of a small birth weight for the metabolic syndrome is increased particularly in families with the metabolic syndrome (Melander *et al*. 2000), suggesting that a low birth weight could be a phenotype for a thrifty gene. In support of this, children with a glucokinase defect, and thereby a decrease in insulin, have a low birth weight (Hattersley *et al*. 1998). This was particularly apparent in children of diabetic mothers, as these children would be expected to have a high birth weight as a consequence of high levels of glucose passing the placenta and thereby stimulating the fetal pancreas to produce increasing amounts of anabolic insulin.

Search for thrifty genes

Two major approaches are being used in the search for thrifty genes, or genes predisposing to (abdominal) obesity. The candidate gene approach aims at the identification of genes based on information of their function. While this approach has been successful in a number of monogenic disorders with known biochemical defects (e.g. phenylketonuria), our knowledge about the underlying defects causing the metabolic syndrome is limited. It is therefore not suprising that the candidate gene approach has not been very successful for the identification of thrifty genes.

The random gene search, also referred to as positional cloning, assumes no knowledge of the underlying defects. Instead, positional cloning aims at localizing the disease gene on the basis of its position in the genome. If a chromosomal region has been linked to the disease, the next step would be the search for attractive candidate genes in the region or narrowing the region by linkage-disequilibrium mapping. This approach has been successful in a number of monogenic disorders (e.g. diastrophic dysplasia; Hästbacka *et al*. 1994) where the relationship between genotype and penetrance of the phenotype is more straightforward than for abdominal obesity. The situation for the metabolic syndrome will be more complicated. Although in one study it was assumed that an autosomal recessive locus would account for 51 % of the variance in visceral fat (Bouchard, 1996), we can assume that common variants in a number of genes will increase susceptibility to the metabolic syndrome and that these genes will act in concert with a number of environmental factors.

Candidate genes for the metabolic syndrome

The thrifty gene hypothesis proposes that efficient storage of energy could have been associated with survival advantage during the evolution (Neel, 1962; Beck-Nielsen & Groop, 1994). Efficient storage of energy must include storage of fat and weight gain. Given the scenario presented earlier, obesity genes could predispose to the metabolic syndrome and thereby to type 2 diabetes. Despite large fluctuations in food intake and energy expenditure, body fat is tightly regulated in humans. A powerful feedback system (also referred to as the lipostat hypothesis) between fat and a satiety/energy expenditure centre in the hypothalamus has been postulated, since damage to this region causes morbid obesity.

Leptin and the leptin receptor

Several obese mouse models have been used to study these interactions, e.g. *ob, db, tubby, agouti, FAT,* etc. The *fa*/*fa* Zucker rat is the counterpart to the *db*/*db* mouse. The *ob*/*ob* mice become obese and insulin resistant when fed a fat diet. When in parabiosis experiments the *ob*/*ob* mouse is joined with a normal animal, it eats less and gains less weight. This finding postulated that the *ob*/*ob* mouse lacks a satiety signal which is supplied by the normal animal. When a *db*/*db* mouse is joined with a normal mouse in parabiosis experiments, the normal mouse dies of starvation. Therefore the *db*/*db* mouse appeared to reflect a defect in the action of the ob protein, which may be a receptor defect (Coleman, 1973).

This field of research has moved into an exciting new era with the discovery of the *ob* gene which codes for a novel protein, leptin, expressed in fat (Zhang *et al*. 1994). A mutation in the *leptin* gene results in the complete absence of the protein in the *ob*/*ob* mouse. The human homologue is located on chromosome 7. Treatment of the *ob*/*ob* mouse with leptin resulted in marked weight loss (Pelleymounter *et al*. 1995). But man is not mouse – obese humans have elevated rather than decreased levels of leptin (Consindine *et al*. 1995), and the leptin levels show a strong positive correlation with the total fat mass (Maffei *et al*. 1995). In this regard, humans resemble the *db*/*db* mouse: the mRNA for leptin is increased in fat tissue (Ogawa *et al*. 1995). One exception, however, has been reported. Two morbidly obese children from consanguine parents had very low circulating leptin levels due to a frameshift mutation involving a deletion of a single guanine nucleotide in codon 133 of the leptin gene (Montague *et al*. 1997). Whereas some progress in treatment of these children with leptin has been reported, treatment of obese persons without mutations in the leptin gene has been less successful.

A defective putative leptin receptor was postulated as the cause of human obesity, and the leptin receptor gene was cloned and mapped to the short arm of human chromosome 1 (Tartaglia *et al*. 1996). The leptin receptor belongs to the cytokine receptor family with high homology with the IL-6 receptor. The receptor exists in several alternatively spliced isoforms with a common extracellular domain and a variable intracellular domain. The *db* mutation is due to an abnormally spliced leptin receptor in the hypothalamus (Lee *et al*. 1996). The mutant protein is lacking the cytoplasmic region, and it is suggested that the defect involves impaired leptin signalling in the hypothalamus. Although the leptin signalling pathway has not yet been described in detail, it has been suggested that the leptin signal leads to inhibition of neuropeptide Y, which stimulates food intake and decreases thermogenesis (Stephens *et al*. 1995).

In one family, early-onset obesity and hypogonadism segregated with mutations in the leptin receptor gene (Clément *et al*. 1998). Instead, screening of the leptin receptor gene in persons with common obesity has given negative results.

Pro-opiomelanocortin, agouti, FAT and melanocortin receptors

In contrast to the previous recessive forms of murine obesity, agouti is a dominantly inherited late-onset form of obesity. It results from ectopic expression of the agouti protein in the hypothalamus. Normally the α-melanocyte-stimulating hormone (α-MSH) binds to the melanocortin 1 receptor (MCR1) in the skin to control pigmentation, and to MCR4 in the hypothalamus to suppress appetite (Fan *et al*. 1997). The agouti protein is normally expressed in the hair follicles where, by antagonizing the effect of α-MSH on MCR1, it causes the characteristic yellow colour of the fur. When the mutated protein is ectopically expressed in the hypothalamus it also antagonizes the effect of α-MSH on MCR4, resulting in uncontrolled appetite. In humans the counterpart of the agouti protein is the agouti-related peptide (AGTR; Ollman *et al*. 1997). Mutations in the MCR4 gene have, in some families, been associated with morbid obesity (Vaisse *et al*. 1998; Yeo *et al*. 1998). The ligand for the melanocortin receptor α-MSH is, together with β-MSH, γ-MSH and ACTH, processed from pro-opiomelanocortin (POMC) by different peptidases. There are several pieces of evidence to support a role of POMC in the pathogenesis of obesity. Neuropeptide Y, which normally stimulates appetite, down-regulates the expression of POMC mRNA in the brain, whereas leptin, which suppresses appetite, up-regulates POMC expression. Linkage to the POMC region on chromosome 2p21 has been reported for leptin levels and resting metabolic rate (Chagnon *et al*. 1997; Comuzzie *et al*. 1997). In one family, mutations in the POMC gene segregated with progressing obesity, ACTH deficiency and red hair (Krude *et al*. 1998).

The FAT mouse represents another form of slowly evolving murine obesity associated with hyperinsulinaemia. The mutation has been mapped to mouse chromosome 8 (syngenic with human chromosome 4), very close to the gene for carboxypeptidase E (CpE; Naggert *et al*. 1995). CpE encodes a protein which processes pro-hormones such as pro-insulin and POMC. Mutations in this gene could therefore result in altered processing of pro-hormones. This was also supported by findings in a woman with obesity and hypogonadotropic hypogonadism associated with elevated serum levels of pro-insulin, cortisol and POMC (Jackson *et al*. 1997). She was found to be composite heterozygous for a Gly → Arg mutation in position 483 resulting in skipping of exon 5 of the endopeptidase prohormone convertase 1.

The POMC and melanocortin receptors represent one of the most promising targets for a novel treatment that could control appetite.

β_2- and β_3-adrenergic receptors

Catecholamines stimulate lipolysis through the β-adrenergic receptors and inhibit lipolysis through α-adrenergic receptors. The β_2-adrenergic receptor (β_2-AR) is expressed in several tissues including the lung and fat tissue. The β_3-adrenergic receptor (β_3-AR) is expressed in brown adipose tissue of rodents, and is considered responsible for thermogenesis (Emorine *et al*. 1989). A lack of brown adipose tissue in transgenic animals results in decreased thermogenesis and obesity (Lowell *et al*. 1993). It was long disputed whether the β_3-AR exists in humans who do not show brown adipose tissue. The demonstration of mRNA from the β_3-AR in visceral fat changed this view (Krief *et al*. 1993). Subsequently it was demonstrated that catecholamine-induced lipolysis was increased in visceral fat from subjects with abdominal obesity due to increased β_3-AR function (Lönnqvist *et al*. 1995). The β_3-AR thereby became a prime candidate gene for abdominal obesity.

A few years ago we and others reported a mutation in the first intracellular loop of the receptor changing a tryptophan in position 64 to arginine (Clément *et al*. 1995; Walston *et al*. 1995; Widèn *et al*. 1995). Mutation carriers showed more abdominal obesity, higher insulin concentrations, more insulin resistance and higher blood pressure than individuals homozygous for the wild type (Trp64Trp), all features of the metabolic syndrome (Widèn *et al*. 1995). Furthermore, they had a lower metabolic rate (Sipiläinen *et al*. 1997) and lower resting sympathetic nervous system

activity (Shihara *et al*. 1999), all features one would expect of a 'thrifty' gene. A number of negative population-association studies have been reported (Gagnon *et al*. 1996; Fujisawa *et al*. 1998), but given the inherent problems in selecting the control group, such association studies must be interpreted with caution. Family-based studies are needed to confirm a potential association between a gene variant and a phenotype (Altsuler *et al*. 1998). In our original analysis we studied sib-pairs who were discordant for the Trp64Arg variant. This finding was later replicated in Mexican American sib-pairs discordant for the Trp64Arg variant of the β_3-*AR* gene but concordant for linkage to a locus on chromosome 2p21–23 (close to the leptin gene; Mitchell *et al*. 1998). The region of the β_3-AR gene on chromosome 8p12–11·1 has also been linked to the quantitative trait of a high BMI in Mexican American families (Mitchell *et al*. 1999). *In vitro*, the mutation appears to be associated with an impairment in catecholamine-stimulated lipolysis (Pietro-Rouxel *et al*. 1997; Hoffstedt *et al*. 1999).

Taken together, the data emphasize the β_3-*AR* gene as a strong candidate for a gene increasing susceptibility to the metabolic syndrome. Its prevalence differs between populations, with an allele frequency of about 10 % in Finns and 30 % in Japanese (Sakane *et al*. 1997).

A Glu → Gln variant at codon 27 of the β_2*AR* gene has been associated with obesity and hypertension (Large *et al*. 1997). Carriers of this variant are resistant to catecholamine-induced down-regulation of the gene (Green *et al*. 1994). A polymorphism in the 5′ leader cistron of the β_2-*AR* gene is in linkage disequilibrium with the codon 27 variant and is associated with obesity and type 2 diabetes in the Japanese (Yamada *et al*. 1999).

Lipases

The breakdown of triglycerides is regulated by several lipases, including the hormone-sensitive lipase (HSL) in adipose tissue, the endothelial lipoprotein lipase (LPL), and hepatic lipase. The genes encoding for these lipases have been widely studied as putative candidate genes for human obesity and the metabolic syndrome. A polymorphism in the *HSL* gene was associated with the form of type 2 diabetes which is characterized by the metabolic syndrome (Klannemark *et al*. 1998*b*). Importantly, this variant was in a transmission disequilibrium test more often transmitted from heterozygous parents to abdominally obese offspring. An Asn291Ser variant in exon 6 of the LPL gene has been associated with high triglycerides, low HDL cholesterol and increased risk of cardiovascular disease (Reymer *et al*. 1995; Knudsen *et al*. 1997). We have found increased frequency of the metabolic syndrome in insulin-resistant normoglycaemic carriers of the Asn291Ser variant (M. Klannemark, unpublished results). A polymorphism at position −514 in the promoter of the hepatic lipase gene has been shown to determine variations in hepatic lipase activity and serum lipoprotein concentrations (Tahvanainen *et al*. 1998).

Uncoupling proteins

Thermogenesis in brown adipose tissue of rodents is largely determined by the activity of the mitochondrial uncoupling proteins (ucp). The uncoupling proteins are proton-channel proteins on the inner mitochondrial membrane, and uncouple oxidative phosphorylation by converting the electrochemical potential of the mitochondria into heat instead of ATP. For a long time only one ucp was known. Somewhat surprisingly, targeted disruption of the *ucp1* gene made the mouse more cold-sensitive but not obese (Enerbäck *et al*. 1997). This led to the postulation and identification of other *ucp* genes, *ucp2* and *ucp3* adjacent to each other on chromosome 11 (Boss *et al*. 1997; Vidal-Puig *et al*. 1997). While *ucp1* is expressed almost exclusively in brown adipose tissue, *ucp2* is expressed in most tissues including white adipose tissue. The *ucp3* gene is a particularly attractive candidate gene as it is strongly expressed in skeletal muscle.

In humans, a polymorphism in the promoter region of the *ucp1* gene on chromosome 4 is associated with weight gain, and this is further accentuated if the patient has the Trp64Arg mutation in the β_3-*AR* gene (Clément *et al*. 1996). So far no mutations in the coding sequences or promoter regions of *ucp2* and *ucp3* have been associated with human obesity, and it is not known for sure whether they are real uncoupling proteins (Klannemark *et al*. 1998*a*; Chung *et al*. 1999).

TNF-α

TNF-α is a cytokine which is overexpressed in adipose and muscle tissue of obese animals and humans (Hotamisigil *et al*. 1993, 1995). TNF-α can induce insulin resistance by inhibition of tyrosine phosphorylation of the insulin receptor β-chain and IRS-1. A positive correlation has also been demonstrated between the level of TNF-α mRNA in fat tissue and the level of hyperinsulinaemia, suggesting a role for this cytokine in the pathogenesis of insulin resistance (Hotamisligil *et al*. 1993). Although we have recently demonstrated a correlation between the plasma concentration of TNF-α and insulin-stimulated glucose metabolism in man (Kellerer *et al*. 1996), it is more likely that this only represents a spillover and that TNF-α exerts a paracrine rather than an endocrine effect in man. In the *ob*/*ob* mouse, neutralizing TNF-α leads to improved insulin sensitivity, but fails to do so in humans (Saghizadeh *et al*. 1996). Two polymorphisms have been identified in the 5′ untranslated region of the *TNF*-α gene, a G → A polymorphism at position 308 and another G → A polymorphism at position 238. Although these polymorphisms appear to influence the host response to infections, discrepant results have been obtained regarding a possible association with human obesity and insulin resistance (Fernandéz-Real *et al*. 1997; Walston *et al*. 1999). It is therefore premature to ascribe the *TNF*-α gene a role in the pathogenesis of human obesity and the metabolic syndrome.

Peroxisome proliferator-activated receptor γ (PPARγ)

There are three forms of PPAR receptors, α, β and γ, which heterodimerize with the retinoid X receptor (RXR) to induce transcription of a number of target genes in adipose tissue. Fatty acids or their derivatives are naturally occurring ligands in addition to drugs such as thiazolidinediones (PPARγ) and fibrates (PPARα). The human PPARγ gene

maps to chromosome 3p24, a region implemented in several genome-wide scans for type 2 diabetes. A rare Pro115Gln mutation in the N-terminal domain of the *PPARγ* gene was described in four morbidly obese subjects (Ristow *et al*. 1998). This mutation leads to inhibition of phosphorylation of the protein at Ser 114 and thereby to a constitutively active PPARγ resulting in increased adipocyte differentiation. Another more common Pro12Ala variant has been associated with low BMI and increased insulin sensitivity (Deeb *et al*. 1998). *In vitro* this mutation leads to decreased PPARγ activity. However, in other studies this variant has been associated with a high BMI (Ek *et al*. 1999). Although the phenotypic correlates of the variant are still uncertain, it has been proposed that it would be associated with increased BMI only in already obese individuals.

Glycoprotein PC-1

The membrane glycoprotein PC-1 was isolated from a patient with extreme insulin resistance and found to inhibit insulin receptor tyrosine kinase activity (Maddux *et al*. 1995). An A121C variant in exon 4 of the *PC-1* gene (Glu → Lys) has been associated with insulin resistance and features of the metabolic syndrome (Pizzuti *et al*. 1999; Feng Gu, unpublished results). *In vitro*, this variant is associated with impaired autophosphorylation of the insulin receptor tyrosine kinase.

Insulin receptor substrate -1 (IRS-1)

The docking protein IRS-1 links the tyrosine-phosphorylated insulin receptor to the downstream part of the insulin-signalling pathway (Sun *et al*. 1991). IRS-1 is phosphorylated on multiple tyrosine residues, and could be a candidate for genetic insulin resistance. Two amino-acid polymorphisms were described in the *IRS-1* gene (Almind *et al*. 1993). These amino-acid substitutions, which were located close to the tyrosine phosphorylation site, were slightly more frequent in type 2 diabetic patients than in control subjects. In obese non-diabetic Danish subjects, the presence of the 972 polymorphism was associated with insulin resistance (Clausen *et al*. 1995). The 972 variant of the *IRS-1* gene seems to be predominantly increased in type 2 diabetic patients with the metabolic syndrome. Several other insulin-receptor substrates (IRS-2, IRS-3 and IRS-4) have been discovered, but no consistent variations in these genes have been found.

Glycogen synthase

Impaired stimulation of glycogen synthesis by insulin is a hallmark of type 2 diabetes and IGT (Beck-Nielsen & Groop, 1994; Eriksson *et al*. 1989). The key enzyme of this pathway, glycogen synthase, could therefore be an important candidate for a genetic defect causing insulin resistance. We have described the genomic structure of the human glycogen synthase gene and assigned it to chromosome 19q21 (Lehto *et al*. 1993; Orho *et al*. 1995). An XbaI polymorphism of the glycogen synthase gene has been associated with type 2 diabetes and insulin resistance, particularly impaired insulin-stimulated glycogen synthesis in skeletal muscle (Groop *et al*. 1993; Majer *et al*. 1996). We recently applied the discordant sib-pair approach to explore this association further. The sibling with the rare *A2* allele had more features of the metabolic syndrome and an increased risk of myocardial infarction compared to siblings with the *A1* allele (Orho-Melander *et al*. 1999). Further support for a role of the glycogen synthase gene in the pathogenesis of insulin resistance comes from studies in the diabetes-prone C57BL/6J mouse (Seldin *et al*. 1994). When fed a diabetogenic diet, this mouse strain becomes obese and insulin-resistant, and develops diabetes. The phenotype of developing hyperglycaemia when fed such a diet was linked to a locus on chromosome 7. This putative susceptibility locus was consistent with that of the glycogen synthase gene, and the diabetes-prone mouse had decreased glycogen synthase activity in muscle even when fed a control diet. However, insulin resistance as demonstrated by elevated plasma insulin levels was seen only when the mouse was fed a diabetogenic diet, suggesting that for insulin resistance to become manifest, an interaction between genetic and environmental factors is necessary.

Genome-wide scan

The advantage of the genome-wide scan approach is that it does not assume any knowledge about the pathophysiological mechanisms leading to the metabolic syndrome; the disadvantages are that it requires large family resources, and that it encompasses a high risk of false-positive results. Several genome-wide scans have been carried out with either obesity or type 2 diabetes as phenotype. Alternatively, BMI, leptin concentration or metabolic rate have been used as quantitative traits. In obese Mexican Americans, linkage was observed between fat mass or leptin concentrations and a region on chromosome 2 (Comuzzie *et al*. 1997). POMC is the most interesting candidate gene in this chromosomal region. In the Quebec family study, linkage was found between percentage body fat and a region on the long arm of chromosome 20. This region is syngenic to a region on mouse chromosome 2, to which linkage was first observed (Lembertas *et al*. 1997). More recently, linkage between basal metabolic rate and a region on chromosome 11 was reported in the Quebec study; this region includes both *ucp2* and *ucp3* genes. Also, in the Pima Indians linkage between percentage body fat and a region on chromosome 11 has been found, but it does not seem to be identical to the ucp2/3 region (Norman *et al*. 1996). In type 2 diabetes, linkage has been reported to several regions: 2q (NIDDM1; Hanis *et al*. 1996), 12q (NIDDM2; Mahtani *et al*. 1996), and 20 q (Zouali *et al*. 1997; Ghosh *et al*. 1999) being the most promising. For NIDDM1 it seems likely that the linkage is due to an intronic variant in a novel gene, emphasizing the problems with identifying the genetic causes of polygenic diseases. No genome-wide scan has been performed using the metabolic syndrome as phenotype.

Conclusions

Despite substantial progress in our understanding of the physiology of the metabolic syndrome, the pathophysiology of the condition remains obscure. So far, mutations in candidate genes can explain only a few rare cases of monogenic

forms of obesity. Abdominal obesity probably results from the interplay between several genes and an affluent environment. Among genes contributing to the metabolic syndrome, genes regulating lipolysis and thermogenesis still remain prime candidates. Our limited knowledge about the pathophysiological events leading to fat accumulation in the abdominal region still emphasizes the need for random approaches. A prerequisite is, however, that the search is focused on families with a clearly defined inherited genetic risk of developing abdominal obesity.

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