# The metabolic syndrome: the crossroads of diet and genetics

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The metabolic syndrome is a very common disease associated with an increased risk of type 2 diabetes mellitus (T2DM) and CVD. The clinical characteristics of the metabolic syndrome include insulin resistance, dyslipidaemia, abdominal obesity and hypertension. The diverse clinical characteristics illustrate the complexity of the disease process, which involves several dysregulated metabolic pathways. Thus, multiple genetic targets must be involved in the pathogenesis and progression of the metabolic syndrome. Nevertheless, the human genome has not changed markedly in the last decade but the prevalence of the metabolic syndrome has increased exponentially, which illustrates the importance of gene–environmental interactions. There is good evidence that nutrition plays an important role in the development and progression of the metabolic syndrome. Indeed, obesity is a key aetiological factor in the development of the metabolic syndrome. Understanding the biological impact of gene–nutrient interactions will provide a key insight into the pathogenesis and progression of diet-related polygenic disorders, including the metabolic syndrome. The present paper will explore the interactions between genetic background and dietary exposure or nutritional therapy, focusing on the role of dietary fatty acids within the context of nutrient regulation of gene expression and individual responsiveness to dietary therapy. Only with a full understanding of gene–gene, gene–nutrient and gene–nutrient–environment interactions can the molecular basis of the metabolic syndrome be solved to minimise the adverse health effects of obesity and reduce the risk of the metabolic syndrome, and subsequent T2DM and CVD.

**Metabolic syndrome: Obesity: Dietary fatty acids: Nutrient regulation of gene expression: Insulin resistance**

The metabolic syndrome represents a heterogeneous group of metabolic disorders, the central component of which is insulin resistance. It can involve multiple metabolic pathways that culminate in dyslipidaemia, abdominal obesity, microalbuminuria and hypertension. The WHO (Alberti & Zimmet, 1998) has defined the metabolic syndrome as impaired insulin sensitivity, glucose intolerance or diabetes mellitus in combination with at least two other metabolic derangements, including abdominal obesity, dyslipidaemia (HDL-cholesterol levels and/or elevated plasma triacylglycerol (TAG) concentrations) and urinary microalbuminuria. The National Cholesterol Education Program Adult Treatment Panel III (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001) have proposed that individuals with the metabolic syndrome have three or more of the following metabolic derangements, including central

obesity (waist girth >1020 mm in men or >880 mm in women), low HDL-cholesterol concentrations (<1·1 mmol/l for men or <1·3 mmol/l for women), hypertriacylglycerolaemia (>1·7 mmol/l), hypertension (blood pressure >130/85 mmHg) or fasting hyperglycaemia (>6·1 mmol/l). Thus, the metabolic syndrome represents a genetically-heterogeneous group of metabolic disorders sharing insulin resistance. The multiple genetic targets involved in the pathogenesis and progression of the metabolic syndrome could relate to pancreatic β-cell function, insulin action, glucose metabolism, hypertension, lipid and lipoprotein metabolism etc. (Groop, 2000; Barroso *et al*. 2003).

In parallel with the increasing incidence of type 2 diabetes mellitus (T2DM), the incidence of obesity and the metabolic syndrome have been rising at an alarming rate worldwide, resulting in increased CVD and premature death (Isomaa *et al*. 2001). Ford *et al*. (2004) have reported
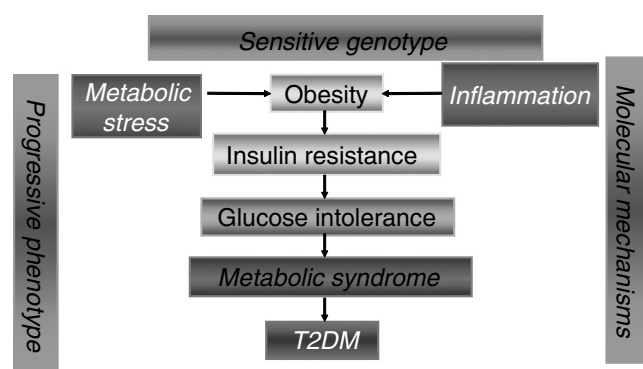
**Fig. 1.** The development and progression of the metabolic syndrome. T2DM, type 2 diabetes mellitus.

an increase in the prevalence of the metabolic syndrome from 23·1 % in the National Health and Nutrition Examination Survey III (1988–1994) to 26·7 % in the National Health and Nutrition Examination Survey (1999–2000). It is important to note that the human genome has not changed markedly in the last 10 years but the prevalence of the metabolic syndrome has increased exponentially, which illustrates the importance of gene–environmental interactions. Fig. 1 illustrates the concept that the metabolic syndrome is a progressive phenotype, whereby obesity and insulin resistance results in the metabolic syndrome and T2DM in individuals who carry the elusive sensitive genotype. Multiple organs are involved; skeletal muscle, liver and adipose tissue become insulin resistant and fail to promote glucose uptake effectively (Le Marchand-Brustel *et al.* 2003). Pancreatic β-cells initially compensate, increasing insulin secretion to maintain euglycaemia, but at some point β-cells fail to respond effectively, leading to glucose intolerance. Impaired insulin action and hyperglycaemia adversely affect peripheral organ function and promote the development of other metabolic abnormalities characteristic of the metabolic syndrome, including elevated NEFA and TAG concentrations, low HDL levels, hyperglycaemia, increased vascular resistance and a prothrombotic state (Saltiel, 2000). The incidence of the metabolic syndrome is age-dependent; an increase from 6·7 % among participants aged 20–29 years to 43·5 % for participants aged 60–69 years has been reported (Ford *et al.* 2002). The progressive and age-dependent nature of the disease makes it much harder to define the genetic components of the metabolic syndrome. Nevertheless, with a greater understanding of the molecular mechanisms involved it will be possible to determine how nutrient-derived metabolic (high-fat diet, elevated plasma non-NEFA levels etc.) and pro-inflammatory stressors, in the presence of obesity, induce insulin resistance and result in the metabolic syndrome.

## Genetic and environmental components of the metabolic syndrome

The global increase in the incidence of the metabolic syndrome and T2DM over the last few decades is a salient example of how the interaction between lifestyle and genotype can dramatically impact on health. The familial nature (Rich, 1990; Freeman *et al.* 2002; Mills *et al.* 2004), the marked difference in the prevalence among various racial groups (Al-Shaer & Abusabha, 2005) and the difference in concordance rates between monozygotic twins (Poulsen *et al.* 2001) is clearly consistent with a genetic component to disease susceptibility. In European populations familial studies show a sibling relative risk of 3·5–4 (Rich, 1990), indicating that siblings of family members with T2DM are up to four times more likely to develop T2DM than the general population. Heritability studies demonstrate a marked genetic influence on several features of the insulin resistance syndrome. One study (Mills *et al.* 2004) has shown that the heritability estimates for fasting glucose concentration and the homeostasis model assessment of pancreatic β-cell function are 0·72 and 0·78 respectively. On the other hand, another study (Freeman *et al.* 2002) has reported heritability estimates for fasting glucose, insulin, TAG and HDL-cholesterol concentrations of 20, 23, 20 and 43 % respectively.

Shared genetic and environmental components almost certainly contribute to the predisposition to disease; nevertheless, determining the relative contribution of each factor is difficult and varies for the different components of the metabolic syndrome. Poulsen *et al.* (2001) have studied the relative impact of genetic *v*. environmental factors for the development of the components of the metabolic syndrome amongst 303 elderly twin pairs. This study has demonstrated that glucose intolerance, obesity and low HDL-cholesterol concentrations are significantly higher among monozygotic twins than among dizygotic twins, indicating a genetic influence on the development on these phenotypes. In contrast, the heritability estimates for hyperinsulinaemia, hypertension and hypertriacylglycerolaemia are low, indicating a more important environmental influence on these components of the metabolic syndrome. These data indicate a major role for genetic susceptibility, whilst also emphasising the important role of the environment. In particular, trends in diet and physical activity, coupled with genetic susceptibility, must account for the recent and dramatic risk in the incidence of the metabolic syndrome and T2DM, demonstrating the important impact of gene–environment interactions.

## Diet-related environmental factors and the risk of the metabolic syndrome

Obesity has been identified as the key aetiological condition that predisposes to the development of the metabolic syndrome. There are at least two ways whereby adipose tissue may influence glucose homeostasis and act as the key aetiological factor in the development of insulin resistance and the metabolic syndrome (Kahn & Flier, 2000). First, excessive adipose tissue energy storage results in increased fatty acid flux to other tissues and increased TAG storage in peripheral tissues, which interferes with insulin signalling, inhibits glucose uptake and promotes insulin resistance. Second, adipose tissue is an important

endocrine organ that secretes several inflammatory factors, collectively known as adipocytokines or adipokines, many of which have a direct effect on insulin sensitivity (Trayhurn & Wood, 2004). There is no doubt that obesity prevention would reduce negative health effects associated with the metabolic syndrome, in particular T2DM and CVD. Nevertheless, current anti-obesity dietary strategies and public health initiatives have been largely unsuccessful, indicating that other strategies are required. Given the increasing prevalence of obesity, there is a high requirement to explore alternative approaches to reduce the impact of the adverse health effects of obesity and the metabolic syndrome.

Several studies have suggested that there may be a link between altered dietary fatty acid composition and risk of the metabolic syndrome. Aside from the obvious link between a high-fat diet and the development of obesity, there is a growing body of evidence that suggests that dietary fatty acid composition could affect insulin-mediated insulin action. The relationship between dietary fat composition and the risk of the metabolic syndrome has been recently reviewed in more detail (Roche, 2005). Briefly, a prospective study (Laaksonen *et al.* 2002) has demonstrated that the proportion of serum esterified and non-esterified saturated fatty acids (SFA) is increased and that of PUFA is decreased in men who after 4 years develop impaired fasting hyperglycaemia or T2DM. Prospective epidemiological evidence (Harding *et al.* 2004) suggests that increased dietary PUFA:SFA (P:S) is associated with reduced risk of T2DM, independent of age, gender, family history of diabetes and other lifestyle factors. Also, the Nurses' Health Study (Tanasescu *et al.* 2004) has shown that higher SFA intake and a low dietary P:S are related to increased CVD risk among women with T2DM. The latter study has also demonstrated that the replacement of 5% of the energy from SFA with MUFA is associated with a 37% lower risk of CVD. A number of dietary intervention studies have investigated the causal nature of the relationship between dietary fatty acid composition and insulin resistance (see Roche, 2005). Most of the earlier dietary intervention studies have shown no association, but these negative results probably reflect poor study design, short study duration, inaccurate measures of insulin sensitivity and/or poor statistical power. More recently, the KANWU study (Vessby *et al.* 2001) has demonstrated that decreasing dietary SFA and increasing MUFA improves insulin sensitivity but has no effect on insulin secretion. It is interesting to note that within each dietary group a second assignment to fish oil supplementation or placebo was completed, but the addition of long-chain (LC) *n*-3 PUFA was found to have no effect on insulin sensitivity. Furthermore, Perez-Jimenez *et al.* (2001) have shown that isoenergetic substitution of SFA by MUFA markedly improves insulin sensitivity. In contrast, Lovejoy *et al.* (2002) have failed to show any marked effect of high-MUFA, high-SFA or *trans*-fatty acid-rich diets on insulin sensitivity. However, when the groups were subdivided according to BMI insulin sensitivity was found to be 24% lower in overweight individuals (BMI 25–30 kg/m$^2$) after the SFA diet, compared with after the MUFA diet. Also, these diets

were low in fat (28% energy); therefore, the effects of dietary fat composition may not be relevant when accompanied by a background low-fat diet.

A number of positive health benefits relevant to the metabolic syndrome have been associated with increased LC *n*-3 PUFA intake, particularly in relation to TAG metabolism (Roche & Gibney, 2000). Animal studies show that feeding LC *n*-3 PUFA has positive effects on glucose metabolism and insulin resistance (Storlien *et al.* 1987; Aguilera *et al.* 2004). However, in human subjects there are no definitive data that consistently show positive effects of LC *n*-3 PUFA on insulin resistance. Human epidemiological data suggest that habitual dietary fish intake is inversely associated with the incidence of impaired glucose tolerance and T2DM (Feskens *et al.* 1985, 1991). While some intervention studies have reported positive effects of LC *n*-3 PUFA supplementation on insulin sensitivity in individuals with impaired glucose tolerance and diabetes (Popp-Snijders *et al.* 1987; Fasching *et al.* 1991), other studies have not (Vessby *et al.* 2001; Brady *et al.* 2004). Clearly, the health impact of increased LC *n*-3 PUFA consumption on insulin resistance in human subjects requires further clarification.

## Approaches for identifying the genetic determinants of the metabolic syndrome

The candidate-gene approach has been the traditional approach for identifying the genes involved in the metabolic syndrome. The candidate genes can be identified according to biological function and/or linkage studies, and association tests for significant differences in their allele frequencies between a patient group and a control population. Given the number of metabolic pathways involved in the metabolic syndrome it is immediately apparent that the number of potential candidate genes is tremendous. Overall, there has not been a high extent of success from candidate-gene studies in terms of defining the genetic determinants of the metabolic syndrome. Genetic-disease-association studies are fraught with difficulties, and a number of the positive results have not been replicated in subsequent studies (Hirschhorn *et al.* 2002). The main reasons for the disparity are inadequate statistical power, multiple hypothesis testing, population stratification, publication bias and phenotypic differences. It is becoming increasingly evident that the identification of true genetic associations in common multi-factorial conditions, such as the metabolic syndrome, requires large studies consisting of thousands rather than hundreds of subjects. Also, the absence of large single-gene effects and the detection of multiple small effects accentuate the need for the study of larger populations in order to reliably identify the size of the effect now expected for complex diseases.

Recent studies (Barak *et al.* 1999; Barroso *et al.* 1999) have suggested guidelines for designing association studies, and implementation of these guidelines has identified apparently promising candidates in T2DM. These studies have focused on variants of the PPARγ gene, which is a strong candidate for conferring susceptibility to the

metabolic syndrome, because PPARγ encodes a transcription factor that regulates adipogenesis, and lipid and glucose metabolism. In the initial study (Deeb *et al*. 1998) it was demonstrated that the Ala allele of the Pro12Ala PPARγ polymorphism is associated with lower BMI, improves insulin sensitivity and thus reduces diabetes risk by 75 %. However, several subsequent reports have failed to show an association (Mancini *et al*. 1999; Ringel *et al*. 1999; Clement *et al*. 2000; Meirhaeghe *et al*. 2000), and those reports that have confirmed the association (Hara *et al*. 2000) have shown that the apparent protective effect of the PPARγ polymorphism is to a much lesser extent, which questions the role of Pro12Ala in diabetes risk. Thus, a large association study of 3000 subjects supported by a meta-analysis of sixteen previously-published studies has been conducted to overcome the inherent limitations of the smaller individual studies (Altshuler *et al*. 2000). This study has confirmed that the common Pro12Ala PPARγ polymorphism is associated with a modest (1·25-fold) but significant risk of T2DM. It is interesting to note that it is the more common Pro allele (approximately 85 % frequency) that is associated with T2DM and, even though the relative risk is modest (approximately1·25), the population attributable risk is high (approximately 20 %; Altshuler *et al*. 2000). As the Pro12Ala polymorphism introduces a missense change in the coding region of the PPARγ gene, it probably represents a functional variant. This hypothesis is supported by *in vitro* studies showing that the Ala allele decreases the DNA-binding affinity of the PPARγ protein and reduces its transcriptional activity (Deeb *et al*. 1998). Also, in human subjects loss-of-function mutations in PPARγ provide compelling genetic evidence that this receptor is important in the control of insulin resistance, T2DM and hypertension (Barroso *et al*. 1999).

Positional cloning involves mapping the susceptibility or causative loci purely on their chromosomal location using multi-generational pedigrees and/or a large number of sibling pairs. This approach allows identification of genes without any previous knowledge of biological function or the disease mechanism. Linkage and linkage disequilibrium analysis relies on the fact that genes with similar chromosome positions will only rarely be separated during genetic recombination, so susceptibility to causative genes can be localised by searching for genetic markers that co-segregate with disease. However, to date this approach has identified relatively few candidate genes relevant to the metabolic syndrome. One of the earliest important linkage peaks was shown to be at chromosome 2q37·3, which has led to the identification of Calpain 10 (*CAPN10*) as a new putative diabetogene (Horikawa *et al*. 2000). The authors implicated an A to G polymorphism in intron 3 of the gene encoding *CAPN10* with greater risk of T2DM. Subsequent to this initial report, several groups have shown modest associations between *CAPN10* polymorphisms and haplotypes with a variety of metabolic phenotypes characteristic of the metabolic syndrome (see Florez *et al*. 2003). However, other groups have failed to show a relationship between *CAPN10* and metabolic traits, or have shown modest associations for other sequence variations and/or haplotype combinations at the *CAPN10* locus that might be

associated with disease (Florez *et al*. 2003). The lack of a consistent gene–phenotype relationship may be related to a number of causes of inconsistency in association studies, including population-specific patterns of linkage disequilibrium, population-specific environmental triggers, gene–gene interactions or gene–nutrient interactions.

## Gene–nutrient interactions: implications for dietary fatty acid intake

Given the complex polygenic nature of the metabolic syndrome, it is clear that understanding the nature of multiple gene–gene and gene–environment interactions is critical to understanding the pathogenesis and progression of the metabolic syndrome. In terms of addressing the gene–environment interactions, the present paper will explore the interactions between genetic background and an individual's nutrient exposure, nutritional therapy and/ or metabolic profile.

As highlighted previously, the PPARγ Pro12Ala polymorphism is a good candidate genetic marker relevant to the metabolic syndrome. This genetic variant also represents an excellent example of the relevance of gene–nutrient interactions in the development of the metabolic syndrome and T2DM. A recent prospective population-based cohort study of the aetiology of T2DM (Luan *et al*. 2001) has shown an important interaction between habitual dietary fat composition and the PPARγ Pro12Ala polymorphism. As the P:S increases there is an inverse relationship for both BMI and fasting insulin concentrations in the Ala carriers but not in the Pro homozygotes. A limited interpretation of this study could be that carriers of the Pro allele, which is associated with greater risk of the metabolic syndrome, are not sensitive to dietary fat composition. Nevertheless, this study presents an important finding in relation to the potential protective effect of the Ala allele, which may be lost in the presence of a high-SFA diet. The Quebéc Family Study (Robitaille *et al*. 2003) has also demonstrated that the PPARγ Pro12Ala polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome. In this study it was found that total fatty acid and SFA intakes are correlated with BMI, visceral adipose tissue area, waist circumference and fasting glucose concentrations in Pro12 homozygotes, but these associations are not observed among carriers of the Ala allele. Also, when the two genotype groups are classified according to quartiles of total fatty acid and SFA intakes, the positive correlation between fat and waist circumference remains in the Pro12 homozygotes, but again there is no relationship in the Ala carriers (Robitaille *et al*. 2003). Thus, there is a disparity between the results of these two studies that have investigated the interaction between the PPARγ Pro12Ala polymorphism and dietary fatty acids. However, the discrepancies may be population specific, as participants carrying the Ala allele had higher BMI than the Pro12 homozygotes in the Robitaille *et al*. (2003) study, an effect not observed in the study by Luan *et al*. (2001). Nevertheless, both studies highlight the need to take account of diet-related environmental exposure and

the relevance of gene–nutrient interactions, particularly in relation to the possible role of dietary fatty acids in the metabolic syndrome. Also, another recent study in an Asian population (Tai *et al*. 2004) has failed to demonstrate this gene–nutrient interaction between the PPARγ Pro12Ala polymorphism and dietary fatty acid composition in a large sample cohort (*n* 2120). Whilst diverse genetic background and/or non-dietary environmental exposure difference between populations could account for differences, the authors suggest that lack of replication of the gene–nutrient interaction argues against the causality of this effect.

The findings of a recent randomised controlled trial (Finnish Diabetes Prevention Study; Lindi *et al*. 2002) suggest that the PPARγ Pro12Ala polymorphism could affect responsiveness to diet and exercise interventions. In this study 522 subjects with impaired glucose tolerance were randomly assigned to an intensive diet and exercise intervention group or a control group. By year 3 of the intervention the odds ratio for the development of T2DM in subjects with the Ala allele was found to be 2·11-fold higher compared with that for the Pro12Pro genotype. This result contrasts with other reports that show that the Ala12 allele is protective against T2DM (Altshuler *et al*. 2000), a discrepancy that may be partly related to differences in study design wherein earlier studies were cross-sectional and the frequency and impact of the Ala allele was compared between T2DM patients and control subjects with normal glucose tolerance. Within the dietary and exercise intervention of the Finnish Diabetes Prevention Study, which aimed to reduce body weight, Ala12 homozygotes were found to lose more weight than the Pro12Pro homozygotes, and none of the Ala12 homozygotes developed diabetes during the trial (Lindi *et al.* 2002).

Gene–nutrient interactions can also be modified by other non-nutrient environmental factors, such as physical activity. It has been proposed (Franks *et al*. 2004) that both dietary fatty acid composition and physical activity may interact with the PPARγ Pro12Ala polymorphism within the context of T2DM risk. Harding *et al*. (2004) have demonstrated that, after adjustment for BMI, physical activity level and P:S are independently associated with lower fasting insulin concentrations and have additive effects on fasting insulin concentrations, a surrogate marker for insulin resistance. In addition, it has been shown that the interaction between P:S and physical activity level on fasting insulin concentrations is altered when individuals are stratified according to PPARγ Pro12Ala genotype (Franks *et al*. 2004) The combined relationship between P:S and physical activity level with fasting insulin is additive in Pro12 homozygotes. In contrast, physical activity level modifies the association between P:S and fasting insulin concentrations in a multiplicative manner.

## Conclusions

The recent global epidemic of the metabolic syndrome is an important illustration of how nutrient exposure and genetic background can dramatically impact on the development of the metabolic syndrome. The diverse clinical characteristics and multiple genetic targets involved in the pathogenesis and progression of the metabolic syndrome illustrate the complexity of the disease process, which involves several dysregulated metabolic pathways. Understanding the biological impact of gene–nutrient interactions is the key to understanding the basis of diet-related polygenic disorders such as the metabolic syndrome. The present paper is limited in that it focuses on the interaction between a single polymorphism in PPARγ and dietary fatty acid exposure, responsiveness to nutritional therapy and physical activity. It highlights the lack of consistency between studies, which may be partly ascribed to variable genetic backgrounds and/or different nutrient and non-nutrient environmental exposures. A more robust and complete understanding of the interactions between multiple genetic targets and dietary fatty acids, within the context of nutrient regulation of gene expression and individual responsiveness to dietary therapy is required. Only with a full understanding of multiple gene–gene, gene–nutrient and gene–nutrient–environment interactions can the molecular basis of the metabolic syndrome be solved in order to reduce the risk and minimise the adverse health effects of obesity, T2DM and CVD.

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