

Perspectives in Diabetes

Genetic Influences of Adiponectin on Insulin Resistance, Type 2 Diabetes, and Cardiovascular Disease.

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## ABSTRACT

Recent evidence points to molecules secreted by the adipose tissue, or adipokines, as possible links between increased adipose mass and metabolic abnormalities. Among these molecules, adiponectin has drawn much attention because of its insulin sensitizing and antiatherogenic actions, suggesting that genetic deficits in its production or action may contribute to insulin-resistance and coronary artery disease (CAD). A meta-analysis of the data published to date supports this hypothesis. Two independent effects, corresponding to the two linkage disequilibrium blocks that can be identified at the adiponectin locus, appear to be present. In the 5' block, the g.-11391G>A variant has a modest but significant effect on adiponectinemia, with a mean difference between genotypes of 1.64 ng/ml (95% CI=0.88-2.41, p=0.01). In the 3' block, the g.+276G>T variant is a strong determinant of insulin-resistance and CAD, with minor allele homozygotes having lower HOMA<sub>IR</sub> index (-0.36 units, 95% CI=0.24-0.47, p<0.0001) and cardiovascular risk (OR=0.55, 95%CI 0.38-0.80) than carriers of other genotypes. No consistent effect on BMI or risk of type 2 diabetes is evident. Polymorphisms in the genes coding for the adiponectin receptors may also influence the risk of insulin-resistance and CAD, but data on these genes are still too sparse to draw firm conclusions. In summary, the studies published to date indicate that polymorphisms at the adiponectin locus are indeed predictors of circulating adiponectin levels, insulin-sensitivity, and atherosclerosis, highlighting the pivotal role of this adipokine in the modulation of metabolism and atherogenesis.

Our view of adipose tissue has undergone dramatic changes over the past fifteen years. Previously believed to be a mere energy depot, adipose tissue is now considered a major endocrine organ regulating whole-body metabolism as well as inflammatory and immune responses (1; 2). These actions are mediated by a number of molecules – collectively known as adipokines – that are secreted by adipocytes and act in an autocrine, paracrine, or endocrine fashion, adapting metabolic fluxes to the amount of stored energy (1; 2). The discovery of such endocrine function of the adipose tissue has prompted the hypothesis that a genetic dysregulation of the adipokine network may contribute to the pathogenesis of insulin resistance and related disorders such as type 2 diabetes and cardiovascular disease. Of all the molecules that have been shown to be produced by the adipose tissue, adiponectin has drawn special attention, largely due to its effects on both insulin sensitivity and inflammation, and the fact that its expression and serum levels can be modulated by PPAR $\alpha$  agonists drugs (Figure 1). In this Perspective article, we will review the evidence that has been thus far gathered on the role of genetic variants in the adiponectin and adiponectin receptors genes as modulators of adiponectin circulating levels and susceptibility to insulin resistance traits. We will also discuss the directions in which research on this topic is heading.

**Adiponectin: a salutary adipokine.**

Adiponectin - also known as adipocyte complement-related protein 30 (Acrp30), gelatin-binding protein 28 (Gbp28), adipose most abundant transcript 1(apM1), or AdipoQ- is exclusively produced by adipocytes (3-6). It is abundantly present in serum, where it circulates in two higher order forms – a low molecular weight dimer of trimers and a larger high molecular weight

complex of 12 to 18 subunits (7). Serum levels are 15% higher in women than in men (8). Data from both animal and human studies indicate that adiponectin has insulin enhancing as well as anti-inflammatory actions (see reference (9) for an extensive review on this topic). Adiponectin levels are markedly reduced in obese/diabetic mice and injection of the adiponectin globular domain to these animals ameliorates insulin resistance – an effect that can be ascribed to an enhancement of fatty acid- $\beta$  oxidation in skeletal muscle and a decrease of hepatic gluconeogenesis mediated by AMP-activated protein kinase (10-13). Furthermore, adiponectin knockout mice show increased susceptibility to diet-induced insulin resistance as well as injury-induced arterial stenosis and neointimal formation (14; 15). Consistent with the results from animal models, several cross-sectional studies have shown an association between low serum adiponectin levels and insulin resistance, type 2 diabetes, and cardiovascular disease in humans (16-19). A similar inverse relationship between adiponectin levels and incidence of insulin resistance, type 2 diabetes, and atherosclerosis has been demonstrated in follow-up studies (20-25). While it cannot be excluded that also in these studies low adiponectin levels are due to underlying disease processes, such prospective data suggest that hypoadiponectinemia is a determinant rather than a consequence of these conditions. The salutary effects of adiponectin on metabolic traits and vascular functions have raised the hypothesis that genetic variants decreasing the production or affecting the function of this molecule or of its receptors may contribute to the etiology of insulin-resistance and the chronic disorders that are frequently associated with this metabolic abnormality.

The gene coding for adiponectin – officially named ADIPOQ - is placed on

chromosome 3q27. This genomic region has been shown to segregate with insulin resistance traits and type 2 diabetes in several family studies (that is, these traits were more similar in relatives who shared this chromosomal region than those who did not), providing a further rationale for studying this gene as a candidate for these conditions (26-29). The ADIPOQ gene includes 3 exons, spanning a total of 16 Kb of genomic sequence (Figure 2). A recent, systematic analysis of this locus in Europeans suggests that this gene is organized in two linkage disequilibrium blocks separated by a region of looser linkage disequilibrium placed in the middle of the first intron (30) (Figure 2). A similar two-block structure has been observed in Chinese (31) and Hispanics, although the between-block boundaries are shifted by a few Kb on the 5' side in the latter population (32).

During the past five years, several polymorphisms at this locus have been repeatedly tested, individually or in combination as haplotypes, for association with low adiponectin levels, measures of adiposity, features of the insulin resistance syndrome, and type 2 diabetes, in both Caucasians and Japanese. Studies have mostly concerned four SNPs, which were among the first to be discovered by targeted resequencing efforts (33; 34). Two of these (g.-11391G>A and g.-11377C>G) are placed in the first LD block, in the immediate 5' flanking region of the gene, the other two (g.+45T>G and g.+276G>T) are placed in the second LD block, in exon 2 and intron 2, respectively. In general, the results of these studies have been contradictory both with regard to whether variability at this locus has an impact on metabolic phenotypes and which polymorphisms are responsible for such an effect (30; 32; 34-57). Some of these discrepancies may relate to the small size of several of these studies, providing limited power to study moderate genetic effects, whereas other may reflect genuine differences

between populations in genetic background or environmental exposures. However, despite these problems, some consistent patterns of association can be identified through meta-analyses as described below for different phenotypes.

Impact of ADIPOQ gene variants on adiponectin circulating levels.

It is estimated that a substantial proportion (between 30 and 70%) of the variability in plasma adiponectin levels is accounted for by genetic factors (54; 56; 58-63). A recent study of 1,700 Caucasian subjects has shown that polymorphisms in both blocks of the ADIPOQ gene contribute to such genetic modulation (30). Other studies, however, have failed to detect such effects, or have provided contradictory results with regard to the polymorphic sites that are involved. In order to summarize the results obtained to date and draw some conclusions, albeit provisional, on the role of adiponectin variants in the control of adiponectin levels, we conducted a systematic meta-analysis of all published data concerning SNPs for which a total of more than 2,000 individuals have been studied. Relevant articles were identified by means of a PubMed search using the phrase "[ADIPOQ or adiponectin polymorphisms] and [adiponectin]". Additional studies were collected by searching the reference lists from the publications identified by the PubMed search. Reports on children and on adults undergoing treatments that could possibly interfere with adiponectin levels were excluded. Three SNPs (g.-11391G>A, g.+45T>G and g.+276G>T) fulfilled these criteria (33; 34; 36; 41; 43; 44; 46; 49; 50; 55; 62-66). For each of these variant, a multivariate, random-effect meta-analysis was performed to estimate the weighted mean differences (WMD) in adiponectin levels between genotypes along with their 95% confidence intervals. Between-studies heterogeneity and the possible presence of

association bias were assessed by the Cochran's Q test and Macaskill's inverse pooled variance weighting method, respectively (67; 68).

A significant associations with adiponectin levels was observed for SNPs g.-11391G>A and g.+276G>T (Table 1). These are probably two independent genetic effects, since the two SNPs belong to different LD blocks (Figure 2). No association was observed for SNP g.+45T>G (Table 1). SNP g.-11391G>A was associated with adiponectin levels according to a dominant model with allele A carriers (i.e., GA +AA) having higher adiponectin levels compared with GG carriers. Although these data must be taken with caution because of a possible publication bias ( $p=0.04$ ) (Table 1), they are compatible with recent in vitro data supporting a biological function of this SNP, with the allele A enhancing the ADIPOQ promoter activity, probably through the displacement of an as yet unidentified transcription factor with inhibitory properties (69).

Results are not as clear-cut for SNP g.+276T>G. Adiponectin levels seem to progressively increase from GG homozygotes to heterozygotes and TT homozygotes, but the statistical significance of this association is marginal and is present only for heterozygotes, probably because of the small number of TT subjects. The lack of significance for TT homozygotes may also be related to the highly significant between-study heterogeneity in the data concerning the comparison between TT and GG carriers ( $p=0.00001$ ) (Table 2), which prompts caution in the interpretation of these findings. The cause of such heterogeneity (observed also for SNP g.+45) is not known, but it may relate to the different levels of circulating adiponectin reported in different studies, reflecting disparities in the criteria used to recruit the study populations and/or in the assays used to measure adiponectin concentrations.

If we assume that SNP g.+276G>T is indeed associated with adiponectin levels, it seems unlikely that this polymorphism is per se responsible for an effect on adiponectin expression, given the results of a recent in vitro study showing no difference between alleles in the binding with adipocyte nuclear factors (69). Thus, SNP g.+276T>G may be a mere marker in linkage disequilibrium with an as yet unidentified functional variant. Of note, SNP g.+276G>T is in moderate to strong LD ( $r^2 > 0.50$ ) with several polymorphisms placed in the 3'UTR – a region known for playing a pivotal role in the control of gene expression by binding proteins that regulate mRNA processing, translation, or degradation (70). In particular, an insertion/deletion polymorphism at position +2019 in the 3'UTR (having an  $r^2$  of 0.58 with g.+276G>T) is a strong candidate for a functional role, given that most of the linkage between ADIPOQ locus and adiponectin circulating levels observed in family study appears to be accounted for by this variant (63). Functional studies in transfected cells examining allelic difference in mRNA stability are needed to investigate the role of 3'UTR SNPs on the modulation of adiponectin levels.

In addition to common SNPs, also several rare variants including G84R, G90S, I164T and R112C have been reported as modulators of adiponectin levels (54; 64; 71). The R112C and I164T substitutions, which are associated with low adiponectin levels, appear to disrupt the assembly of adiponectin trimers, which may, in turn, impair adiponectin secretion from adipocytes (72). By contrast, both the G84R and G90S variants have been shown to hamper the formation of high molecular weight multimers, which are deemed to be essential for adiponectin action (72). The functional link between these sequence differences and adiponectin levels, if any, must be further investigated by functional studies in transfected adipose cells.

Although the results of the meta-analysis indicate a role for variants in the ADIPOQ gene in the modulation of adiponectin secretion, it must be emphasized that variability at this locus explains only a small proportion (~2-8%) of serum adiponectin variance (30; 54; 56; 60; 62; 63). If one considers that between 30 and 70% of this variance is accounted for by genetic factors, other loci are likely to play a bigger role. Consistent with such scenario is the recent observation of a major locus for adiponectin levels on 3q27 that appears to be distinct from the ADIPOQ gene (60). The existence of additional genetic modulators is also supported by the observation of linkage with adiponectin levels at locations other than 3q27 (58; 59; 61-63). Among these, the region of 14q13 is especially interesting since linkage has been detected at this location in two independent studies (59; 62). Of note, the linkage at this locus is not attenuated by adjustment for BMI, suggesting that the effect of this gene on adiponectin levels is not secondary to an effect on adiposity (60-62). By contrast, the linkage signal observed on chromosome 15 in Chinese decreases significantly after BMI adjustment, indicating that the gene(s) at this locus may modulate adiponectin levels through an effect on adipose mass (58). Genes with an effect on adiponectin levels may also include known modulators of insulin sensitivity such as PPAR $\alpha$  and TNF- $\alpha$ , although conflicting results have been obtained in different populations (73-77).

Impact of ADIPOQ gene variants on BMI, insulin-resistance, and type 2 diabetes.

Low plasma adiponectin levels have been consistently associated with body-weight, insulin-resistance, and increased risk of type 2 diabetes in cross-sectional as well as prospective studies (16-18; 20-22). Furthermore, adiponectin has been shown in animal models to decrease body-weight and to

independently increase insulin-sensitivity (11; 13). Thus, one would expect that the same polymorphisms that are associated with adiponectin levels would also display an association with metabolic traits. Such prediction has not been unequivocally confirmed. In the first LD block, a haplotype characterized by a G at positions g.-11391 and g.-11377 was found to be associated with type 2 diabetes in a case-control study of more than 2,000 French Caucasians (54; 55). This association has been replicated with regard to SNP g.-11377C>G in studies from Japan and Sweden populations (39; 47), but not in other studies (37; 56). In the second block, positive findings have been reported for SNPs at positions g.+45, g.+276, g.+712, g.+3639 and g.+4545, which are in strong linkage disequilibrium with each other ( $r^2 > 0.8$  for SNPs g.+45, + g.+712 and g.+2019;  $r^2 > 0.8$  for g.+276 and g.+3696, Figure 2), as well as for less frequent variants such as G84R, G90S, I164T and R112C. These sequence differences have been variably associated with increased BMI, insulin-resistance related traits, and type 2 diabetes in cross-sectional studies of populations of different origin (34; 35; 38; 40-43; 45; 46; 51; 53). Some of these variants have also been reported to influence weight gain, impaired glucose tolerance, and type 2 diabetes risk in prospective studies (31; 36; 50; 78). Also in this case, however, results have been far from uniform, being characterized by the same lack of replication displayed by genetic studies of other complex traits.

To better understand whether ADIPOQ variants are genuine determinants of metabolic traits, we conducted a meta-analysis using an approach similar to that described above for adiponectin levels. Applying the same criterion of having access to at least 2,000 subjects per SNP-phenotype combination, sufficient data were available in the literature for SNPs g.-11391G>A and g.-11377C>G with regard to type 2 diabetes (34; 36; 37; 39; 40; 47; 54-56; 79), and for SNPs

g.+45T>G and g.+276G>T with regard to BMI, insulin resistance, and type 2 diabetes (34; 36-41; 43; 45-47; 51-57; 65; 79-82). The only evidence for a significant association was observed between SNP g.+276G>T and insulin resistance, as defined by the surrogate marker HOMA<sub>IR</sub> (Table 2). This index was higher in +276 GG carriers as compared to TG and TT subjects, indicating higher insulin sensitivity in carriers of allele T – the same allele that showed an association, albeit marginal, with increased adiponectin levels (Table 2). All the studies were homogeneous and no publication bias was present (Table 2). No significant global effects were observed between any of the SNPs and risk of type 2 diabetes (Table 3) or higher BMI (Table 4) although these data must be taken with caution since evidence of between-studies heterogeneity and/or publication bias was detected for some SNP-phenotype combinations (Tables 3 and 4). Such negative results are in apparent contrast with those reported by recent prospective studies on the role of SNP g.+45T>G on weight gain (36) and SNPs g.-11391G>A, g.-11377G>C and g.+45T>G on type 2 diabetes (50; 78). The reasons for such discrepancies are unknown, but in addition to the obvious possibility of false positives due to chance or bias, one should also consider the role of differences among populations in environmental exposures that may be critical for genetic effects to become manifest. Differences in study design (i.e., cross-sectional vs. prospective) must also be taken into account, given the possibility of survival bias obscuring genuine genetic effects in cross-sectional studies.

Impact of ADIPOQ gene variants on the risk of coronary artery disease.

Another notable area of investigation is the relationship between genetic variants in the ADIPOQ gene and cardiovascular risk. In addition to modulating atherogenesis through

its insulin-sensitizing actions, adiponectin has direct anti-atherogenic effects on the arterial wall by inhibiting monocyte adhesion to the endothelium, smooth muscle cell proliferation, and foam cell formation (9; 14; 15). Administration of recombinant adenovirus expressing human adiponectin to apoE deficient animals causes a 30% reduction in the formation of atherosclerotic lesions in the absence of any effects on metabolic traits (83; 84). Consistent with these findings, men with adiponectin levels in the highest quartile have been shown to have a significantly lower incidence of cardiovascular than men with adiponectin levels in the lowest quartile, independently of other cardiovascular risk factors or systemic inflammation markers (85; 86).

Studies of the association between adiponectin polymorphisms and cardiovascular outcomes have not been as numerous as those concerning adiponectin levels or metabolic traits, but the overall number of study subjects is such that some preliminary conclusions can be drawn. Four populations – all consisting of subjects with type 2 diabetes – have been examined. The first one – recruited by Lacquemant et al. in Switzerland and France – is a cross-sectional sample of 162 cases with coronary artery disease (CAD) and 315 controls without significant CAD (87). The second one is a similar set of 142 CAD-positive cases and 234 CAD-negative controls recruited in Southern Italy (88). The last two consist of incident CAD cases and controls from the Nurses Health Study (989 women) and the Health Professional Follow-up Study (879 men), both of which are being conducted in the US (89; 90). In the French-Swiss population, SNP g.+45 was found to be a significant predictor of coronary artery disease, with an OR of 1.9 (95% CI 1.2-2.9) (87) This effect, however, was not confirmed in the other three populations, which instead showed a significant association with SNP g.+276 (88-90). When the data from the four populations

are considered together in a meta-analysis (including a total of 827 CAD-positive cases and 1,887 CAD-negative controls), individuals homozygous for allele T at g.+276 have about half the cardiovascular risk of allele G carriers (OR=0.55, 95% CI 0.4-0.8) (89). While this result will have to be confirmed in additional populations, it is noteworthy that the pattern of association is consistent with that that is found for this SNP with regard to insulin-resistance and adiponectin levels.

ADIPOQ gene variants as possible predictors of thiazolidinediones efficacy.

The PPAR $\alpha$  agonists thiazolidinediones (TZDs) are potent insulin sensitizers that are increasingly used for the treatment of type 2 diabetes. Several studies have shown that treatment with TZDs increases serum adiponectin, raising the hypothesis that some of the anti-diabetic actions of these agents are due to their effects on adiponectin levels (91-94). The hypoglycemic response to TZDs varies in the population, with 30-40% of individuals who have type 2 diabetes or are at risk for type 2 diabetes failing to respond to this therapy (95-99). Given the possible links between the anti-diabetic action of TZDs and the effect of these drugs on adiponectin levels, genetic variation in the ADIPOQ gene might account for such differences in TZDs responsiveness. Studies exploring this hypothesis are in their infancy. In the only published report to date, Kang et al. have found that patients with type 2 diabetes carrying the G allele at either position g.+45 or position g.+276 were less responsive to the TZD rosiglitazone in terms of both reduction of fasting plasma glucose and HbA1c and increase of circulating adiponectin levels (80). This result, however, has been obtained in a very small cohort of type 2 diabetic patients. If confirmed in larger populations, these findings may translate into clinical tools, allowing the identification of the best candidates for TZDs

treatment, with obvious implications for devising more cost-effective approaches to diabetes care.

Impact of genetic variability in the adiponectin receptors on insulin resistance, type 2 diabetes, and coronary artery disease.

The molecules mediating the biological functions of adiponectin were not known until 2003, when adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) were cloned by Yamauchi et al. (100). In addition to AdipoR1 and AdipoR2, T-cadherin also appears to function as an adiponectin receptor (101). Both AdipoR1 and AdipoR2 are seven transmembrane domain proteins belonging to a new class of molecules with homology to proteins functioning as progesterin receptors (progesterin/adiponectin/adipoQ receptor, or PAQR, family) (100). In humans, AdipoR1 is ubiquitously expressed, whereas AdipoR2 is predominantly expressed in skeletal muscle and liver (100). Glucose-tolerant Mexican-American subjects with a family history of type 2 diabetes were found to have significantly lower skeletal muscle levels of AdipoR1 and AdipoR2 mRNA as compared to individuals with no family history of diabetes (102). In the same study, the expression of both receptors was positively correlated with the glucose disposal rate as measured by the euglycemic hyperinsulinemic glucose clamp technique, and in the case of AdipoR2 alone, with plasma adiponectin levels (102). Interestingly, the expression of both receptors in human macrophages is stimulated by TZDs (103). Taken together, these data, although preliminary, suggest that downregulation of AdipoR1 and/or AdipoR2 might contribute to the development of insulin resistance in peripheral tissues.

The genes coding for AdipoR1 and AdipoR2 are placed on chromosomes 1q32.1 and 12p13.33, respectively (100). Both have been studied as candidate genes for insulin

resistance and related disorders, although less extensively and with even more conflicting results than for the ADIPOQ gene. Wang et al. screened the ADIPOR1 gene for sequence variants in Caucasian and African-American subjects, but failed to find associations between any of the identified polymorphisms and either insulin resistance or type 2 diabetes (104). Similarly, negative results were obtained by Hara et al, who failed to observe any association between ADIPOR1 or ADIPOR2 gene variants and type 2 diabetes in a French Caucasian population (105). More recently, however, three SNPs at the ADIPOR2 locus have been reported to confer a modest increase in the risk of type 2 diabetes in a French population (106). Multiple polymorphisms in both ADIPOR1 and ADIPOR2 have been also associated with type 2 diabetes and related traits in Old Order Amish (107), and two SNPs in the ADIPOR1 gene (i.e. g.-8503G>A in the promoter and g.-1927T>C in intron 1) have been associated with insulin resistance features and amount of fat in the liver in Caucasians from Germany (108). The association between ADIPOR1 gene variants and insulin resistance features, but not with the risk of type 2 diabetes has been confirmed also in Finnish (109) and Mexican Americans (110). ADIPOR2 gene variants (i.e. g.+795G>A, g.+870C>A and g.+963C>T) have also been recently associated as a haplotype with plasma adiponectin levels (111). Finally, recent data from our laboratories indicate that polymorphisms in the 3'UTR and 3' flanking region of ADIPOR1 may play a major role in the modulation of atherogenesis, conferring a three-fold increase in the risk of coronary artery disease among individuals with type 2 diabetes (112). This effect seems to be related to a lower ADIPOR1 expression in carriers of the at risk genotypes, possibly determining a deficit of the antiatherogenic effects of adiponectin on vascular cells (112).

Despite this encouraging evidence, the number of studies published to date is still too small to draw firm conclusions on the role of variability in ADIPOR1 and/or ADIPOR2 in predicting insulin-resistance and related disorders. In particular, the strong effect that we have demonstrated on cardiovascular risk must be confirmed in prospective studies before ADIPOR1 polymorphisms can be considered as clinically useful predictors of increased susceptibility to coronary artery disease.

#### Conclusions and future directions.

Many reports have been published to date concerning the metabolic effects of variation in the gene coding for Adiponectin (30-32; 34-36; 38-47; 50-55; 62-66; 69; 71; 78; 79; 81; 82; 87-90; 110). Meta-analyses of these studies support the hypothesis that variability in this gene contribute to the modulation of circulating adiponectin levels and the risk of insulin-resistance and coronary artery disease. Two independent genetic effects, corresponding to the two distinct linkage disequilibrium blocks observed at this locus, appear to be present (30). The effect corresponding to the 5' block, epitomized by SNP g.-11391G>A, can be demonstrated for plasma adiponectin levels, although its statistical significance is not especially striking, considering that these data were derived from more than 2,000 individuals. The effect of the 3' block, captured by SNP g.+276, is especially strong for insulin-resistance and coronary artery disease, whereas it is only marginally significant for adiponectin levels. No consistent effect on BMI or risk of type 2 diabetes can be demonstrated in either block. Genetic variants in the genes coding for adiponectin receptors may also influence the risk of insulin-resistance and cardiovascular disease (106-112), but data on these genes are still too sparse to draw meaningful conclusions.

Despite the progress in this field, several aspects remain unresolved. One of these concerns the significant evidence of heterogeneity that emerges from several of the meta-analyses. At this stage, it is unclear whether these differences in association across studies reflect differences in genetic background or modifying environmental factors between populations, are the result of discrepancies in ascertainment criteria or phenotyping methods, or simply represent statistical fluctuations. Large collaborative studies including thousands of individuals from a few different ethnic groups, recruited and phenotyped according to standard protocols and genotyped for the same set of SNPs, are clearly needed to conclusively address this matter.

One aspect that deserves further investigation is the inconsistency between the results concerning the association with adiponectin levels and those concerning the association with metabolic traits. For instance, SNP g.-11391G>A displays the strongest association with adiponectin levels, yet it does not seem to be associated with type 2 diabetes or coronary artery disease. Conversely, SNP g.+276 shows a rather weak association with adiponectin levels, yet it appears to have a strong effect on the risk of insulin resistance and coronary artery disease. The reasons for such discrepancies are not clear at this time, but an important point to consider is that serum levels may not necessarily reflect the overall amount of adiponectin in the body or its concentration in the interstitial space where the targets for the insulin-sensitizing or antiatherogenic effect of this adipokine are located. Thus, studies considering the impact of SNPs on adiponectin production (determined for instance by measuring adiponectin expression in adipocytes) rather than its serum concentrations may be more revealing.

Another unresolved issue concerns the identities of the functional variants that are

responsible for the association with adiponectin levels or metabolic phenotypes. While the genetic effect in the 5' block seems to be secondary to differences in promoter activation due to SNP g.-11391G>A (69), the situation is more complex for the 3' block where the marker displaying association with metabolic traits, i.e. SNP g.+276, does not have obvious functions. It is important to consider, however, that only a small proportion of ADIPOQ variants have been analyzed to date, and is therefore possible that other, as yet uninvestigated polymorphisms are responsible for the associations with metabolic traits. Thus, there is the need for a thorough characterization of the repertoire of polymorphisms in either block followed by in vitro studies systematically investigating the biological function of each sequence difference in strong LD with the two markers associated with metabolic phenotypes.

As these additional studies proceed, new insights are expected by the completion of the whole genome association studies that are currently in progress in a variety of populations. These efforts are expected to lead to the identification of additional genes involved in the regulation of serum adiponectin levels and explaining the proportion of genetic variance of this trait that is not accounted for by SNPs at the adiponectin and adiponectin receptor loci. With this novel knowledge, studies investigating the interaction between the ADIPOQ and other genes in the etiology of the insulin-resistance syndrome and related disorders will become a reality.

Another area of research that is expected to grow concerns the role of specific variants at the adiponectin and adiponectin receptor loci in modulating inter-individual differences in the response to TZDs treatment. While the data available in the literature are promising (80), more evidence must be gathered from properly designed pharmacogenetic studies evaluating the

outcome of TZDs treatment in carriers of different genotypes. If positive and replicable data are obtained, it may be possible to use the genetic information concerning these loci to build algorithms allowing physicians to tailor anti-diabetic therapy to the patients' genetic features, advancing diabetes care into the realm of personalized medicine.

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