Diabetologia (2006) 49:2317–2328
DOI 10.1007/s00125-006-0384-3

ARTICLE

Association between variants in the genes for adiponectin and its receptors with insulin resistance syndrome (IRS)-related phenotypes in Mexican Americans

D. K. Richardson · J. Schneider · M. J. Fourcaudot · L. M. Rodriguez · R. Arya · T. D. Dyer · L. Almasy · J. Blangero · M. P. Stern · R. A. DeFronzo · R. Duggirala · C. P. Jenkinson

Received: 19 December 2005 / Accepted: 12 June 2006 / Published online: 29 August 2006

Abstract
Aims/hypothesis The aim of this study was to examine whether genetic variation in *ADIPOQ*, *ADIPOR1* and *ADIPOR2* may contribute to increased susceptibility to components of the insulin resistance syndrome (IRS).
Materials and methods We genotyped single-nucleotide polymorphisms (SNPs) in *ADIPOQ*, *ADIPOR1* and *ADIPOR2* in Mexican American subjects (N=439) and performed an association analysis of IRS-related traits.
Results Of the eight SNPs examined in the *ADIPOQ* gene, rs4632532 and rs182052 exhibited significant associations with BMI (p=0.029 and p=0.032), fasting specific insulin (p=0.023 and p=0.026), sum of skin folds (SS) (p=0.0089 and p=0.0084) and homeostasis model assessment of insulin sensitivity (HOMA-%S) (p=0.015 and p=0.016). Two other SNPs, rs266729 and rs2241767, were significantly associated with SS (p=0.036 and p=0.013). SNP rs7539542 of *ADIPOR1* was significantly associated with BMI, SS and waist circumference (p=0.025, p=0.047 and p=0.0062). Fourteen of the *ADIPOR2* SNPs were found to be significantly (p<0.05) associated with fasting plasma triglyceride concentrations. Four of these SNPs (rs10848569, rs929434, rs3809266 and rs12342) were in high pairwise linkage disequilibrium (r^2=0.99) and were strongly associated with fasting triglyceride levels (p=0.00029, p=0.00016, p=0.00027 and p=0.00021). Adjusting for the effects of BMI and HOMA-%S on triglyceride concentrations increased significance to p=0.000060 for SNP rs929434. Bayesian quantitative trait nucleotide analysis was used to examine all possible models of gene action. Again, SNP rs929434 provided the strongest statistical evidence of an effect on triglyceride concentrations.
Conclusions/interpretation These results provide evidence for association of SNPs in *ADIPOQ* and its receptors with multiple IRS-related phenotypes. Specifically, several genetic variants in *ADIPOR2* were strongly associated with decreased triglyceride levels.

Keywords Adiponectin · Adiponectin receptors · Association study · Genetics of type 2 diabetes mellitus · Insulin Resistance Syndrome · Single-nucleotide polymorphisms

Abbreviations

AMPK	adenosine 5′-monophosphate-activated protein kinase
BQTN	Bayesian quantitative trait nucleotide
FPG	fasting plasma glucose
FSI	fasting specific insulin
HOMA-%β	homeostasis model assessment of β-cell insulin secretion
HOMA-%S	homeostasis model assessment of insulin sensitivity
HWE	Hardy–Weinburg equilibrium

D. K. Richardson (✉) · M. J. Fourcaudot · L. M. Rodriguez · R. A. DeFronzo · C. P. Jenkinson
Division of Diabetes MC 7886, Department of Medicine,
The University of Texas Health Science Centre at San Antonio,
7703 Floyd Curl Drive,
San Antonio, TX 78229-3900, USA
e-mail: richardsond2@uthscsa.edu

J. Schneider · T. D. Dyer · L. Almasy · J. Blangero · R. Duggirala · C. P. Jenkinson
Southwest Foundation for Biomedical Research,
San Antonio, TX, USA

R. Arya · M. P. Stern
Division of Clinical Epidemiology, Department of Medicine,
University of Texas Health Science Centre at San Antonio,
San Antonio, TX, USA

IRS	insulin resistance (metabolic) syndrome
LD	linkage disequilibrium
LEP	fasting leptin
MAF	minor allele frequency
MGA	measured genotype approach
PAI-1	plasminogen activator inhibitor-1
SAFDS	San Antonio Family Diabetes Study
SNP	single-nucleotide polymorphism
SS	sum of skin fold thickness
TRL	triglyceride-rich lipoproteins
WC	waist circumference

Introduction

Type 2 diabetes mellitus is a chronic metabolic disorder characterised by hyperglycaemia, insulin resistance, impaired beta cell function [1, 2] and a variety of cardiovascular risk factors, including hypertension, elevated triglycerides, reduced HDL cholesterol, increased plasminogen activator inhibitor-1 (PAI-1) levels, and endothelial dysfunction [3, 4]. Collectively, these metabolic and cardiovascular risk factors have been referred to as the insulin resistance syndrome (IRS) or metabolic syndrome. Insulin resistance in type 2 diabetes results from impaired insulin action in skeletal muscle and liver [5, 6]. Also, adipocytes are resistant to the anti-lipolytic action of insulin [5, 7], and the resultant increase in plasma NEFA exacerbates the insulin resistance in muscle and liver [5–9].

The prevalence of type 2 diabetes and the IRS is rapidly and relentlessly increasing and, although major risk factors for these disorders have been identified [1], the genetic factors responsible for type 2 diabetes mellitus and the IRS remain unclear. Genome-wide scans have demonstrated linkage between a number of chromosomal regions and diabetes or diabetes-related traits [10, 11]. Candidate gene studies and a number of single-nucleotide polymorphisms (SNPs) have also been associated with type 2 diabetes and/or related diseases, including obesity and insulin resistance [12, 13].

Adiponectin (encoded by the gene *ADIPOQ*) is a hormone that is expressed only by adipocytes [14] and regulates energy homeostasis and glucose and lipid metabolism [15]. Rodent and human studies have demonstrated that adiponectin levels are decreased in insulin-resistant states, such as type 2 diabetes and obesity [16–19]. Administration of adiponectin to rodents with obesity and diabetes had insulin-sensitising effects [18] and reduced triglyceride accumulation in muscle [19]. Adiponectin acts through its receptors, ADIPOR1 and ADIPOR2. It was initially thought that *ADIPOR1* was primarily expressed in skeletal muscle, whereas *ADIPOR2* was predominantly expressed in liver [20]. However, we recently showed that *ADIPOR2* is highly expressed in human muscle and may be the predominant isoform through which adiponectin exerts its insulin-sensitizing effects in skeletal muscle [21]. Adiponectin receptors mediate enhanced fatty acid oxidation and glucose uptake, increase adenosine 5′-monophosphate-activated protein kinase (AMPK) activity, and interact with peroxisome proliferator-activated receptor (PPAR) pathways [22, 23].

Common polymorphisms in the *ADIPOQ* gene have been studied in several populations, including Japanese [24] and Europeans [25]. These studies suggest that genetic variation in the *ADIPOQ* gene may predispose humans to insulin resistance and/or components of the IRS. Additionally, SNP +45 and SNP +276 polymorphisms of the *ADIPOQ* gene have been shown to predict conversion from impaired glucose tolerance to type 2 diabetes in the Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) [26]. In contrast, common *ADIPOQ* polymorphisms were not associated with type 2 diabetes in Pima Indians [27]. Studies of SNPs in the *ADIPOR1* and *ADIPOR2* genes failed to find any association with type 2 diabetes in northern Europeans or African Americans [28], or in the Japanese population [29]. However, genetic variants in both *ADIPOR1* and *ADIPOR2* genes were found to be associated with type 2 diabetes in the Old Order Amish population [30]. More recently, it was reported that variation in *ADIPOR1* may affect insulin sensitivity and liver fat content in Europeans [31].

To date, no data have been published on the effects of genetic variation in the *ADIPOQ*, *ADIPOR1* and *ADIPOR2* genes on type 2 diabetes and the IRS in Mexican Americans, a group at high risk of these diseases [32]. In this study, we conducted a preliminary survey of these three candidate genes, selecting SNPs from the public SNP database (dbSNP) and from a review of the literature. We chose a subset of SNPs in the *ADIPOQ* gene and all available SNPs in the recently discovered *ADIPOR1* and *ADIPOR2* genes. We genotyped these common polymorphisms in a Mexican American population sample, and performed association analyses with IRS-related phenotypes.

Subjects and methods

The San Antonio Family Diabetes Study

All study participants were Mexican Americans from the San Antonio Family Diabetes Study (SAFDS) [10, 33]. Briefly, probands for the SAFDS were identified by diabetes status in an earlier epidemiological survey, the San Antonio Heart Study, which has been extensively described elsewhere [34]. All first, second and third degree relatives of the probands, aged from 18–98 years, were

invited to participate in the study. Metabolic, anthropometric, demographic and medical history information was obtained on 439 individuals (116 with type 2 diabetes) distributed across 27 low-income Mexican American pedigrees [10, 33]. All procedures were approved by the Institutional Review Board of the University of Texas Health Science Centre at San Antonio, and all subjects gave informed consent prior to their participation.

For this study, we used phenotypic information from those subjects without diabetes (n=323 individuals, depending on availability of data), to avoid metabolic derangements secondary to type 2 diabetes or treatment for type 2 diabetes. The following ten IRS-related phenotypes, which have been described in detail elsewhere [10], were considered for the present study: fasting plasma glucose (FPG), fasting specific insulin (FSI), homeostasis model assessment of β-cell function (HOMA-%β) and insulin sensitivity (HOMA-%S, an inverse measure of insulin resistance), fasting HDL cholesterol, fasting triglyceride concentrations, fasting leptin (LEP), BMI, waist circumference (WC) and sum of skin fold thickness (SS). The sum of eight skinfold measures was used as a measure of overall subcutaneous adiposity. Phenotype-specific distinct outliers were excluded from the analyses (e.g. triglyceride values >9 mmol/l). Where indicated, the data were log-transformed to normalise distributions.

SNP genotyping

Genomic DNA was isolated from whole blood (Qiagen, Chatsworth, CA, USA). SNPs were selected from the NCBI dbSNP database and from previous studies that reported association with diabetes, obesity and/or IRS-related traits. SNPs were obtained by either the Assay-on-Demand or Assay-by-Design service and genotyped according to manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Alleles were scored using the allelic discrimination software Sequence Detection System v2.1 (Applied Biosystems). All SNPs were tested for Mendelian pedigree inconsistencies. For all SNPs genotyped, our mean rate of success for genotyping was >98%. Primers and probes used for the SNP genotyping are available on request.

Statistical genetic analysis

We performed association analysis in our complex pedigree-based data using the measured genotype approach (MGA) within the variance components (VC) analytical framework implemented in the program SOLAR (http://www.sfbr.org/solar/, last accessed in July 2006) [35]. The VC-based approach accounts for the non-independence among family members. In this analytical technique, VCs are modelled as random effects (e.g. additive genetic effects and random environmental effects), whereas the effects of measured covariates such as age and sex are modelled as fixed effects on the trait mean. The VCs, the association parameters, and the other covariate effects (e.g. age and sex terms) were estimated, simultaneously, by maximum likelihood techniques. A likelihood function based on multivariate normal density was numerically maximised to obtain parameter estimates.

Prior to performing MGA, the quantitative transmission disequilibrium test [36], as implemented in SOLAR, was used to examine hidden population stratification, using the notation of within (w) and between (b) family components. In MGA, generally, the marker genotypes were incorporated in the mean effects model as a measured covariate, assuming additivity of allelic effects [37, 38]. Using the notation of within (w) and between (b) family components, a significant test of b=w vs b=0 and w=0 reflects a significant difference between the genotypic means (Table 1). All analyses included age and sex terms as covariates, if found to be significant. The tests of association and population stratification and the related hypothesis testing are detailed in Table 1. The nested models were compared using the likelihood ratio test. Twice the difference between the log-likelihoods of these models yields a test statistic that is asymptotically distributed, approximating a χ^2 distribution with 1 df. A p value ≤0.05 is considered significant. Using SOLAR, linkage disequilibrium (LD) between SNP pairs was estimated using the absolute value of the correlation coefficient $|\rho|$. However, for the purpose of discussion, r^2 values are reported to describe the pairwise LD patterns.

The Bayesian quantitative trait nucleotide model

The Bayesian quantitative trait nucleotide (BQTN) analytical technique is employed to analyse SNPs to find the responsible nucleotide variants (the QTNs [39, 40]) influencing a given phenotype. Given complete SNP data for a gene, this statistical technique can be used to identify the sequence variants that are either potentially functional or that exhibit the highest disequilibria with such potential functional sites. The BQTN model is a simple extension of the classical variance component model, which aims to disentangle the genetic architecture of a quantitative trait [41]. This technique has been described in detail elsewhere [41].

Bayesian model selection/model averaging

Since a candidate gene may contain a number of SNPs that could generate several possible competing models of QTN action, we employed a Bayesian model selection/model

Table 1 Association/linkage disequilibrium and population stratification analyses and hypothesis testing

Test	Hypothesis	Inference
Population stratification	b, w (both parameters are estimated) vs $b=w$ (parameters are estimated to be equal)	Significant $b\neq w$ indicates the presence of population stratification
MGA	$b=w$ (parameters are estimated to be equal) vs $b=0$, $w=0$ (both parameters are fixed to 0)	Significant $b=w$ refers to significant difference between genotypic means

averaging approach to analyse the SNP data simultaneously in order to estimate the probability that each SNP has a direct effect on the phenotype. This technique has been described in detail elsewhere [41, 42].

The BQTN analysis can be extended to analyse the haplotype data. For a given individual, the most probable haplotypes were generated using the program MERLIN [36], as implemented in SOLAR. The BQTN analytical procedures are implemented in the computer program SOLAR [41].

Conditional linkage/QTN analysis

To examine whether the associated SNP(s) found by the QTN analysis can account for our reported linkage of triglyceride concentrations at chromosome 12p13.31, which is close to the *ADIPOR2* locus (chromosome 12p13.33), we combined the QTN analysis with our identity-by-descent (IBD)-based variance component linkage analysis. If a variant, or set of variants, in the *ADIPOR2* gene is responsible for the observed linkage signal, linkage analysis conditional on a fixed-effect MGA of the polymorphism will yield an expected logarithm of the odds ratio in favour of linkage (LOD) score near zero. Alternatively, if the associated polymorphism is in less than complete linkage disequilibrium with the true functional site, linkage analysis will generally yield a non-zero LOD score. This method has been described in detail elsewhere [43].

Results

The clinical characteristics of the subjects are shown in Table 2. We genotyped 59 SNPs (*ADIPOQ*=9, *ADIPOR1*=12 and *ADIPOR2*=38), and, of these, 38 (*ADIPOQ*=8, *ADIPOR1*=6, and *ADIPOR2*=24) were polymorphic and in Hardy–Weinberg equilibrium (HWE) in our dataset. SNPs that were not in HWE were discarded from further analysis. Schema of genomic structure and variants of the *ADIPOQ*, *ADIPOR1* and *ADIPOR2* genes are shown in Fig. 1. The minor allele frequencies (MAFs) of the SNPs, categorised by diabetes status, are shown in Table 3. The frequency of the minor alleles varied from less than 1 to 49%. There were no significant associations with type 2 diabetes (Table 3).

We performed association analysis using MGA, after finding no statistical evidence of hidden population stratification. The significant results ($p\leq0.05$) of the association analyses are summarised in Table 4. Of the eight SNPs examined in the *ADIPOQ* gene, four exhibited significant associations with several IRS-related phenotypes. The minor allele for SNPs rs4632532 and rs182052 were significantly associated with an increase in BMI ($p=0.029$ and $p=0.032$), FSI ($p=0.023$ and $p=0.026$) and SS ($p=0.0089$ and $p=0.0084$), and a decrease in HOMA-%S ($p=0.015$ and $p=0.016$). Two other SNPs, rs266729 and rs2241767, were significantly associated with SS ($p=0.036$ and $p=0.013$). For all SNPs genotyped in the *ADIPOQ* gene, the pairwise LD (r^2) ranged from 0 to 0.98. The highest pairwise LD found among the eight *ADIPOQ* SNPs was between SNP pair rs4632532–rs182052 ($r^2=0.98$), followed by SNP pair rs2241766–rs2241767 ($r^2=0.92$), as shown in Fig. 2a. The average pairwise LD among the *ADIPOQ* SNPs was 0.37.

SNP rs7539542 of *ADIPOR1* was significantly associated with BMI, SS and WC ($p=0.025$, $p=0.047$ and $p=0.0062$, respectively). The minor allele was associated with an increase in BMI, SS and WC, as shown in Table 4. Correlations between SNP pairs were estimated and are shown in Fig. 2b. The pairwise LD in the *ADIPOR1* gene (r^2) ranged from 0 to 0.87. The highest LD was between SNP pair rs2275737–rs2275738 ($r^2=0.87$), as shown in Fig. 2b. The average pairwise LD among the *ADIPOR1* SNPs was 0.39.

Of note, 14 of the 24 *ADIPOR2* SNPs were significantly ($p<0.05$) associated with fasting plasma triglyceride concentrations. The mean triglyceride concentrations per genotype are depicted in Table 4 for the significantly associated SNPs. The majority of the minor alleles, with the exception of rs1029629 and rs12582624, were associated with decreased triglyceride levels. Figure 2c shows the overall pattern of LD in the *ADIPOR2* gene, and Table 4 highlights SNPs that were in strong LD with each other. The average proportion of shared variation between SNPs, measured by the pairwise correlation, was 0.31, with SNP-specific values ranging from 0 to 0.99. Of particular note, four of these SNPs (rs10848569, rs929434, rs3809266 and rs12342) were in high pairwise LD ($r^2=0.99$) and were strongly associated with triglyceride levels ($p=0.00029$, $p=0.00016$, $p=0.00027$ and $p=0.00021$).

Table 2 Characteristics of the SAFDS subjects without diabetes distributed across 27 families

Variable	Sample size	Mean±SD or %	Distributional properties of the phenotypes	
			Skewness	Kurtosis
Number	323	–	–	–
Women	187	58%	–	–
Age (years)	323	38.3±15.4	–	–
Fasting specific insulin (pmol/l)	293	138±86	1.1	0.9
Fasting glucose (mmol/l)	323	4.9±0.6	0.7	1.0
ln HOMA-%S	290	3.8±0.7	0.0	−0.9
ln HOMA-%β	293	5.1±0.5	−0.0	−0.6
ln Triglycerides	311	4.9±0.6	0.4	−0.3
HDL cholesterol (mmol/l)	309	1.0±0.3	0.6	0.3
Leptin (ng/ml)	292	22.2±17.6	1.4	1.6
BMI (kg/m^2)	317	29.4±6.6	1.0	1.2
Sum of skinfold thickness (mm)	319	174.0±56.3	0.2	−0.4
Waist circumference (mm)	317	946±171	0.7	0.5

Given our previous findings of common genetic influences on IRS-related phenotypes, including obesity, insulin resistance and triglyceride concentrations [10], we verified whether adjustment for measures of obesity and insulin resistance may affect the observed patterns of association between variants in *ADIPOR2* and triglyceride concentrations. Thus, we repeated the association analysis of triglyceride and *ADIPOR2* SNP data after accounting for the effects of BMI and HOMA-%S. All of the initial findings were observed again, but with increased levels of significance, most notably for SNP rs929434 (p=0.000060), with the exception of SNP rs1468491, where the p value changed from 0.026 to 0.032.

Following the findings of the association analysis of the SNPs in *ADIPOR2* using MGA (Table 4), we performed the BQTN analysis using ten of the 24 SNPs in *ADIPOR2* to obtain a more plausible model, given the data. The 14 SNPs that exhibited redundancy owing to relatively high pairwise LDs (r^2 >0.80) were excluded from the BQTN analysis. The remaining ten non-redundant SNPs resulted in the examination of 1,024 possible additive gene action models. The model with only SNP rs929434 provided the strongest evidence for the observed associations, with estimated posterior probability of >0.82. The BQTN analysis was extended to analyse the predicted *ADIPOR2* haplotypes. Of the 35 observed haplotypes, 13 haplotypes had frequencies >1%, with a cumulative frequency of 89%. The 35 haplotypes generated 59,536 possible models to be examined by BQTN analysis; however, haplotypic information did not improve the association.

To determine whether rs929434 has relevance to our reported linkage of fasting triglyceride concentrations at chromosome 12p13.31 [11], which is close to the *ADIPOR2* locus (chromosome 12p13.33), we re-analysed the original triglyceride data using information from our recent genome scan data generated by the Centre for Inherited Disease Research (CIDR). Using the total fasting triglyceride dataset, for which SNP rs929434 data were also available, we found modest evidence for linkage (LOD=1.85) of plasma triglyceride levels to a genetic location between markers D12S374 and GATA49D12 at 12p13.31. When we re-analysed the data, conditional on SNP rs929434, the LOD score was reduced to 0.98. Thus, about 47% of the evidence for linkage at 12p13.31 was explained by the sequence variation at rs929434.

Discussion

We have previously shown that the Mexican American population is at relatively high risk of obesity, type 2 diabetes and the IRS. The genetic variation in candidate genes *ADIPOQ*, *ADIPOR1* and *ADIPOR2* could influence variation in such disease conditions and related traits. In the present study we provide evidence that *ADIPOR2* polymorphisms are strongly associated with decreased plasma triglyceride levels. Of the polymorphisms that we genotyped in the *ADIPOR2* gene, our strongest association was with fasting triglyceride levels, most notably SNPs rs10848569, rs929434, rs3809266 and rs12342, where we observed high pairwise correlation between all SNPs (r^2=0.99). These four SNPs with relatively high MAF of 44.3–45.1% appear to have substantial influence on variation in circulating triglyceride levels, and the evidence for association continues to be significant after Bonferroni's correction for multiple testing. Given that there is some correlation between phenotypes and between SNPs, the Bonferroni correction is rather conservative. To further investigate the potential functional relevance of the variants in the *ADIPOR2* gene, we performed a BQTN analysis, in which we compared all possible models of gene action.

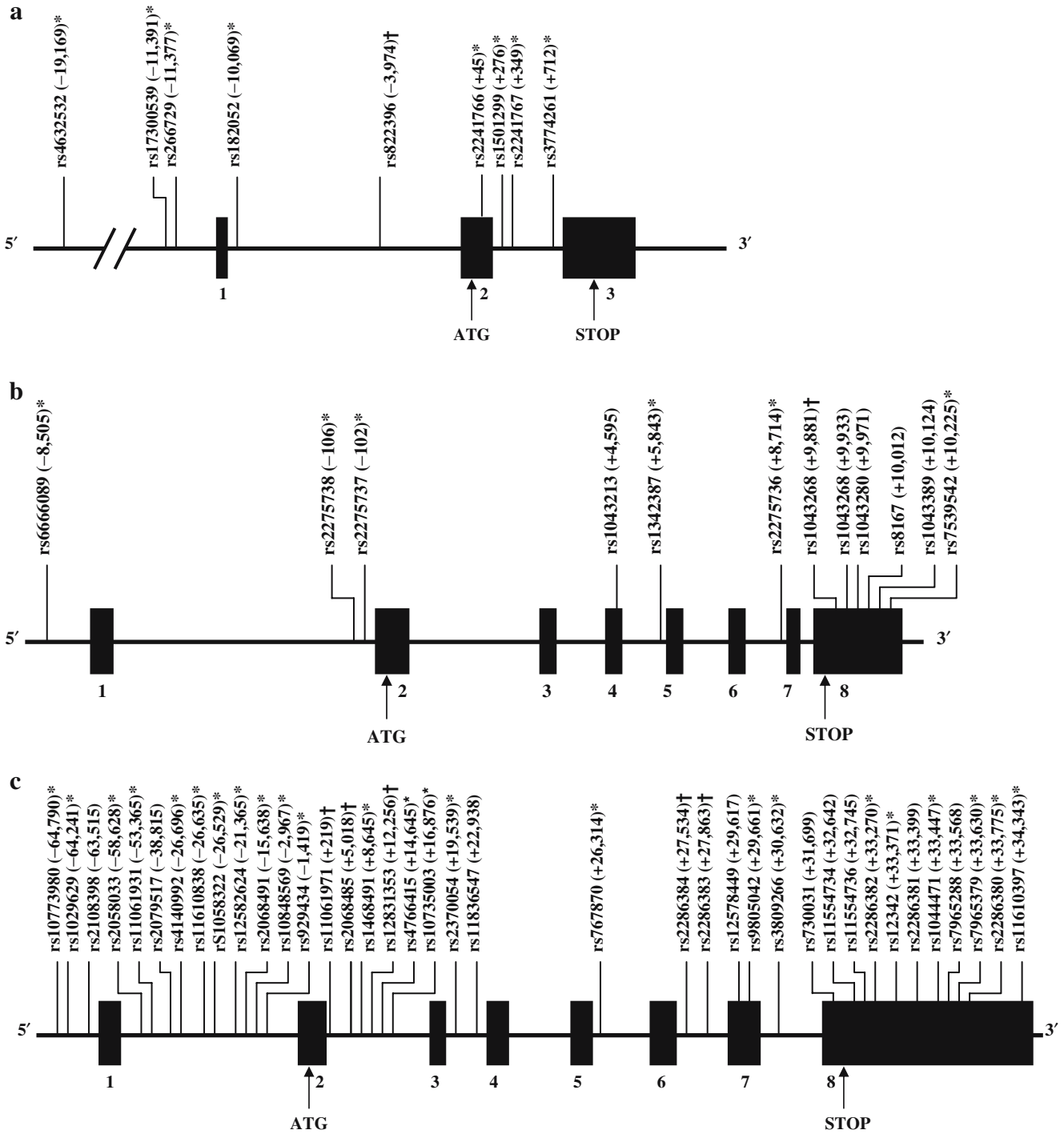

Fig. 1 Genomic structure, and location of SNPs in the ADIPOQ (**a**), *ADIPOR1* (**b**) and *ADIPOR2* (**c**) genes. Exons are shown as *boxes*, and introns as *lines connecting the boxes*. *Numbers in parenthesis* indicate location relative to the A of the ATG of the start codon. The *asterisks* (*) denote SNPs that were polymorphic in this population (MAF >0.01). The SNPs that were not in HWE are denoted by a *dagger* (†)

This analysis provided strong statistical support for a single SNP model (i.e. rs929434) for the observed association with triglyceride concentrations.

Genetic variants in the *ADIPOR2* gene were found to be associated with type 2 diabetes in the Old Order Amish population [30]. Of the eight SNPs typed (rs10773980, rs1029629, rs11061971, rs10735003, rs9805042, rs12342, rs1044471 and rs2286380), three (rs1029629, rs12342 and rs1044471) in the Amish population were significantly associated with combined type 2 diabetes and impaired

Table 3 Minor allele frequencies of SNPs by diabetes status

Gene	SNP rs no.	Position relative to ATG start[a]	Major/minor allele	Frequency % (with diabetes)[b]	Frequency % (without diabetes)[b]
ADIPOQ	rs4632532	−19,169	C/T	46.6	48.4
ADIPOQ	rs17300539	−11,391	C/T	3.6	2.8
ADIPOQ	rs266729	−11,377	C/G	33.3	30.5
ADIPOQ	rs182052	−10,069	A/G	46.5	48.7
ADIPOQ	rs2241766	45	T/G	10.7	10.8
ADIPOQ	rs1501299	276	G/T	28.3	29.9
ADIPOQ	rs2241767	349	A/G	12.7	14.9
ADIPOQ	rs3774261	712	G/A	40.7	46.8
ADIPOR1	rs6666089	−8,505	G/A	15.8	19.4
ADIPOR1	rs2275738	−106	G/A	42.3	37.3
ADIPOR1	rs2275737	−102	C/A	41.8	40.7
ADIPOR1	rs1342387	5,843	A/G	36.5	42.4
ADIPOR1	rs2275736	8,714	T/A	3.0	4.1
ADIPOR1	rs7539542	10,225	C/G	32.0	34.9
ADIPOR2	rs10773980	−64,790	C/T	40.4	36.5
ADIPOR2	rs1029629	−64,241	A/C	48.9	46.6
ADIPOR2	rs2058033	−63,515	C/A	21.2	24.0
ADIPOR2	rs11061931	−53,365	T/A	6.1	6.6
ADIPOR2	rs4140992	−26,696	T/C	35.2	32.4
ADIPOR2	rs11610838	−26,635	T/C	6.0	7.0
ADIPOR2	rs1058322	−26,529	C/T	38.0	33.2
ADIPOR2	rs12582624	−21,365	C/G	46.6	46.5
ADIPOR2	rs2068491	−15,638	A/G	41.0	37.9
ADIPOR2	rs10848569	−2,967	A/G	45.7	44.3
ADIPOR2	rs929434	−1,419	A/G	45.8	45.1
ADIPOR2	rs1468491	8,645	C/G	4.5	5.4
ADIPOR2	rs4766415	12,256	A/T	37.1	35.4
ADIPOR2	rs10735003	16,876	C/T	39.8	35.9
ADIPOR2	rs2370054	19,539	A/G	0	0.4
ADIPOR2	rs767870	26,314	C/T	6.2	8.5
ADIPOR2	rs9805042	29,661	C/T	6.1	7.2
ADIPOR2	rs3809266	30,632	T/G	45.8	44.6
ADIPOR2	rs2286382	33,270	G/A	6.0	7.7
ADIPOR2	rs12342	33,371	T/C	45.8	44.3
ADIPOR2	rs1044471	33,447	C/T	36.2	32.6
ADIPOR2	rs7965379	33,630	C/T	6.1	5.9
ADIPOR2	rs2286380	33,775	A/T	4.6	6.1
ADIPOR2	rs11610397	34,343	G/A	6.8	7.4

[a]Numbers indicate location relative to the A of the start codon (ATG), which is +1
[b]The allele frequencies were not significantly ($p<0.05$) different between the subjects with and without diabetes based on a chi squared test

glucose tolerance, while in the present study, these SNPs were associated with fasting triglyceride concentrations. Interestingly, the minor allele for rs12342 in the present study, which was significantly associated with decreased fasting triglyceride, was found to be the major allele in the Amish population. In contrast, a study in a Japanese population demonstrated no association between variants (including five that we examined: rs2058033, rs12582624, rs10848569, rs12342 and rs1044471) in *ADIPOR2* and type 2 diabetes mellitus [29]. Likewise, no association between variants (including five that we examined: rs10773980, rs767870, rs12578449, rs9805042 and rs730031) in *ADIPOR2* and glucose and lipid metabolic parameters were found in a European population [31].

We previously reported modest evidence for linkage of fasting triglyceride concentrations to a genetic location on chromosome 12p, which is close to the *ADIPOR2* locus (chromosome 12p13.33) [11]. To determine whether rs929434 could account for our linkage signal, we re-evaluated linkage on chromosome 12p, conditional on the measured genotype effect. By including rs929434 genotypes as a covariate in the model, the LOD score for fasting

Table 4 Significant associations between genetic polymorphisms in *ADIPOQ*, *ADIPOR1* and *ADIPOR2* genes and IRS-related phenotypes

Gene	SNP rs no.	Phenotype	Major/major[a]	Major/minor[a]	Minor/minor[a]	Direction of change	*p* value
ADIPOQ	rs4632532	BMI (kg/m^2)	28.8±0.9	30.0±0.6	31.3±0.8	↑	0.029
ADIPOQ	rs182052	BMI (kg/m^2)	28.8±0.9	30.1±0.6	31.4±0.8	↑	0.032
ADIPOQ	rs4632532	FSI	108.7±10.4	126.1±6.7	143.5±10.0	↑	0.023
ADIPOQ	rs182052	FSI	108.8±10.3	125.9±6.6	143.0±10.0	↑	0.026
ADIPOQ	rs4632532	HOMA-%S	3.96±0.08	3.81±0.05	3.67±0.08	↓	0.015
ADIPOQ	rs182052	HOMA-%S	3.96±0.08	3.81±0.05	3.67±0.08	↓	0.016
ADIPOQ	rs4632532	SS (mm)	140.3±7.1	151.5±5.5	162.7±6.8	↑	0.0089
ADIPOQ	rs182052	SS (mm)	139.8±7.1	151.2±5.5	162.7±6.8	↑	0.0084
ADIPOQ	rs266729	SS (mm)	157.4±6.1	147.7±5.9	137.9±8.7	↓	0.036
ADIPOQ	rs2241767	SS (mm)	147.8±5.6	162.2±6.7	176.6±11.2	↑	0.013
ADIPOR1	rs7539542	BMI (kg/m^2)	28.8±1.7	30.5±1.6	32.2±1.9	↑	0.025
ADIPOR1	rs2275736	BMI (kg/m^2)	28.9±1.4	26.6±1.8	–[b]	↓	0.026
ADIPOR1	rs7539542	SS (mm)	138.8±6.7	150.3±5.9	161.8±9.4	↑	0.047
ADIPOR1	rs7539542	WC (mm)	967.5±24.3	1020.8±22.1	1074.0±33.5	↑	0.0062
ADIPOR2	rs10773980[c]	ln TG	5.27±0.07	5.16±0.06	5.06±0.08	↓	0.030
ADIPOR2	rs1029629[d]	ln TG	5.08±0.07	5.20±0.06	5.31±0.07	↑	0.016
ADIPOR2	rs4140992	ln TG	5.25±0.06	5.14±0.06	5.03±0.08	↓	0.019
ADIPOR2	rs12582624[d]	ln TG	5.06±0.07	5.19±0.06	5.31±0.07	↑	0.0044
ADIPOR2	rs10848569[e]	ln TG	5.33±0.07	5.17±0.05	5.02±0.07	↓	0.00029
ADIPOR2	rs929434[e]	ln TG	5.34±0.07	5.18±0.05	5.02±0.07	↓	0.00016
ADIPOR2	rs1468491[f]	ln TG	5.21±0.06	4.99±0.10	4.77±0.19	↓	0.026
ADIPOR2	rs4766415[c]	ln TG	5.25±0.06	5.14±0.06	5.03±0.08	↓	0.020
ADIPOR2	rs10735003[c]	ln TG	5.28±0.07	5.17±0.06	5.05±0.08	↓	0.016
ADIPOR2	rs767870	ln TG	5.22±0.06	5.01±0.09	4.80±0.17	↓	0.013
ADIPOR2	rs3809266[e]	ln TG	5.32±0.07	5.17±0.05	5.01±0.07	↓	0.00027
ADIPOR2	rs12342[e]	ln TG	5.33±0.06	5.17±0.05	5.02±0.07	↓	0.00021
ADIPOR2	rs1044471	ln TG	5.26±0.06	5.15±0.06	5.05±0.08	↓	0.027
ADIPOR2	rs2286380[e]	ln TG	5.21±0.06	5.02±0.09	4.83±0.17	↓	0.028
ADIPOR2	rs2058033	HOMA-%S	3.75±0.06	3.89±0.06	4.02±0.11	↑	0.046

TG Fasting triglyceride concentrations
[a]Mean trait concentrations by genotype category, after adjusting for age and sex effects, using the measured genotype approach [41]
[b]No minor/minor homozygotes are observed in our model
[c]Three markers are in strong LD with each other ($r^2 \geq 0.92$)
[d]Two markers are in strong LD with each other ($r^2 = 0.93$)
[e]Four markers are in strong LD with each other ($r^2 = 0.99$)
[f]Two markers are in strong LD with each other ($r^2 = 0.99$)

triglyceride concentrations was reduced from 1.85 to 0.98. Thus, about 47% of the evidence for linkage at 12p13.31 was explained by the sequence variation at rs929434, in turn suggesting that this may be one of several functional variants or in strong LD with the true functional variant(s). The majority of the minor alleles for the polymorphisms in *ADIPOR2* were associated with decreased fasting triglyceride and, thus, may provide some protection against developing the IRS and atherosclerosis. Two *ADIPOR2* SNPs, rs1029629 and rs12582624, that were in strong LD with each other (r^2=0.93), were associated with increased fasting triglyceride. This seemingly paradoxical finding is not unexpected, given that independent variants in the same gene may have independent effects on traits.

The molecular mechanisms by which adiponectin exerts its insulin-sensitising effects on liver and muscle appear to be related to an increase in fatty acid oxidation, an effect that is mediated, at least in part, by its activation of AMPK [23]. In skeletal muscle, adiponectin acts through its receptors (ADIPOR1 and ADIPOR2) to phosphorylate AMPK, which, in turn, phosphorylates acetyl CoA carboxylase. This leads to an increase in fatty acid oxidation [22, 23] and a reduction in toxic intracellular lipid metabolites that inhibit insulin signalling. Adiponectin also decreases hepatic glucose output by reducing the expression of enzymes involved in gluconeogenesis in the liver [44, 45], thereby contributing to the regulation of whole-body glucose homeostasis. As a result of these processes, a reduction in tissue triglycerides and toxic lipid metabolites is observed in both skeletal muscle and liver [19, 22, 23], and this contributes to improved insulin signal transduction. The effects of adiponectin on muscle and liver were recently reviewed elsewhere [46], and it was suggested that liver is the primary site of adiponectin bioactivity.

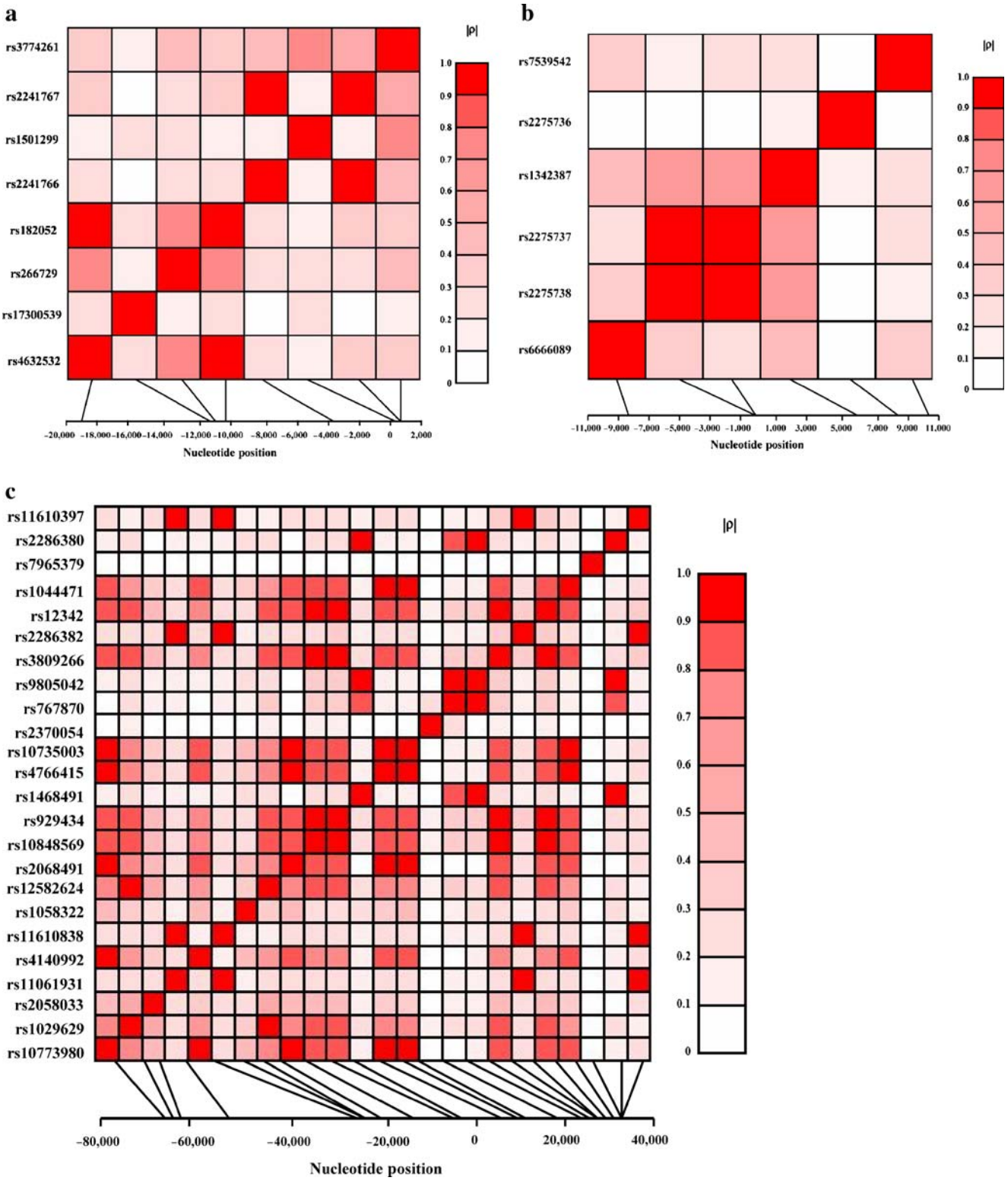

Fig. 2 Linkage disequilibrium (LD) between SNP pairs within *ADIPOQ* (**a**), *ADIPOR1* (**b**) and *ADIPOR2* (**c**) genes. SNPs are labelled on the *y*-axis, and the locations (bp) within the gene are shown on the *x*-axis. Pairwise LD is estimated using the correlation coefficient $|\rho|$, and depicted in the figure by the colour intensity of the shaded box, as shown in the legend. The diagonal represents a comparison of each SNP against itself (i.e. $|\rho|=1.0$)

ADIPOR2 is abundantly expressed in human liver [20], and an important question raised by our results is whether adiponectin acts through the hepatic *ADIPOR2* to influence triglyceride biosynthesis and homeostasis.

There has been considerable interest in the role of kinetic defects in triglyceride-rich lipoprotein (TRL) metabolism in the development of atherosclerosis and dyslipidaemia (e.g. increased fasting triglycerides and low HDL cholesterol), as it relates to type 2 diabetes and the IRS [47, 48]. It has been suggested that adiponectin concentration may play an independent role in regulating TRL metabolism, aside from its link with abdominal fat, insulin resistance and dyslipidaemia [47]. These observations have relevance to a recent finding where a significant correlation was demonstrated between the expression of *ADIPOR1* and *ADIPOR2* mRNA in human skeletal muscle and in vivo parameters of glucose and lipid metabolism [49]. *ADIPOR2* mRNA levels correlated positively and independently only with fasting triglyceride concentrations [49], whereas *ADIPOR1* mRNA expression was positively correlated with serum insulin and C-peptide concentrations, first-phase insulin secretion, and elevated plasma triglyceride and cholesterol concentrations [49]. Given this independent association of *ADIPOR2* gene expression with fasting triglyceride concentrations, but not with BMI, insulin or glucose, and our previous findings of common genetic influences on IRS-related phenotypes (e.g. obesity, insulin resistance and fasting triglyceride) [10], we examined whether adjustment for measures of obesity and insulin resistance may affect the observed patterns of association between variants in *ADIPOR2* and fasting triglyceride. The sample size was reduced (N=273) because of the requirement of common covariate information. All the initial findings were observed again, but with increased levels of significance (with the exception of SNP rs1468491, as noted above). For example, the p value for association of SNP rs929434 with fasting triglyceride concentrations was 0.000060. These results, together with previous findings [49], indicate that adiponectin and its receptors, especially *ADIPOR2*, play an important role in regulating fasting plasma triglyceride levels, and that they could have a potential role in VLDL metabolism.

Several studies have shown that the *ADIPOQ* gene is associated with IRS-related phenotypes [24–27]. Of particular note, a haplotype including two SNPs at positions −11,391 (rs17300539) and −11,377 (rs266729), both located in the promoter sequence of the *ADIPOQ* gene, was shown to be strongly associated with adiponectin concentrations and type 2 diabetes in the French population [50]. Our results provide additional evidence that variants of the *ADIPOQ* gene are significantly associated with IRS-related traits.

We genotyped 12 SNPs in the *ADIPOR1* gene (Fig. 1), and six of these were polymorphic and in HWE in our dataset. Of the six polymorphic SNPs, one was significantly associated with IRS-related traits. Interestingly, SNP rs7539542 of *ADIPOR1* was significantly associated with three obesity traits: SS, BMI and waist circumference. Similar to our findings, variants in *ADIPOR1* were found to be associated with type 2 diabetes in the Old Order Amish population [30]. The following five SNPs were evaluated in both studies: rs6666089, rs2275738, rs2275737, rs1342387 and rs7539542. In the Amish population, allele/genotype frequencies of SNPs rs2275737 and rs1342387 differed significantly between subjects with type 2 diabetes *vs*. those with normal glucose tolerance. However, there were no significant associations between *ADIPOR1* SNPs and type 2 diabetes, insulin sensitivity or insulin secretion in northern Europeans, African Americans [28] or a Japanese population [29]. In contrast, a recent study in a European population demonstrated significant association between several SNPs (including the two we examined, rs666089 and rs1342387) and insulin sensitivity/liver fat [31].

In conclusion, this study demonstrates that polymorphisms in *ADIPOR1*, *ADIPOR2* and *ADIPOQ* are associated with several IRS-related phenotypes in Mexican Americans, including plasma triglyceride levels, SS, BMI and waist circumference. Of particular note, multiple genetic variants in *ADIPOR2* were strongly associated with decreased triglyceride levels. Our findings of multiple strong associations between *ADIPOR2* polymorphisms and plasma triglyceride concentrations may have important implications for atherogenesis and/or dyslipidaemia, owing to the potential influence of *ADIPOR2* genetic variation on triglyceride-rich lipoprotein metabolism.

Acknowledgements We thank the participants of SAFDS and are grateful for their participation and cooperation.

Duality of interest We do not have any conflicts of interest with respect to the study.

References

1. DeFronzo RA (1997) Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. Diabetes Rev 5:177–269
2. Bays H, Mandarino L, DeFronzo RA (2004) Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. J Clin Endocrinol Metab 89:463–478
3. Reaven GM (1995) Pathophysiology of insulin resistance in human disease. Physiol Rev 75:473–486
4. DeFronzo RA (1997) Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis. Neth J Med 50:191–197
5. Groop LC, Bonadonna RC, Del Prato S, Ratheiser K, Zych K, DeFronzo RA (1989) Effect of insulin on oxidative and non-

oxidative pathways of glucose and FFA metabolism in NIDDM. Evidence for multiple sites of insulin resistance. J Clin Invest 84:205–213
6. Bergman RN, Finegood DT, Kahn SE (2002) The evolution of beta-cell dysfunction and insulin resistance in type 2 diabetes. Eur J Clin Invest 32:35–45
7. Arner P (2001) Free fatty acids—do they play a role in insulin resistance? Diabetes Obes Metab 3:11–19
8. Groop L, Bonadonna R, Simonson DC, Petrides A, Hasan S, DeFronzo RA (1992) Effect of insulin on oxidative and non-oxidative pathways of glucose and free fatty acid metabolism in human obesity. Am J Physiol 263:79–84
9. McGarry JD (2001) Banting Lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes 51:7–18
10. Duggirala R, Blangero J, Almasy L et al (2001) A major locus for fasting insulin concentrations and insulin resistance on chromosome 6q with strong pleiotropic effects on obesity-related phenotypes in nondiabetic Mexican Americans. Am J Hum Genet 68:1149–1164
11. Duggirala R, Blangero J, Almasy L et al (2000) A major susceptibility locus influencing plasma triglyceride concentrations is located on chromosome 15q in Mexican Americans. Am J Hum Genet 66:1237–1245
12. Elbein SC (2002) Perspective: the search for genes for type 2 diabetes in the post-genome era. Endocrinology 43:2012–2018
13. McCarthy M (2003) Growing evidence for diabetes susceptibility genes from genome scan data. Curr Diab Rep 3:159–167
14. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (Adipose Most abundant Gene transcript 1). Biochem Biophys Res Commun 221:286–289
15. Berg AH, Combs TP, Scherer PE (2002) ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 13:84–89
16. Weyer C, Funahashi T, Tanaka S et al (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 86:1930–1935
17. Bajaj M, Suraamornkul S, Piper P et al (2004) Decreased plasma adiponectin concentrations are closely related to hepatic fat content and hepatic insulin resistance in pioglitazone-treated type 2 diabetic patients. J Clin Endocrinol Metab 89:200–206
18. Heilbronn LK, Smith SR, Ravussin E (2003) The insulin-sensitizing role of the fat derived hormone adiponectin. Curr Pharm Des 9:1411–1418
19. Yamauchi T, Kamon J, Waki H et al (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7:941–946
20. Yamauchi T, Kamon J, Ito Y et al (2003) Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423:762–769
21. Civitarese AE, Jenkinson CP, Richardson DK et al (2004) Adiponectin receptors gene expression and insulin sensitivity in nondiabetic Mexican Americans with or without a family history of Type 2 diabetes. Diabetologia 47:816–820
22. Gil-Campos M, Canete RR, Gil A (2004) Adiponectin, the missing link in insulin resistance and obesity. Clin Nutr 23:963–974
23. Yamauchi T, Kamon J, Minokoshi Y et al (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8:1288–1295
24. Nakatani K, Noma K, Nishioka J et al (2005) Adiponectin gene variation associates with the increasing risk of type 2 diabetes in nondiabetic Japanese subjects. Int J Mol Med 15:173–177
25. Gu HF, Abulaiti A, Ostenson CG et al (2004) Single nucleotide polymorphisms in the proximal promoter region of the adiponectin (APM1) gene are associated with type 2 diabetes in Swedish Caucasians. Diabetes 53:31–S35
26. Zacharova J, Chiasson JL, Laakso M; STOP-NIDDM Study Group (2005) The common polymorphisms (single nucleotide polymorphism [SNP] +45 and SNP +276) of the adiponectin gene predict the conversion from impaired glucose tolerance to type 2 diabetes: the STOP-NIDDM trial. Diabetes 54: 893–899
27. Vozarova de Courten B, Hanson RL, Funahashi T et al (2005) Common polymorphisms in the adiponectin gene *ACDC* are not associated with diabetes in Pima Indians. Diabetes 54:284–289
28. Wang H, Zhang H, Jia Y et al (2004) Adiponectin receptor 1 gene (*ADIPOR1*) as a candidate for type 2 diabetes and insulin resistance. Diabetes 53:2132–2136
29. Hara K, Horikoshi M, Kitazato H et al (2005) Absence of an association between the polymorphisms in the genes encoding adiponectin receptors and type 2 diabetes. Diabetologia 48:1307–1314
30. Damcott CM, Ott SH, Pollin TI et al (2005) Genetic variation in adiponectin receptor 1 and adiponectin receptor 2 is associated with type 2 diabetes in the Old Order Amish. Diabetes 54: 2245–2250
31. Stefan N, Machicao F, Staiger H et al (2005) Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. Diabetologia 48: 2282–2291
32. Stern MP, Gaskill SP, Hazuda HP, Gardner LI, Haffner SM (1983) Does obesity explain excess prevalence of diabetes among Mexican Americans? Results of the San Antonio Heart Study. Diabetologia 24:272–277
33. Stern MP, Mitchell BD, Blangero J et al (1996) Evidence for a major gene for type II diabetes and linkage analyses with selected candidate genes in Mexican-Americans. Diabetes 45:563–568
34. Stern MP, Rosenthal M, Haffner SM, Hazuda HP, Franco LJ (1984) Sex difference in the effects of sociocultural status on diabetes and cardiovascular risk factors in Mexican Americans. The San Antonio Heart Study. Am J Epidemiol 120:834–851
35. Almasy L, Blangero J (1998) Multipoint quantitative trait linkage analysis in general pedigrees. Am J Hum Genet 62:1198–1211
36. Abecasis GR, Cookson WOC, Cardon LR (2000) Pedigree tests of transmission disequilibrium. Eur J Hum Genet 8:545–551
37. Boerwinkle E, Chakraborty R, Sing CF (1986) The use of measured genotype information in the analysis of quantitative phenotypes in man. Ann Hum Genet 50:181–194
38. Almasy L, Blangero J (2004) Exploring positional candidate genes: linkage conditional on measured genotype. Behav Genet 34:173–177
39. Long AD, Lyman RF, Langley CH, Mackay TF (1999) Two sites in the *Delta* gene region contribute to naturally occurring variation in bristle number in *Drosophila melanogaster*. Genetics 149:999–1017
40. Phillips PC (1999) From complex traits to complex alleles. Trends Genet 15:6–8
41. Blangero J, Goring HH, Kent JW Jr et al (2005) Quantitative trait nucleotide analysis using Bayesian model selection. Hum Biol 77:541–559
42. Curran JE, Jowett JB, Elliott KS et al (2005) Genetic variation in selenoprotein S influences inflammatory response. Nat Genet 37:1234–1241
43. Almasy L, Blangero J (2004) Exploring positional candidate genes: linkage conditional on measured genotype. Behav Genet 34:173–177

44. Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L (2001) Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest 108:1875–1881
45. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 7:947–953
46. Trujillo ME, Scherer PE (2005) Adiponectin—journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. J Intern Med 257:167–175
47. Chan DC, Watts GF, Ng TW et al (2005) Adiponectin and other adipocytokines as predictors of markers of triglyceride-rich lipoprotein metabolism. Clin Chem 51:578–585
48. Hamsten A, Silveira A, Boquist S et al (2005) The apolipoprotein CI content of triglyceride-rich lipoproteins independently predicts early atherosclerosis in healthy middle-aged men. J Am Coll Cardiol 45:1013–1017
49. Staiger H, Kaltenbach S, Staiger K et al (2004) Expression of adiponectin receptor mRNA in human skeletal muscle cells is related to in vivo parameters of glucose and lipid metabolism. Diabetes 53:2195–2201
50. Populaire C, Mori Y, Dina C et al (2003) Does the −11377 promoter variant of *APM1* gene contribute to the genetic risk for Type 2 diabetes mellitus in Japanese families? Diabetologia 46:443–445