ARTICLE

The adiponectin gene is associated with adiponectin levels but not with characteristics of the insulin resistance syndrome in healthy Caucasians

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Low concentrations of adiponectin, the protein product of the *APM1* gene, have been reported to be associated with obesity and insulin resistance. However, contrasting results have been described on the genetic variability in *APM1* and characteristics of the metabolic syndrome and adiponectin serum concentrations. In the present study, we investigated the association of the two most well-known SNPs of *APM1* (+45T>G and +276G>T) and their haplotypes, with serum adiponectin concentrations, metabolic parameters and intima-media thickness of the carotid arteries in 1745 well-phenotyped asymptomatic unrelated Caucasian subjects of the SAPHIR cohort. The common T-allele (88.5%) of SNP +45T>G and the common G-allele (70.5%) of SNP +276G>T were associated with significantly lower serum adiponectin levels ($P=0.0008$ and $P=0.00005$, respectively). The most frequent haplotype TG (59.0%) defined by both loci showed a strong association with lower serum adiponectin concentrations ($P=0.000000002$). A clear effect per copy of the respective haplotype was observed. This association was most pronounced in lean and insulin-sensitive subjects. The two less common haplotypes TT (29.5%) and GG (11.5%) were associated with higher serum adiponectin levels in a dose-dependent association. Interestingly, no significant association between the adiponectin 45–276 haplotypes and the majority of parameters of the metabolic syndrome or intima-media thickness of the carotid arteries was found in our study. In summary, we replicated a strong association of the adiponectin 45–276 genotypes and haplotypes with adiponectin levels in healthy Caucasians. However, we could not confirm an association of this gene locus with metabolic parameters of the insulin resistance syndrome.

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Introduction

Recent studies point to the adipose tissue as a highly active endocrine organ secreting a range of hormones. There is great interest in clarifying the role of adipose tissue-derived peptides (adipokines) as possible mediators between body fat distribution and insulin sensitivity.

Adiponectin, the major adipocyte secretory protein, has been shown to improve insulin sensitivity and to possess anti-inflammatory and antiatherosclerotic properties.^{1–8} Hypoadiponectinaemia has been found to be associated with insulin resistance,^{9,10} obesity^{4,11} and other features of the metabolic syndrome,^{12–14} as well as type 2 diabetes^{15,16} and cardiovascular disease.^{5–8} In patients with anorexia nervosa¹⁷ and chronic renal failure,^{10,18} plasma adiponectin levels are increased. Weight loss and therapy with thiazolidinediones enhance endogenous adiponectin production in humans.¹⁹

Two common nucleotide changes in the adiponectin gene, SNPs +45T>G and +276G>T, have been shown to be associated with obesity and insulin sensitivity,^{12,20} type 2 diabetes,^{21–23} and coronary artery disease in type 2 diabetic patients,^{24–26} and serum adiponectin levels.^{12,14,21,22,26–31} A haplotype of these two SNPs showed even higher associations with obesity and other components of the metabolic syndrome.¹² However, the data are inconsistent and many of the described associations above could not be confirmed in other studies.^{32–36} Most studies investigated the association between the *APM1* gene and adiponectin levels in diabetic or obese subjects. Only few studies focused on healthy individuals from the general population.

In the present study, we investigated the association of adiponectin 45–276 genotypes and haplotypes with serum adiponectin concentrations and metabolic parameters of the insulin resistance syndrome, in a large ethnically homogeneous and well-phenotyped healthy Austrian population.

Subjects and methods

Study participants and clinical investigations

A total of 1745 subjects were included in the present study. The cohort consisted of 1098 male subjects aged between 39 and 66 years and 647 female subjects aged between 47 and 67 years, who were participants of the 'Salzburg Atherosclerosis Prevention program in subjects at High Individual Risk' (SAPHIR), a prospective study investigating the role of various genetic and metabolic factors for progression of atherosclerotic vascular disease.³⁷ All individuals were of Caucasian origin and the population was homogeneous with regard to ethnic background. Subjects with established coronary artery, cerebrovascular or peripheral arterial disease, congestive heart failure, valvular heart disease, chronic alcohol (more than three drinks a day) or drug abuse, morbid obesity (BMI > 40 kg/m²) and pregnant women were excluded. At baseline all study participants were subjected to a thorough screening program that included assessment of a detailed personal and family history, physical examination, determination of anthropometric indices and measurement of various biochemical parameters and several other procedures,

including measurement of the intima-media thickness (IMT) of the carotid arteries by B-mode ultrasound. Subjects were classified as type 2 diabetics if they were on hypoglycemic medications or when their fasting glucose concentrations exceeded 126 mg/dL. Informed consent was obtained from each participant and the study was carried out in accordance with the local ethics committee.

IMT of carotid arteries was measured by high-resolution B-mode ultrasound (HDI 3000 CV from ATL, Munich, Germany) according to the protocol published by the ACAPS' investigators.³⁸ All measurements were performed by the same ultrasound operator. Additionally, a B-score describing morphological alterations of the carotid arteries was formed as follows: 0, no alterations; 1, wall thickness > 1 mm; 2, plaque < 2 mm; 3, plaque 2–3 mm; 4, plaque > 3 mm; 5, total occlusion of the lumen.

A 24 h ambulatory blood pressure measurement was performed using the TM 2430 PC monitoring system from BOSO (Bosch + Sohn, Jungingen, Germany). Body composition was determined by body impedance analysis (BIA 2000-S, Data Input, Darmstadt, Germany).

Laboratory measurements

Venous blood was collected after an overnight fast, and plasma and serum samples were either used immediately for analysis or were stored frozen at –80°C. Total serum cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, Lp(a), apolipoprotein A-I, apolipoprotein B, urinary albumin/creatinine ratio, HbA1c and fasting glucose were determined using commercially available assays (Hoffmann-LaRoche GmbH, Vienna, Austria). Fasting insulin was measured using the IMX insulin assay (Abbot, Vienna, Austria). Insulin sensitivity was determined using the short insulin tolerance test (k-ITT). Insulin sensitivity was also estimated by the homeostasis model assessment (HOMA-IR) indices calculated using the formula: fasting blood glucose (mg/dl) × fasting insulin (μU/ml)/405. Glucose tolerance was evaluated according to the 1985 World Health Organization criteria (oral glucose tolerance test, OGTT). Serum adiponectin levels were measured using the human adiponectin ELISA kit (BioCat GmbH, Heidelberg, Germany). Subjects on lipid-lowering drugs were excluded from statistical analysis of lipids.

Genetic analysis

Genomic DNA was isolated from white blood cells, using Puregen DNA purification kit (Gentra Systems, MN, USA) and stored at –20°C. Genotyping was performed with two different methods in the entire cohort. First, for the SNP +45T>G, a 305-bp DNA fragment containing the polymorphic site was amplified by polymerase chain reaction (PCR) using forward primer 5'-TGT GTG TGT GGG GTC TGT CT-3' and reverse primer 5'-TGT GAT GAA AGA GGC CAG AA-3'. The annealing temperature was 62°C. After digestion with the restriction enzyme Ava I (incubation

temperature 37°C), fragments were separated on 2% agarose gels. For the SNP +276G>T, a 107-bp DNA fragment containing the polymorphic site was amplified by PCR using forward primer 5'-CTA CAC TGA TAT AAA CTA TAT GGA G-3' and reverse primer 5'-CCC CAA ATC ACT TCA GGT TG-3'. The annealing temperature was 55°C. After digestion with the restriction enzyme Hinf I (incubation temperature 37°C), fragments were separated on 15% polyacrylamide gels. Bands were visualized by ethidium bromide staining. Second, genotypes of both SNPs were re-evaluated using MALDI-TOF-based mass spectrometry of allele specific primer extension products (Mass Array™, Sequenom, San Diego, CA, USA) as described previously.³⁹ These two methods showed no major differences. Additionally, 15% of the samples were genotyped in duplicate for quality control, using MALDI-TOF-based mass spectrometry. Altogether, we found 13 discordant genotypes in 4908 duplicates of recent genotyping projects leading to a discordance rate of 0.26%. Moreover, 2% of negative and positive controls are added routinely.

Statistical analysis

Haplotypes were statistically reconstructed using the EM-algorithm implemented in PROC HAPLOTYPE by SAS®. Hardy–Weinberg equilibrium was tested using the χ^2 test. The association of the genotypes and the haplotypes with adiponectin concentrations was estimated using a general linear regression model. Further, we estimated and tested the change in adiponectin concentrations per copy of one allele/haplotype. Adiponectin concentrations were analysed on a transformed scale ($\log(\text{adiponectin} + 1)$) in order to obtain a normally distributed variable. A trend per copy of the allele was ascertained by viewing the mean adiponectin concentrations in three groups: subjects with two, one or zero copies of the rare allele or haplotype. All analyses were adjusted for gender, age and BMI. Two-tailed tests were performed with the significance level set at 0.05.

The association between haplotypes and serum adiponectin concentrations was also evaluated in four clinical subgroups: (1) L-IS - lean and insulin sensitive (BMI < median, k-ITT $\geq$ median, $n = 532$), (2) L-IR - lean and insulin resistant (BMI < median, k-ITT < median, $n = 335$), (3) O-IS - obese and insulin sensitive (BMI $\geq$ median, k-ITT $\geq$ median, $n = 318$), (4) O-IR - obese and insulin resistant (BMI $\geq$ median, k-ITT < median, $n = 560$).

In a secondary analysis, the parameters BMI, waist, fat mass, HbA1c, k-ITT values, total cholesterol, HDL and LDL cholesterol, triglycerides, apolipoprotein A-I, apolipoprotein B, Lp(a), systolic and diastolic blood pressure, IMT of the common carotid artery (CCA) and the internal carotid artery (ICA), the albumin–creatinine ratio, HOMA-IR, fasting glucose and fasting insulin concentrations as well as the OGTT parameters (glucose and insulin concentrations 1/2, 1 and 2 h after OGTT) were tested using a

general linear regression model with regard to their association with the APM1 +45 and +276 loci. Adiponectin concentrations, all insulin concentrations, triglycerides, Lp(a), the albumin–creatinine ratio and the HOMA-IR index were logarithmically transformed in order to more closely fulfill the requirements of a normal distribution.

Results

Clinical characteristics of 1098 male and 647 female participants of the SAPHIR study are summarized in Table 1. The proportion of subjects with type 2 diabetes was 3.8% among males and 2.3% among females.

In Table 2, frequencies of genotypes and haplotypes are summarized. The rare allele G of the APM1 +45 locus showed a minor allele frequency (MAF) of 11.5%. The rare allele T of the locus +276 had an MAF of 29.5%. Lewontin's D' of these two loci was unity; the correlation between the alleles was 0.23 with the minor G allele of the +45 locus being correlated with the major G allele of the +276 locus. The TG haplotype was the most common haplotype, with a frequency of 59%, among the chromosomes. The other two haplotypes in this sample were the TT (29.5%) and the GG (11.5%).

The table further states the mean (95% confidence intervals) serum adiponectin concentrations per genotype/haplotype. These rescaled means were derived via a general linear model adjusting for age and sex performed on $\log(\text{adiponectin} + 1)$. The P -values testing for association of a single nucleotide polymorphism (SNP) locus or for association of a haplotype with adiponectin concentrations without and with assuming a trend per copy of the allele/haplotype are given. It can be seen that there is a highly significant association of both the +45 and the +276 locus ($P = 0.002$ and 0.0002 without assuming a

Table 1 Clinical characteristics of the SAPHIR study population

	Males ($n = 1098$)	Females ($n = 647$)
Age (years)	49.2 $\pm$ 5.5	56.2 $\pm$ 4.3
BMI (kg/m ²)	26.92 $\pm$ 3.72	26.59 $\pm$ 4.74
Smokers (%)	22.9	13.3
Diabetes (%)	3.8	2.3
HbA1c (%)	5.60 $\pm$ 0.59	5.66 $\pm$ 0.46
Fasting glucose (mg/dl)	94.4 $\pm$ 18.3	92.1 $\pm$ 17.1
Fasting insulin (μ U/ml)	7.57 $\pm$ 5.56	7.23 $\pm$ 4.27
Total cholesterol (mg/dl)	226 $\pm$ 40	234 $\pm$ 41
LDL cholesterol (mg/dl)	146 $\pm$ 36	145 $\pm$ 38
HDL cholesterol (mg/dl)	55.2 $\pm$ 13.4	67.1 $\pm$ 16.4
Triglycerides (mg/dl)	137 $\pm$ 100	106 $\pm$ 53
SBP (mmHg)	135 $\pm$ 13	131 $\pm$ 13
DBP (mmHg)	82 $\pm$ 8	81 $\pm$ 8

The values are given as mean $\pm$ standard deviation. For the dichotomously coded smokers and diabetics, percentages are given.

Table 2 Association of the +45T>G and +276G>T polymorphisms with adiponectin concentrations

	% (n)	n	Serum adiponectin ($\mu\text{g/ml}$) mean (95% CI)	Overall	P-values Trend per copy
SNP+45T> G					
T/T	77.88 (1359)	1349	7.52 (7.34, 7.71)	0.002	0.0008
T/G	21.20 (370)	368	8.07 (7.70, 8.45)		
G/G	0.92 (16)	16	10.05 (8.07, 12.47)		
Minor allele frequency = 11.5%					
SNP +276G> T					
G/G	50.60 (883)	876	7.37 (7.15, 7.60)	0.0002	0.00005
G/T	39.83 (695)	693	7.82 (7.56, 8.09)		
T/T	9.57 (167)	164	8.54 (7.97, 9.15)		
Minor allele frequency = 29.5%					
TG Haplotype					
0 copy	16.62 (290)	287	8.46 (8.03, 8.91)	0.00000001	0.000000002
1 copy	48.77 (851)	847	7.85 (7.62, 8.10)		
2 copies	34.61 (604)	599	7.04 (6.78, 7.30)		
Haplotype frequency = 59.0%					
GG haplotype					
0 copy	77.88 (1359)	1349	7.52 (7.34, 7.71)	0.002	0.0008
1 copy	21.20 (370)	368	8.07 (7.70, 8.45)		
2 copies	0.92 (16)	16	10.05 (8.07, 12.47)		
Haplotype frequency = 11.5%					
TT Haplotype					
0 copy	50.60 (883)	876	7.37 (7.15, 7.60)	0.0002	0.00005
1 copy	39.83 (695)	693	7.82 (7.56, 8.09)		
2 copies	9.57 (167)	164	8.54 (7.97, 9.15)		
Haplotype frequency = 29.5%					

The analysis was performed on the normally distributed transformed variable $x = \log(\text{adiponectin} + 1)$ and adjusted for age and gender, using a general linear model. Values given are genotype/haplotype frequencies, including the number of subjects, re-transformed age- and sex-adjusted mean adiponectin levels including 95% confidence intervals (ie $\exp(\text{mean}(x)) - 1$), and — for each SNP and each haplotype — *P*-values testing for overall association and a *P*-value testing for a trend per copy of the allele/haplotype.

trend per copy) with adiponectin levels. A clear multiplicative trend of increasing adiponectin levels per copy of the rare allele can be observed (7.52, 8.07, 10.05 for the T/T, the T/G and the G/G genotype in the +45 locus, *P*-values for testing for trend = 0.0008, and 7.37, 7.82, 8.54 for the G/G, G/T and the T/T genotype in the +276 locus, *P*-value for testing for trend = 0.00005). The highest association was observed when analyzing both SNPs together; the haplotype with the major allele in both loci (TG) was most highly associated with decreased adiponectin concentrations showing a clear trend per copy (8.46, 7.85 and 7.04 for zero, one and two copies of TG, $P = 0.00000001$ without trend and $P = 0.000000002$ with trend). The other two haplotypes (TT and GG) with one major and one minor allele were associated with increased adiponectin levels. The haplotype with both minor alleles (GT) did not occur in this sample.

All analyses were adjusted for age and sex. Adjustment for age did neither change mean adiponectin levels nor the gene effect. Adjustment for sex did affect the mean values as adiponectin levels differ between male and female subjects. However, the gene effect did not depend upon

sex, and thus the genotype/haplotype associations were not stated separately for male and female subjects. Adjustment for BMI did slightly reduce the strength of the association and the difference of the mean adiponectin levels between the genotype/haplotype groups, but the effect of adjusting for BMI was relatively small compared to the strong gene effect of adiponectin (*P*-value reaching 0.000000002 with a trend per copy).

We also evaluated association of the TG haplotype with serum adiponectin levels in four subgroups: (1) L-IS, lean insulin-sensitive subjects, (2) L-IR, lean insulin-resistant, (3) O-IS, obese insulin-sensitive, (4) O-IR, obese insulin-resistant individuals (Table 3). The association of the TG haplotype with decreasing adiponectin concentrations was most pronounced in the lean insulin-sensitive subjects ($P \leq 0.0001$ assuming a trend per copy). The association was weaker in the lean insulin-resistant subjects ($P = 0.005$), and it was the least obvious in the obese insulin-sensitive subjects ($P = 0.04$) and in the obese insulin-resistant subjects ($P = 0.03$). It can clearly be seen that the differences in adiponectin levels between the homozygous common haplotype and the homozygous rare haplotype,

Table 3 Association of the 45–276 haplotype and adiponectin concentrations in BMI + k-ITT categorized subgroups

Groups	Lean		Obese	
	Insulin sensitive N = 532	Insulin resistant N = 335	Insulin sensitive N = 318	Insulin resistant N = 560
<i>Mean values and 95% confidence interval^a</i>				
BMI (kg/m ²)	23.7 (23.4, 23.9)	23.7 (23.4, 24.0)	29.1 (28.7, 29.4)	30.3 (30.1, 30.6)
k-ITT (%/min)	5.30 (5.24, 5.37)	3.31 (3.23, 3.40)	5.05 (4.96, 5.13)	2.97 (2.91, 3.04)
Adiponectin (μg/ml)	8.59 (8.28, 8.92)	8.15 (7.77, 8.54)	7.66 (7.29, 8.04)	6.60 (6.36, 6.86)
<i>Haplotype frequency:</i>				
TG/TG n (%)	183 (34.4)	125 (37.3)	108 (34.0)	188 (33.6)
TG/X n (%)	258 (48.5)	153 (45.7)	153 (48.1)	287 (51.2)
X/X n (%)	91 (17.1)	57 (17.0)	57 (17.9)	85 (15.2)
<i>Mean serum adiponectin^a by TG haplotype group</i>				
TG/TG	8.01 (7.51, 8.54)	7.89 (7.23, 8.59)	7.50 (6.90, 8.13)	6.61 (6.20, 7.04)
TG/X	9.45 (8.95, 9.98)	8.92 (8.26, 9.65)	8.41 (7.85, 9.00)	7.00 (6.65, 7.37)
X/X	10.33 (9.46, 11.28)	9.64 (8.50, 10.92)	8.51 (7.62, 9.50)	7.44 (6.79, 8.15)
<i>P-value without trend</i>	<i>P</i> ≤ 0.0001	<i>P</i> = 0.02	<i>P</i> = 0.06	<i>P</i> = 0.10
<i>P-value with trend per TG copy</i>	<i>P</i> ≤ 0.0001	<i>P</i> = 0.005	<i>P</i> = 0.04	<i>P</i> = 0.03

L-IS - lean insulin sensitive. (BMI < median, k-ITT ≥ median); L-IR - lean insulin resistant (BMI < median, k-ITT < median); O-IS - obese insulin-sensitive (BMI ≥ median, k-ITT ≥ median); O-IR - obese insulin-resistant (BMI ≥ median, k-ITT < median). All median are agegroup- and sex-specific.

X - denotes any non-TG haplotype.

^aMeans are adjusted for 5-year-age groups and sex, using a general linear model.

in each of these subgroups became smaller with insulin resistance and obesity (ranging from 2.3 μg/ml in the lean insulin-sensitive individuals to about 0.8 μg/ml in the obese insulin-resistant individuals). In the full group using the general linear model as before and including an interaction of the TG haplotype effect with obesity (BMI > median) or insulin resistance (k-ITT < median), the interaction term was highly significant with *P* < 0.0001. This further underscores the effect modification of the genotype impact on adiponectin concentrations by obesity and insulin resistance.

In a secondary analysis, a series of biochemical and clinical parameters (BMI, waist, fat mass, HbA1c, k-ITT values, total, HDL and LDL cholesterol, triglycerides, apolipoprotein A-I, apolipoprotein B, Lp(a), systolic and diastolic blood pressure, IMT-CCA, IMT-ICA, the albumin-creatinine ratio, HOMA-IR, fasting glucose and fasting insulin concentrations as well as glucose and insulin concentrations 1/2, 1 and 2 h after OGTT) were analyzed with respect to their association with the TG haplotype (Table 4). Only apolipoprotein A-I and the albumin-creatinine ratio showed a *P*-value of 0.03, which cannot be deemed significant in the light of the multiple comparisons made (27 parameters tested). IMT of carotid arteries measured by B-mode ultrasound did not show significant differences or even trends in individuals with various 45–276 haplotypes. There was a tendency towards increased glucose concentrations after oral glucose tolerance test (OGTT) with each copy of the TG haplotype (Table 4). Furthermore, the detectable effect

size with 80% power for the given sample size is stated in Table 4.

Discussion

Several susceptibility loci for type 2 diabetes have been identified by genome wide linkage scans.⁴⁰ Among these regions, 3q27 appears to be of special interest, because it harbours the *APM1*-gene-encoding adiponectin, which is known to modulate insulin sensitivity and glucose homeostasis. A series of SNPs in the adiponectin gene have been reported. Two common SNPs, a silent T > G substitution in exon 2 (+45T > G) and a G > T substitution in intron 2 (+276G > T), have been the subject of interest in several investigations.^{12,14,20-22,26-31,33,41} From these studies, it was concluded that both SNPs are related to obesity, insulin resistance and type 2 diabetes. Even stronger associations with traits of the insulin resistance syndrome were reported when the two SNPs were considered together as haplotypes. The haplotype TG defined by these two SNPs has been found to be associated with higher plasma insulin and lower adiponectin levels in healthy individuals.¹² How these two SNPs and the respective haplotypes are involved in the observed association has to be determined. It is conceivable that the two SNPs are in strong linkage disequilibrium with other SNPs that might by themselves explain the observed association with adiponectin concentrations. It is interesting to note that the TG haplotype is in almost complete linkage disequilibrium with an insertion/deletion polymorphism in the 3' UTR (position 2019).¹² As the 3' UTR region plays a

Table 4 Association of clinical and metabolic characteristics of the SAPHIR study population with adiponectin 45–276 haplotype TG (X denoting any other haplotypes): stated are mean values and standard deviations

	Haplotype 45–276				Detectable effect with 80% power ^b
	X/X (n = 290)	TG/X (n = 851)	TG/TG (n = 604)	P-value ^a	
Full sample (n = 1745)					
BMI (kg/m ²)	26.7 (4.3)	26.7 (3.8)	26.9 (4.4)	0.65	0.6
Waist (cm)	94.5 (13.1)	94.7 (11.8)	94.6 (13.2)	0.82	1.8
Fat mass (kg)	19.3 (9.5)	18.7 (8.7)	19.5 (9.33)	0.69	1.3
HbA1c (%)	5.60 (1.10)	5.60 (1.08)	5.61 (1.08)	0.96	0.08
k-ITT (%/min)	4.22 (1.34)	4.14 (1.34)	4.16 (1.31)	0.59	0.2
Total cholesterol (mg/dl)	226 (42)	229 (39.4)	230 (41)	0.66	6
LDL-cholesterol (mg/dl)	144 (39)	146 (36)	146 (37)	0.74	5
HDL-cholesterol (mg/dl)	59.2 (16.7)	59.1 (15.3)	60.5 (15.6)	0.30	2.2
Triglycerides (mg/dl)	105 (1.8)	108 (1.7)	104 (1.7)	0.30	8
Apolipoprotein A-I (mg/dl)	154 (28)	155 (26)	158 (28)	0.03	4
Apolipoprotein B (mg/dl)	113 (26)	115 (24)	115 (25)	0.57	4
Lp(a) (mg/dl)	17.8 (3.0)	17.2 (2.9)	18.0 (3.0)	0.76	2.8
SBP 24 h (mmHg)	133 (13)	134 (13)	134 (13)	0.51	2
DBP 24 h (mmHg)	81.3 (8.3)	82.1 (7.9)	82.0 (7.5)	0.31	1
IMT-CCA (mm)	0.76 (0.13)	0.76 (0.13)	0.77 (0.12)	0.14	0.02
IMT-ICA (mm)	0.83 (0.14)	0.82 (0.13)	0.83 (0.13)	0.34	0.02
Albumin/creatinine	0.54 (3.12)	0.56 (3.07)	0.49 (2.89)	0.03	0.07
HOMA-IR	1.45 (1.94)	1.42 (1.90)	1.42 (1.91)	0.44	0.13
Fasting glucose (mg/dl)	93.0 (19.7)	93.5 (17.5)	93.8 (17.4)	0.98	2.5
Fasting insulin (μU/ml)	6.42 (1.79)	6.26 (1.78)	6.17 (1.77)	0.15	0.54
Subsample with OGTT (n = 651)					
30 min BG (mg/dl)	150 (33)	153 (36)	157 (36)	0.15	8
60 min BG (mg/dl)	135 (44)	142 (54)	145 (50)	0.20	12
120 min BG (mg/dl)	97 (32)	102 (39)	105 (39)	0.10	9
30 min insulin (μU/ml)	60.4 (2.1)	57.0 (1.9)	53.5 (1.8)	0.25	7.5
60 min insulin (μU/ml)	68.1 (2.2)	66.0 (2.1)	67.5 (2.0)	0.86	11.0
120 min insulin (μU/ml)	32.6 (2.5)	30.9 (2.5)	35.1 (2.3)	0.78	7.6

To comply with normality assumption, the following variables were log-transformed: all insulin measures, HbA1c, TG, HOMA-IR, albumin/creatinine and Lp(a); for these, the geometric mean and geometric standard deviation are given to report on the original scale.

IMT-CCA=intima media thickness of the common carotid arteries; IMT-ICA=intima media thickness of the internal carotid arteries; HOMA=homeostasis model assessment for insulin resistance; k-ITT=short insulin tolerance test; BG=blood glucose concentration; OGTT=oral glucose tolerance test.

^aP-values were from generalized linear regression adjusted for age, sex and BMI (no adjustment for BMI for the phenotypes BMI, waist, fat mass), no adjustment for multiple testing.

^bFor the log-transformed variables, the effect size δ is computed on the log-scale and retransformed by reporting the average between $\exp(\text{mean}+\delta)$ - $\exp(\text{mean})$ and $\exp(\text{mean}-\delta)$ - $\exp(\text{mean})$, where 'mean' is the mean of the variable on the log-scale.

key role in the control of gene expression by binding proteins that regulate mRNA processing, translation or degradation, it could have a strong influence on the investigated protein and related diseases.⁴²

Haplotype data from 1745 individuals were available for analysis in our study. The most important finding of our study is the highly demanded replication of an association between the adiponectin +45T allele and the +276G allele as well as the 45–276 haplotype TG with significantly lower serum adiponectin levels. It is the highest association for adiponectin levels ever reported, and it is gene-dose-dependent. Two important points have to be discussed in this context.

Genetics of adiponectin levels in a healthy population

We consider it as a major strength of the SAPHIR study, that it consists of quite a healthy working population and

excluded by definition individuals such as those with established atherosclerotic events, congestive heart failure or morbid obesity. Therefore, associations observed between the *APM1* gene and adiponectin levels as observed in our study can be considered not simply as a companion of a disturbed metabolism of such patients but can already be observed long before the development of such diseases. In order to study in more detail, the association between the TG haplotype and insulin sensitivity, we analysed the impact of the TG haplotype in four subgroups, according to BMI as well as k-ITT values as a measure of insulin sensitivity. The association between the 45–276 haplotype and serum adiponectin levels was most pronounced in the subgroup of lean insulin-sensitive individuals ($P < 0.0001$). This is supported by a significant interaction of the TG haplotype with obesity and insulin resistance. One possible explanation for this finding might be that the effect of the

adiponectin gene locus on serum adiponectin levels is partially over-ruled by environmental factors such as diet-induced obesity. The mentioned subgroup analysis might even explain why we found the strongest associations between genetic variability of these two loci and adiponectin levels, ever reported and which might be strongly related to the generally quite healthy condition of the entire group investigated. In this context, it appears of interest that Filippi *et al*³² recently reported an association between the adiponectin 276G>T genotype and insulin resistance determined by HOMA-IR in a study of 253 nondiabetic subjects. This association was only found in the group of participants with a BMI below the median of 26.2 kg/m² with a relative risk of 9.7 and a wide 95% confidence interval (1.32–87.7).

APM1 and aspects of the metabolic syndrome

Data from Menzaghi *et al*¹² in 413 nondiabetic individuals suggested that the TG haplotype is strongly associated with insulin resistance parameters. Although we observed a strong association between genetic variations in the APM1 gene and adiponectin levels, we could not detect an association between the 45–276 TG haplotype and numerous metabolic parameters related to insulin resistance. Several circumstances might have contributed to this observation. First, there might be several genes, which have an influence on adiponectin levels and the APM1 gene is only one gene regulating adiponectin concentrations. Second, metabolic parameters of the insulin resistance syndrome are not only determined by serum adiponectin levels, but are influenced by other multiple genetic and environmental factors. Therefore, genetic variations within one gene, explaining a particular amount of an intermediate phenotype such as adiponectin levels, will only be responsible for a very small fraction of end-phenotypes of the metabolic syndrome. To detect this small fraction will need an enormous number of study participants. Power calculations, however, revealed that our study would have been able to detect relatively small and clinically relevant differences between the various haplotypes as shown in Table 4. Third, as mentioned above, the SAPHIR Study consists of a relatively healthy and young study population (average age of 49 and 56 years for men and women, respectively) with a frequency of diabetes mellitus of 3.8% and 2.3% for male and female subjects, respectively. Therefore, less marked differences in the variability of the metabolic parameters could be an explanation. We have to bear in mind, that probably a large segment of this population, quite young and healthy at the time of recruitment, has still the potential to develop characteristic features of the metabolic syndrome. A recently published study by Daimon *et al*¹⁶ identified a lower serum adiponectin level as an independent risk factor for progression to type 2 diabetes. During a 5-year follow-up period, subjects with

serum adiponectin concentrations in the lowest tertile (<6.46 µg/ml) had an almost 10-fold higher risk for development of type 2 diabetes than that for subjects in the upper tertile (>10.56 µg/ml). Results from the ARIC study point still in the same direction but with a less pronounced association.¹⁵ In light of those studies a difference of 1.42 µg/ml between TG homozygotes and non-TG haplotype carriers as observed in our study might be considered as clinically significant and results in increased risk for diabetes development. A long-term follow-up of our study population is needed to prove this assumption.

In summary, our data show a very strong association of the adiponectin 45–276 haplotypes with adiponectin levels in healthy Caucasians but no association with metabolic parameters of the insulin resistance syndrome.

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