Original Article

Genetic Architecture of the *APM1* Gene and Its Influence on Adiponectin Plasma Levels and Parameters of the Metabolic Syndrome in 1,727 Healthy Caucasians

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The associations of the adiponectin (*APM1*) gene with parameters of the metabolic syndrome are inconsistent. We performed a systematic investigation based on fine-mapped single nucleotide polymorphisms (SNPs) highlighting the genetic architecture and their role in modulating adiponectin plasma concentrations in a particularly healthy population of 1,727 Caucasians avoiding secondary effects from disease processes. Genotyping 53 SNPs (average spacing of 0.7 kb) in the *APM1* gene region in 81 Caucasians revealed a two-block linkage disequilibrium (LD) structure and enabled comprehensive tag SNP selection. We found particularly strong associations with adiponectin concentrations for 11 of the 15 tag SNPs in the 1,727 subjects (five *P* values <0.0001). Haplotype analysis provided a thorough differentiation of adiponectin concentrations with 9 of 17 haplotypes showing significant associations (three *P* values <0.0001). No significant association was found for any SNP with the parameters of the metabolic syndrome. We observed a two-block LD structure of *APM1* pointing toward at least two independent association signals, one including the promoter SNPs and a second spanning the relevant exons. Our data on a large number of healthy subjects suggest a clear modulation of adiponectin concentrations by variants of *APM1*, which are not merely a concomitant effect in the course of type 2 diabetes or coronary artery disease. *Diabetes* 55:375–384, 2006

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CCA, common carotid artery; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; MAF, minor allele frequency; SAPHIR, Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk; SNP, single nucleotide polymorphism.

The metabolic syndrome is a cluster of disorders with increasing prevalence mainly due to behavioral risk factors (1). However, it remains to be shown to what extent genetic components play a role in the pathogenesis of this major health problem.

One of the most interesting candidate genes with respect to the metabolic syndrome and type 2 diabetes is the *APM1* gene encoding adiponectin. Adiponectin plasma concentrations play an important role in modulating insulin sensitivity and glucose homeostasis (2–4) and have been found to be decreased in humans with type 2 diabetes (5), coronary artery disease (6), and obesity (7). Morbidly obese subjects loosing weight following a gastric partition surgery consequently exhibited a raise in adiponectin concentrations, while glucose and insulin levels decreased (8). Injection experiments in various animal models revealed that adiponectin is sufficient to reduce blood glucose levels without affecting plasma insulin levels, suggesting that adiponectin directly improves insulin sensitivity (9–11). Low plasma adiponectin levels are further associated with other components of the metabolic syndrome, such as hypertension (12) and dyslipidemia (13).

APM1 maps to chromosome 3q27, a region with evidence for linkage with type 2 diabetes (14) and the metabolic syndrome (15). However, despite the key role of adiponectin plasma concentrations in the pathogenesis of the major metabolic disorders and an abundance of literature regarding the *APM1* gene locus, direct evidence for the association of certain SNPs with disease outcomes are still conflicting or are not in line with associations published for adiponectin concentrations. One of the reasons for this may be that most of the studies to date were designed to estimate associations with a certain disease outcome in a case-control design and a limited number of subjects with measured adiponectin concentrations.

It has thus been the aim of this study to provide a systematic investigation involving high-density SNPs covering the *APM1* gene in a large group of healthy Caucasians in order to clarify whether the gene variants show associations with plasma adiponectin concentrations and parameters of the metabolic syndrome independently from the development of obesity, type 2 diabetes, or coronary artery disease.

RESEARCH DESIGN AND METHODS

The Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) study is an observational study conducted in 1999–2002 involving 1,770 healthy unrelated subjects (663 women 50–70 years of age and 1,107 men 40–60 years of age). The differential age range between the sexes was chosen to match the cardiovascular risk, which is lower for women when compared with men of the same age but matches the risk of men aged ≥10 years. Study participants were recruited by health screening programs in large companies in and around the city of Salzburg. All individuals were of Caucasian origin. 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; or morbid obesity (BMI >40 kg/m^2) and pregnant women were excluded. Informed consent was obtained from each participant.

Phenotyping. Details of the study including phenotyping are given elsewhere (16). At baseline, all study participants were subjected to a comprehensive program; detailed personal and family history was assessed via standardized questionnaires. A physical examination included measurement of anthropometric parameters such as BMI, waist circumference, and percentage body fat. Venous blood was collected after an overnight fast, and plasma samples were either used immediately for analysis or were stored frozen at −80°C. Adiponectin concentrations were measured by an enzyme-linked immunosorbent assay kit from BioCat (Heidelberg, Germany). A complete lipoprotein profile including fasting triglycerides, HDL and LDL cholesterol, glucose, insulin levels, and HbA_{1c} (A1C) were determined. Additional measurements included blood pressure, intima-media thickness of common carotid arteries (CCAs) by high resolution B-mode ultrasound, and glucose and insulin levels post–oral glucose tolerance test (30, 60, and 120 min) in a subgroup of 643 individuals. Subjects were classified as having type 2 diabetes (n = 57) if on hypoglycemic medication or if fasting plasma glucose concentrations exceeded 126 mg/dl.

Selection of tag SNPs and genotyping. From the known polymorphisms in the NCBI (National Center for Biotechnology Information) database dbSNP build 124 (NCBI version 35.1) and in the literature, 53 SNPs of the *APM1* gene with an average distance of 0.7 kb and including SNPs in the promoter and 5′-flanking region, the coding and the noncoding regions of the exons have been identified at the start of this project in February 2003. All of these were genotyped by restriction enzyme assays in 81 randomly selected subjects from the 1,770 SAPHIR study participants. From these 53 SNPs, those with a minor allele frequency (MAF) exceeding 1% and without violation of the Hardy-Weinberg equilibrium (HWE) were eligible for genotyping in the full sample of 1,770 subjects.

To identify the haplotype tagging SNPs, the eligible SNPs were entered into the tag SNP program (17) with one known SNP causing an amino acid exchange (Y111H) being forced into the selection. Briefly, for a given number of SNPs that can afford to be typed in the full sample, the subset of those SNPs is derived, which maximizes the R^2 (i.e., the proportion of the variance of haplotypes reconstructed based on the subset compared with the variance of haplotypes reconstructed based on all SNPs) and thus minimizes the uncertainty in the prediction of the common haplotypes (17). We chose the number of SNPs to be genotyped in the full sample sufficiently large enough to ensure an R^2 of 95% for common haplotypes (frequency ≥5%), but we also attempted a sensible prediction of the rare haplotypes (1–5% frequency) of at least 90%, where possible. Very rare haplotypes (frequencies <1%) were not the focus of this investigation.

Genotyping for the 18 haplotype tag SNPs was achieved by primer extension of multiplex PCR products with detection of the allele-specific products by matrix-assisted laser desorption ionization time-of-flight (MALDI_TOF) mass spectroscopy (18).

Statistical analysis. Tests for violation of the HWE (χ^2 or exact test depending on allele frequency) were performed. The relationship between pairs of SNPs was viewed by the correlation coefficient and Lewontin's D′ (PROC ALLELE, SAS version 9.0, released 2004).

To estimate the association of the variants in the SNPs with changes in plasma adiponectin concentrations, we applied a general linear regression model with log(adiponectin + 1) as the outcome and each SNP entered separately in the model as the explanatory variable. For each SNP, we tested the association of any of the genotypes with adiponectin levels without and with assuming a trend per copy of the minor allele.

The expected number of copies of each haplotype for all subjects in the full sample was estimated via the expectation-maximization (E-M) algorithm (PROC HAPLOTYPE, SAS) based on the tag SNPs. The best-guess number of haplotypes per subject (i.e., the haplotype pair with the highest probability) was assigned to each subject. Haplotypes with frequency ≤1% were collected into one group ("very rare haplotypes"). For haplotype association analyses, all haplotypes were included into the regression model testing the association of each haplotype against the reference group and adjusting for the other haplotypes. The initial reference group contained the subjects with two copies of the most common haplotype.

All analyses were adjusted for age and sex. The impact of additional adjustment for BMI was explored. Because adiponectin concentrations are known to be modulated by antidiabetic medications, we performed a sensitivity analysis excluding subjects with type 2 diabetes. The proportion of variance in adiponectin explained by the SNPs (haplotypes) was computed via R^2. A subset of SNPs (haplotypes) maximizing R^2, while minimizing the number of SNPs (haplotypes), was given using a backward selection.

In a secondary analysis, SNP and haplotype association analysis with other phenotypes correlated with adiponectin concentrations and linked to the metabolic syndrome were performed.

RESULTS

Selection of tag SNPs and analysis of the block structure. To provide a systematic investigation of the *APM1* gene polymorphism and their haplotypes, 53 narrow-spaced polymorphisms spanning the whole gene were genotyped in 81 unrelated healthy subjects. A large number of SNPs were monomorph in the 162 chromosomes, including six of the eight SNPs with reported amino acid substitutions. Only one known SNP resulting in an amino acid substitution in Caucasians (Y111H) was polymorphic, showing an MAF >1%. Details are given in the online-only appendix (available from http://diabetes.diabetesjournals.org). Excluding *1*) the 17 monomorph SNPs, *2*) one SNP with severe violation of HWE ($P < 0.01$, −9330), and *3*) three SNPs with a minor allele frequency <0.05 (−11423, 1161, and 1245) left 32 SNPs eligible for tag SNP selection, which were entered into the tag SNP program. The Y111H was forced into the selection despite the MAF of 3.7% due to its potentially functional importance.

For analysis of the linkage disequilibrium (LD) block structure, we used 27 of the 32 SNPs (5 excluded due to MAF <10%). We observed two LD blocks (D′ >0.8 for pairs of consecutive SNPs) with a block boundary between −2049 and −450 (Fig. 1, *upper part*). The correlation between the alleles also supported a two-block structure, as there was no notable correlation between any SNP of block 1 with a SNP of block 2 (Fig. 1, *lower part*). Groups of highly correlated SNPs (r^2 >0.8) are specified in the online appendix.

From the 32 SNPs in the tag SNP program, 10 tag SNPs were selected for block 1, 7 tag SNPs for block 2, and 1 SNP in between (for details, see the online appendix). Interestingly, selecting the tag SNPs based on a one-block assumption would have yielded the same SNPs.

Characteristics of the 18 tag SNPs genotyped in 1,770 subjects. The characteristics of the 18 SNPs genotyped in the full sample of 1,770 subjects are summarized in Table 1. Two SNPs (−4522 and −2063) severely violated the HWE ($P < 0.0001$) and were thus removed from analysis. The genotyping success rate was >95% except for −18003 (success rate 79%), which was therefore discarded to assure high-quality data, leaving 15 SNPs in the association analysis.

Association of *APM1* variants with adiponectin concentrations in 1,727 subjects. Exclusion of subjects without measured adiponectin (n = 17) or <50% of the SNPs successfully genotyped (n = 26) yielded an analysis sample of 1,727 subjects. Subject characteristics are given in Table 2, indicating the excess of men in this study. The higher age of women and the relatively low diabetes prevalence in both men and women are due to study design (see RESEARCH DESIGN AND METHODS). Adiponectin levels were markedly higher in women. There was no

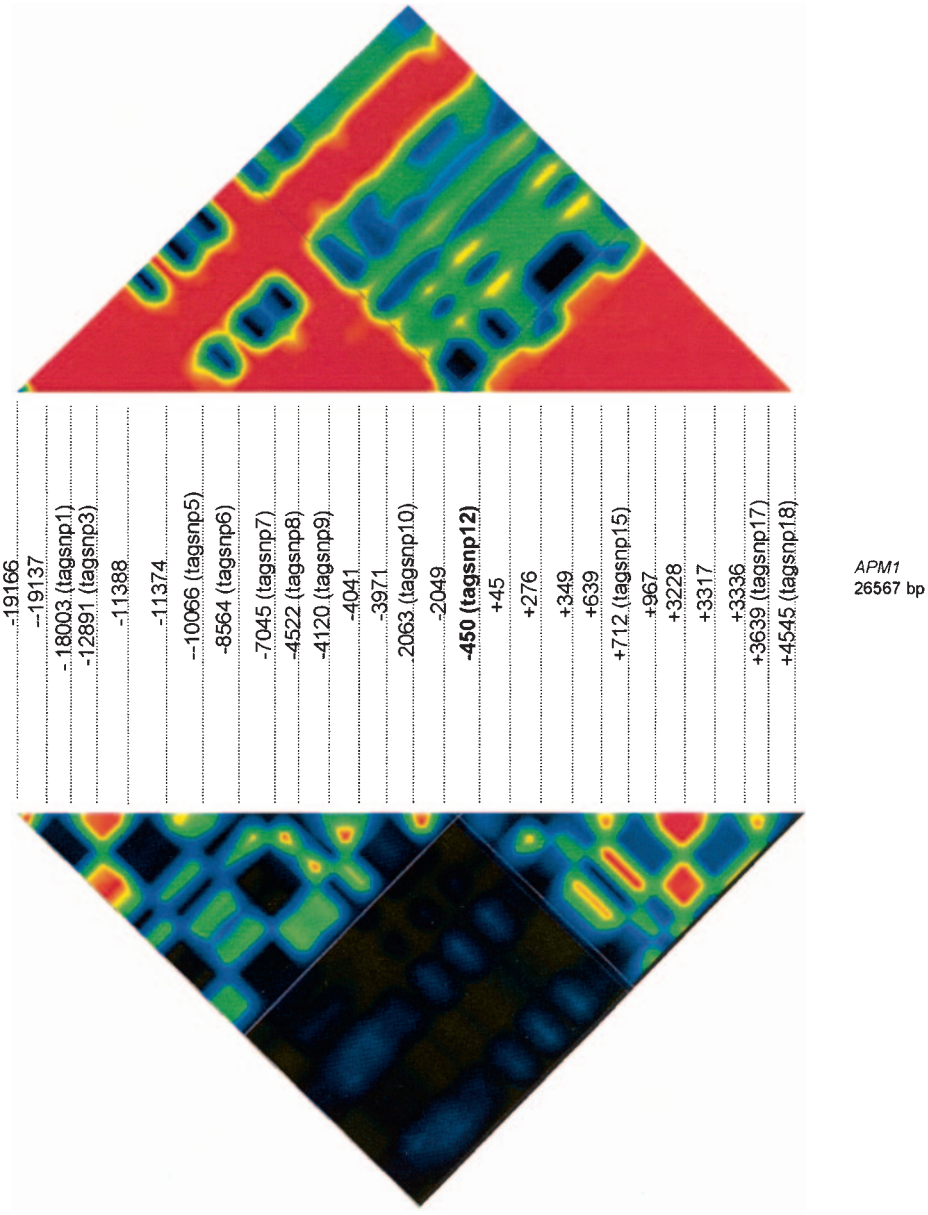

FIG. 1. LD measures (GOLD plot) for 27 of the 32 SNPs genotyped in 81 subjects with MAF >10%. *Top triangle*: D′ (red = 1.0, yellow = 0.7–0.9, green = 0.4–0.6, blue = 0.2–0.3, and black = 0.0–0.1). *Bottom triangle*: R^2 (red = 1.0, yellow = 0.7–0.9, green = 0.4–06, blue = 0.1–0.3, black = 0.0). Block boundary between −2049 and −450.

trend of decreasing adiponectin by age if differentiated between the sexes.

Figure 2 summarizes the results from the SNP association and gives the *P* values for overall difference in mean adiponectin levels in the three or two genotype groups per SNP. It illustrates significant associations for 11 of the 15 SNPs analyzed. These results remained highly significant, except for those of Y111H, even when considering the number of tests performed. Most of these associations were consistent with an additive inheritance model yielding even stronger *P* values for association, assuming a trend per copy (−11388, −10066, −8564, −450, +45, +276, and +712).

Note that there were correlated SNPs showing the same association signal, partly due to their correlation: *1*) −14811 and −11388, *2*) −10066 and −8564, *3*) 45 and 4545, and *4*) 276, 712, and 3639. There was not a significant interaction between −11388 and −10066, and the two SNPs' effect estimates were independent and additive.

All analyses were adjusted for age and sex. All associations were consistent in both men and women when analyzed separately and when excluding subjects with type 2 diabetes ($n = 57$). Additional adjustment for BMI did not markedly change the estimates or the precision, which excluded the possibility of confounding of the results by BMI. We also did not find a modulation of the slope between BMI and adiponectin by the *APM1* SNPs strongly associated with adiponectin levels.

Haplotype analysis. Including all subjects with full information on adiponectin concentration and the 15 SNPs yielded an analysis sample of 1,506 subjects for the haplotype association analysis. A total of 124 different haplotypes were reconstructed. We analyzed the 18 haplotypes with frequency >1% and the construct of "very

TABLE 1
Characteristics of 18 SNPs genotyped in 1,770 subjects

SNP no.	rs number of SNPs	Genome position*	Gene position†	Literature name	Gene region	Common allele	Rare allele	Success rate‡	MAF	Genotype frequency§		
										0	1	2
1	rs2117985	188035547	−18003		5′ region	A	G	0.79	0.06	0.88	0.11	0.01
2	rs822387	188038739	−14811		5′ region	T	C	0.97	0.09	0.83	0.16	0.01
3	rs860291	188040659	−12891		5′ region	C	T	0.98	0.11	0.80	0.19	0.01
4	rs17300539	188042162	−11388	−11391, −11379	Promoter	G	A	0.98	0.10	0.82	0.17	0.01
5	rs182052	188043484	−10066		Intron 1	A	G	0.98	0.34	0.43	0.47	0.11
6	rs822388	188044986	−8564		Intron 1	T	G	0.96	0.30	0.49	0.42	0.09
7	rs822391	188046505	−7045		Intron 1	T	C	0.96	0.18	0.67	0.30	0.03
8	rs822393	188049028	−4522		Intron 1	C	T	0.98	0.22	0.57	0.43	0.001
9	rs822394	188049430	−4120		Intron 1	C	A	0.97	0.17	0.69	0.28	0.03
10	rs7649121	188051487	−2063		Intron 1	A	T	0.98	0.21	0.65	0.27	0.08
11	rs2036373	188052893	−657		Intron 1	T	G	0.98	0.06	0.89	0.11	0.01
12	rs9882205	188053100	−450		Intron 1	G	A	0.95	0.29	0.50	0.42	0.08
13	rs2241766	188053594	45		Exon 2	T	G	0.99	0.12	0.78	0.21	0.01
14	rs1501299	188053825	276	IVS2G62T	Intron 2	G	T	0.99	0.30	0.51	0.40	0.10
15	rs3774261	188054261	712		Intron 2	G	A	0.94	0.42	0.32	0.51	0.17
16	rs17366743	188054791	1242	Y111H, T111H, 331T>C	Exon 3	T	C	0.95	0.03	0.94	0.06	0.001
17	rs4686804	188057188	3639		Exon 3	A	G	0.98	0.45	0.30	0.50	0.20
18	rs1063539	188058094	4545		Exon 3	G	C	0.97	0.11	0.80	0.19	0.01

*Relating to the NCBI version build 35; †relating to the first position of the translation starting point ATG; ‡portion of successfully typed subjects; §coded genotypes: 0 = homozygote for common allele, 1 = heterozygote, and 2 = homozygote for rare allele.

rare haplotypes" (frequency ≤1%). Subjects with a very rare haplotype and one copy of the most common haplotype were added to the reference group because these haplotypes exhibited similar mean adiponectin concentrations.

Table 3 summarizes the 18 haplotypes sorted by their strength of association (i.e., sorted by the change in adiponectin concentration per copy of the haplotype). Of the 17 haplotypes tested, 9 showed significant associations with adiponectin concentrations. It was apparent that the haplotypes H1 and H2 containing the minor alleles of −14811 and −11388 yielded higher adiponectin concentrations than the reference. The haplotype H4 with only a minor allele in −11388 also showed rather high adiponectin concentrations. Subjects with the minor allele in −10066 and −8564 exhibited lower adiponectin concentrations independent from −11388 or −14811 ($P = 0.0001$, when testing all haplotypes with G-G against haplotypes with A-T independent from the haplotypes H1, H2, and H4).

In block 2, the haplotypes containing ATGGTGG (H12 and H20) or, less strong, ATGGTAG (H17, H19, and H11) were associated with decreased adiponectin concentration (e.g., for H12, $P = 0.0000008$). The haplotypes with GTTATGG as well as AGGATGC showed increased levels. With the exception of the −450, the SNP associations in block 2 were explained by these haplotypes. Another remarkable haplotype is H21, which contains the rare allele of the amino acid exchange Y111T, otherwise resembles the reference, and is associated with increased adiponectin concentrations.

The variance of adiponectin concentrations (on the transformed scale) explained by the significantly associated *APM1* SNPs was 8% for men and 7% for women. The portion of variance explained by all haplotypes was 8% for men and 9% for women. Note that 18% of the adiponectin variance was explained by sex alone. The SNPs −11388, −10066, −450, 45, 712, 1242, Y111H, and 3639 make up the minimal subset of SNPs explaining the largest portion of adiponectin variance yielded via backward selection. The haplotypes H12, H20, H8, H16, H9, H21, H15, H4, H2, and H1 were the respective subset of haplotypes.

Association of adiponectin levels and APM1 variants with parameters of the metabolic syndrome. The associations between adiponectin concentrations and parameters of the metabolic syndrome were strong. We found one unit of increase in adiponectin concentrations that was associated with −0.25 BMI points ($P < 0.0001$, by linear regression adjusted for sex and age). Additionally adjusting for BMI, one unit of increase in adiponectin concentrations was associated with +1.3 units in HDL cholesterol ($P < 0.0001$), −2.9 units in triglycerides ($P <$

TABLE 2
Clinical characteristics of the analyzed 1,727 subjects of the SAPHIR study

	Men	Women
n	1,085	642
Age (years)	49.2 ± 5.4	56.2 ± 4.3
BMI (kg/m²)	26.9 ± 3.7	26.6 ± 4.7
Adiponectin (μg/ml)	7.1 ± 3.3	11.1 ± 5.3
Diabetes (%)	3.9	2.3
A1C (%)	5.6 ± 0.6	5.7 ± 0.5
Fasting glucose (mg/dl)	94.3 ± 18.3	92.1 ± 17.1
Fasting insulin (μU/ml)	7.6 ± 5.6	7.2 ± 4.2
HDL cholesterol (mg/dl)	55.2 ± 13.4	67.2 ± 16.4
Triglycerides (mg/dl)	136.6 ± 100.5	105.7 ± 52.4
SBP (mmHg)	135.1 ± 12.6	131.3 ± 13.4
DBP (mmHg)	82.4 ± 8.0	81.0 ± 7.6

Data are means ± SD unless otherwise indicated. DBP, diastolic blood pressure; SBP, systolic blood pressure.

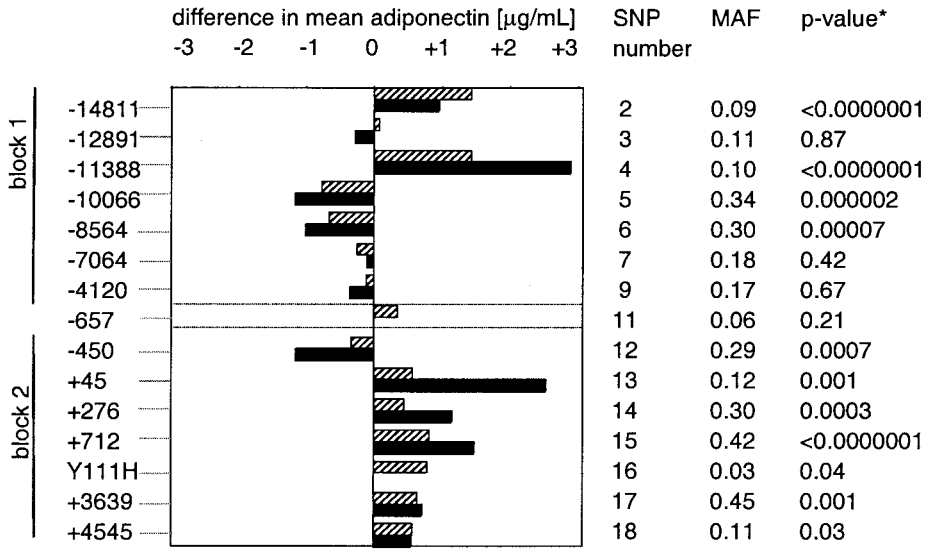

FIG. 2. SNP association analysis showing differences in mean adiponectin levels (rescaled and adjusted for age and sex) for those subjects with one copy of the minor allele (▨) and for those with two copies (■) compared with zero copies. MAF and *P* values are also shown. *Testing for overall differences of means in the genotype groups, adjusted for age and sex.

0.0001), −0.0002 units in CCA ($P = 0.0004$), no changes in blood pressure, −0.05 homeostasis model assessment index points ($P < 0.0001$), −0.008 units in A1C ($P = 0.003$), and an 8.8% decrease in the odds of type 2 diabetes ($P = 0.008$, via logistic regression). For men and women, respectively, adiponectin concentrations explained 5 and 7% of the variance of BMI, 4 and 8% of waist circumference, 4 and 3% of waist-to-hip ratio, 3 and 5% of percent body fat, 1.1 and 1.6% of A1C, 7 and 6% of the homeostasis model assessment index, 5 and 12% of post–1-h oral glucose tolerance test levels, 5 and 6% of fasting triglycerides, 10 and 19% of HDL concentrations, and 1.1 and 1.2% of CCA. When adjusting for BMI, the explained variances dropped to ≤1%.

Figure 3 summarizes the *P* values from the SNP association with the parameters related to the metabolic syndrome. Given the large number of tests performed in this secondary analysis, we would not deem any of the associations as statistically significant. The *P* values >0.01 (indicated by two arrows in the Fig. 3) were found for the outcome A1C for −7064 and −4120, two correlated SNPs of the few SNPs not showing association with adiponectin

TABLE 3
Common and rare (frequency >1%) haplotypes across 15 SNPs in 1,506 subjects sorted by change in adiponectin concentrations for those carrying one copy of the haplotype compared with those with two copies of the most common haplotype (H22) from the general linear regression model with log(adiponectin + 1) as the outcome adjusted for age and sex and with corresponding *P* value testing for the difference of this change from zero

	Haplotypes				
	Block 1 SNP number: 2 3 4 5 6 7 9	Block 2 SNP number: 1 1 1 1 1 1 1 1 1 2 3 4 5 6 7 8	Frequency*	Δadiponectin for 1 (2) haplotype(s) (μg/ml)	*P*†
H12	T-C-G-A-T-T-C	-T-A-T-G-G-T-G-G	0.023 ± 0.003	−2.0	0.0000008
H20	T-C-G-G-G-T-C	-T-A-T-G-G-T-G-G	0.019 ± 0.002	−0.9	0.04
H17	T-C-G-G-G-C-C	-T-A-T-G-G-T-A-G	0.011 ± 0.002	−0.6	>0.05
H19	T-C-G-G-G-T-C	-T-A-T-G-G-T-A-G	0.031 ± 0.003	−0.4	>0.1
H11	T-C-G-A-T-T-C	-T-A-T-G-G-T-A-G	0.066 ± 0.003	−0.3	>0.1
H23	T-C-G-G-G-T-C	-T-G-T-T-A-T-G-G	0.039 ± 0.004	−0.1	>0.1
H22	T-C-G-G-G-T-C	-T-G-T-G-G-T-A-G	0.124 ± 0.006	Reference	
H24	T-C-G-G-T-C-A	-T-G-T-G-G-T-A-G	0.044 ± 0.004	0	>0.1
H26	T-T-G-A-T-C-A	-T-G-T-G-G-T-A-G	0.096 ± 0.005	0.3 (0.9)	>0.1
H6	T-C-G-A-G-T-C	-T-A-T-T-A-T-G-G	0.010 ± 0.002	0.5	>0.1
H8	T-C-G-A-T-T-C	-G-G-T-T-A-T-G-G	0.054 ± 0.004	0.6	0.06
H16	T-C-G-A-T-T-C	-T-G-T-T-A-T-G-G	0.100 ± 0.005	0.8 (2.6)	0.0005
H9	T-C-G-A-T-T-C	-T-A-G-G-A-T-G-C	0.066 ± 0.005	0.8	0.007
H21	T-C-G-G-G-T-C	-T-G-T-G-G-C-A-G	0.025 ± 0.003	0.9	0.03
H15	T-C-G-A-T-T-C	-T-G-T-G-G-T-A-G	0.093 ± 0.005	0.9 (1.5)	0.0003
H4	T-C-A-A-T-T-C	-T-G-T-T-A-T-G-G	0.019 ± 0.002	1.6	0.002
H2	C-C-A-A-T-T-C	-T-G-T-T-A-T-G-G	0.053 ± 0.004	1.8	0.00000002
H1	C-C-A-A-T-T-C	-T-A-G-G-A-T-G-C	0.016 ± 0.002	2.5	0.00002

*Expected haplotype frequency and SE; †assuming a trend per copy, adjusted for age and sex.

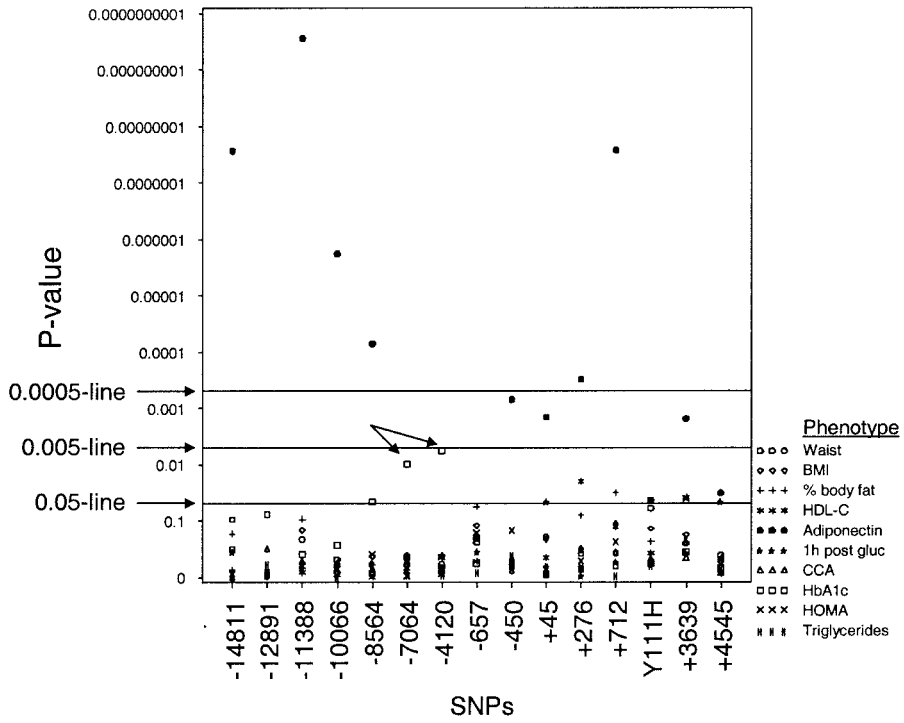

FIG. 3. *P* values from SNP association with all quantitative phenotypes repeating the *P* values from the association with adiponectin concentrations (compare with Fig. 2).

concentrations. A *P* value of 0.02 was found for the association between +276 with HDL cholesterol (60.7, 59.6, and 58.3 ml/mg), which showed a trend per copy of the minor allele consistent with the trend in adiponectin concentrations (compare with Fig. 2). Further, the direction of the changes in parameters of body composition (BMI, waist, and percent body fat), even if not significant, was as expected from the SNP association with adiponectin levels for the SNPs −11388 (waist 94.8, 93.8, and 91.0 cm; BMI 26.8, 26.6, and 25.1 kg/m^2; and percent body fat 19.3, 18.1, and 18.7% for 0, 1, or 2 copies of the minor allele, respectively) and Y111H (waist 94.8 and 92.4 cm, BMI 26.9 and 26.2 kg/m^2, and percent body fat 19.1 and 18.1 for 0 or 1 minor allele).

DISCUSSION

We have systematically analyzed fine-mapped SNPs covering the full range of *APM1* and underlying haplotypes in a large group of 1,727 healthy Caucasians. We observed a two-block structure of the *APM1* gene as well as many and particularly strong associations of SNPs and haplotypes with adiponectin concentrations. We have not found a significant association of the *APM1* gene with parameters of the metabolic syndrome.

Finemapping, block structure, haplotypes, and genealogy. To date, none of the reported studies have applied the approach of fine mapping in combination with selecting haplotype tagging SNPs to represent the underlying haplotypes spanning the whole gene. Menzaghi et al. (19) and Gu et al. (20) described four tag SNPs for the nine common SNPs described by Vasseur et al. (21) in 2002. In contrast, we based our tag SNP selection on genotyping 53 known SNPs in 81 subjects from our study sample and selecting haplotype tagging SNPs to sufficiently predict all haplotypes with frequency >1%. This resulted in genotyping 18 tag SNPs in 1,770 subjects. By this two-step approach described by Stram et al. (17), we have yielded optimal haplotype prediction while minimizing genotyping resources.

LD blocks define recombination-free regions and delineate potential causal regions for an observed association signal (22). Gibson and Froguel (23) reported the *APM1* as a locus of high haplotype diversity and estimated the population recombination rate to be 0.092, which is high compared with the average value of 0.0004 (24). Our data on the 27 SNPs with MAF >10% of the prescreening in 81 subjects suggest a two-block structure of *APM1* gene with a block boundary between −2049 and −450 ($D' = 0.16$) (Fig. 1). Therefore, part of the high recombination rate in *APM1* may be explained by a recombination "hot spot" between the two blocks. Very recently, Sutton et al. (25) described a two-block structure of *APM1* in 91 Hispanics from obese families with a block boundary between −7950 and −4120. Such a shift in block boundaries was observed previously between European populations, particularly between South and Central Europeans (26).

Our haplotype analysis showed clear stratification of adiponectin concentrations suggesting that either the combination of certain alleles or rare latent mutations picked up by the respective haplotypes may be part of the functional genetic mechanism modulating circulating adiponectin levels (27). In block 1, the promoter SNPs −11388 and −11374 (highly correlated to the analyzed −10066) seem to act independently and additively on adiponectin concentrations, which was underscored by a between-SNP interaction model as well as by haplotype association analysis. In block 2, the direct impact of one SNP alone was more difficult to distinguish because loci 45, 276, 712, 3639, and 4545 were moderately correlated and one could have picked up part of the signal of the other in the SNP association, which calls for haplotype analysis. Functional effects have been speculated for the

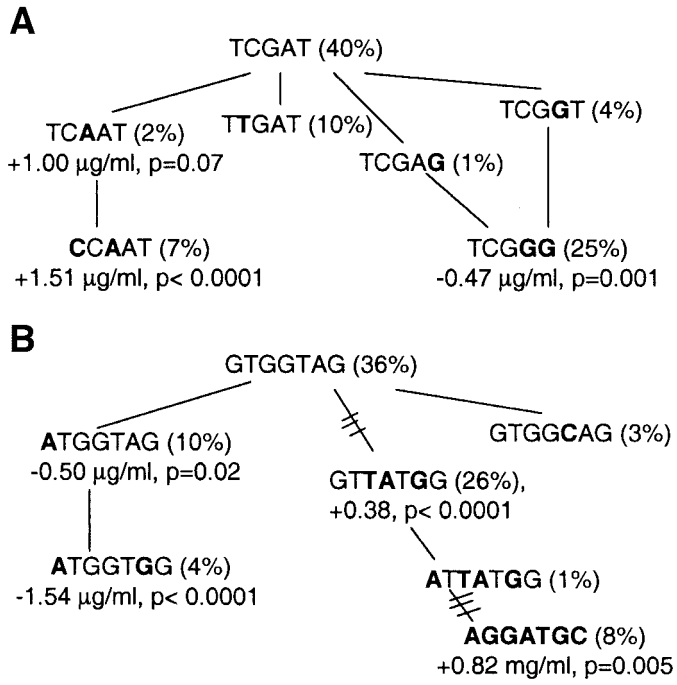

FIG. 4. Haplotypes from Table 3 in block 1 (*A*) and block 2 (*B*) with the line indicating the distance between two haplotypes of one mutation and a line with three marks indicating three mutations (haplotype frequency in parentheses). Shown is the change in circulating adiponectin for subjects having one copy of the haplotype and the corresponding *P* value if $P < 0.1$ is given.

promoter SNPs as well as the rare mutations in the coding region of exon 3 (21,28). This speculation would thus be supported by our finding of the two-block structure, indicating at least two causal regions of the *APM1* gene because the promoter SNPs located in block 1 and the haplotypes in block 2 may represent latent causal mutations in the relevant exons.

Based on a two-block assumption, our haplotypes suggested a possible genealogy, as depicted in Fig. 4. For block 1, the haplotype TCAAT is just one mutation away from the wild-type TCGAT; the haplotype CCAAT is two mutations away from the wild type. Both haplotypes were associated with increased levels of adiponectin in a dose-dependent manner. Similarly, for block 2, the haplotypes ATGGTAG (one mutation) and ATGGTGG (two mutations away) account for decreased levels and are also dose dependent. It was most intriguing that the "bad haplotypes" tended to gather on one branch and the "good" ones on another.

Strong association of *APM1* variants with adiponectin concentrations. Overall, we found more associations with markedly smaller *P* values than those published thus far. A reason for the exceptionally clear associations might be the large sample size and the fact that the SAPHIR study involves particularly healthy subjects, all working ("healthy worker effect") (29) and without symptomatic cardiovascular disease (exclusion criterion). We speculate that in healthy subjects, the primary genetic effect of *APM1* variants on adiponectin concentrations is better detected as no counter regulations from disease processes occurred (30–32). Our findings are in line with associations in French obese and type 2 diabetic subjects reported by Vasseur et al. (21) in one of the largest studies to date, analyzing 922 subjects with measured adiponectin levels. Our findings extend their results by showing more and stronger associations and by underscoring that the modulation of adiponectin concentrations from variants in the *APM1* is not merely a concomitant effect of obesity or type 2 diabetes.

Reviewing the literature on the *APM1* gene revealed conflicting results on the presence and direction of associations of adiponectin levels with particular SNPs. Furthermore, it was difficult to judge whether the reported SNP associations with parameters of the metabolic syndrome were consistent with the direction of their association with adiponectin concentrations; most reported studies were case-control studies comparing patients with type 2 diabetes (20,21,23,33–36), hyperglycemia (20,37), coronary artery disease (34,38,39), or obesity (40) with control subjects. Many of these studies have measured adiponectin levels only, if at all, in a small subsample of subjects. Only a few studies (21,23,41) have apparently aimed to investigate adiponectin concentrations as the primary outcome. An overview of the studies on *APM1* with adiponectin measured in at least 100 unrelated individuals is provided in Table 4. There were only five studies (19,21,23,41,42) including >500 subjects with adiponectin levels measured. Two of those (41,42) involved different ethnicities than in our study, and only one study (19) investigated healthy Caucasian subjects.

Association with components of the metabolic syndrome. There is strong evidence in the literature linking low levels of adiponectin with the development of the metabolic syndrome as well as with its consequences, type 2 diabetes and coronary artery disease (7,43). Adiponectin is even deemed a potential therapeutic agent (44). Animal models have shown that adiponectin is a potent insulin enhancer, regulating energy homeostasis and glucose tolerance (9–11). Mice fed a high-fat diet experienced profound weight loss when chronically treated with a proteolytic fragment of adiponectin (10). However, the direct contribution of the *APM1* variant-induced changes of adiponectin levels on measures of adiposity, lipids, or parameters of insulin resistance and the role of type 2 diabetes in humans is less clear.

Although we found strong associations of adiponectin plasma concentrations with type 2 diabetes and several parameters of the metabolic syndrome, we could not find significant associations of the *APM1* gene variants with these phenotypes. This might be explained by the observation that the *APM1* gene variants in our data explained ~8% of adiponectin concentration variance and adiponectin concentrations explained 1–10% of the variance of the parameters of the metabolic syndrome. Considering that this leaves a maximum of 1% of the metabolic syndrome parameter variance directly explained by the *APM1*, it could well be that there is a causative link; in this case it would not be surprising that we could not detect an association of the *APM1* gene variants with these phenotypes. This underscores that studying gene products as intermediate phenotypes closely related to the gene itself might be more fruitful than concentrating on phenotypes further down the pathway.

Conclusion. The present study on the *APM1* gene is the first one exceeding 1,500 subjects with measured adiponectin levels. The study sample represents a particularly healthy Caucasian population. Further, the *APM1* gene was, to our knowledge, never analyzed with the approach of similarly narrow dense SNPs covering the full gene. These prerequisites—large sample, healthy population, and fine-mapped SNPs—enabled the discovery of many

TABLE 4
All studies on unrelated subjects on the association of *APM1* polymorphisms with measured adiponectin concentrations

Ref.	Study population	*n*	*n* with adiponectin	Investigated SNPs	Association of SNP with adiponectin concentrations	Other major outcome	Consistency with our study
Bacci et al. 2004 (34)	Case-control, Caucasian T2DM	142 CAD patients, 234 control subjects	≤376 control	45,276	None	276: CAD ↓	
Berthier et al., 2005 (40)	Cross-sectional, Caucasian	270 obese, non-T2DM men	≤270	−13752,* −13702,* and 45,276	45: ↑ (P = 0.04)	276: LDL C ↑, (P = 0.02) HDL triglyceride ↓ (P = 0.01); all SNPs: BMI ⇆	45 yes, 276 no: increased LDL contrasts the increased adiponectin concentrations in our data
Fumeron et al., 2004 (37)	Case-control, Caucasian	229 hyper- and 229 normoglycemic patients	229 case and 229 control	−11388, −11374, and 45,276	Only −11388: ↑ (P = 0.02 men, <0.0001 women)	−11388: hyperglycemia ↑ (P = 0.007); 45: hyperglycemia ↑ (OR 2.7, P = 0.007)	No: increased hyperglycemia risk contradicts increased adiponectin concentrations in our data
Gibson and Froguel, 2004 (23)	Case-control, Caucasian	812 T2DM and 1,044 control subjects (532 healthy, 512 obese)	≤1,854 (≤532 healthy)	−11423, −11388, −11374, −4041, −3971, 45,276, and 712	−11423: ⇆	−11423: T2DM ↑ (P = 0.03), HOMA ⇆	
Hara et al., 2002 (42)	Case-control, Japanese	384 T2DM and 480 control subjects	≤384 and ≤480	−11423, −11388, −11374, −4041, −3971, 45,276, 349, 712, 2017, and mutations in exon3	276: ↑ (P = 0.01)	45: T2DM ↑ (P = 0.01); 276: T2DM ↓ (P = 0.007), HOMA ↓ (P = 0.001)	276 yes, 45 no
Kondo et al., 2002 (35)	Case-control, Japanese	218 T2DM and 452 control subjects	≤218 case and ≤452 control	R112C, I164T, R221S, and H241P	I164T: ↓	I164T: ↑ T2DM (P = 0.01)	I164T not present in Caucasians
Menzaghi et al., 2004 and 2002 (19,28)	Cross-sectional, Caucasian	413 healthy representative subjects	≤413	−11374, −4041, and 45,276	276: ↑ (P = 0.03); −11374: ↓ (P = 0.1)	45: SBP ↓ (P = 0.02), weight, waist, fasting glucose, insulin, cholesterol ↓ (NS); 276T: similar as for 45, HOMA ↓ (P = 0.05)	Yes
Ohashi et al., 2004 (39)	Case-control, Japanese	383 CAD and 368 control subjects	≤383 case and ≤368 control	I164T and 45,276	I164T: ↓ (P < 0.0001); 45,276: ⇆	I164T: CAD ↑ (P < 0.05); 45,276: CAD ⇆	I164T not present in Caucasians
Vasseur et al., 2002 (21)	Case-control, Caucasian	743 T2DM and 1,373 obese control subjects	922	−11423, −11388, −11374, −11153, −11040, −4041, −3971, 45,276, 349, 712, 2017, and mutations in exon3	−11388 (P < 0.0001), 45 (P = 0.01), 276 (P = 0.01), 2017 (P < 0.0001): ↑ ; −11374 (P = 0.0003), Y111H (P = 0.08): ↓	−11374: T2DM ↑ (P = 0.04); HOMA: ⇆; −4041: T2DM ↑ (P = 0.09); HOMA: ⇆	Yes: −11388, −11374, 45,276, and 2017, with same direction in adiponectin; no: Y111H other direction in adiponectin
Vozarova et al., 2005 (41)	Pima Indian	1,338 morbidly obese subjects	≤550	−12823, −12137, −11365, 45,276, 3187, 3267, 3286, and 3336	−12823: ↓ (P = 0.002)	None	No: no associations with adiponectin; yes: no finding in other parameters
Yoshioka et al., 2004 (36)	Case-control, Japanese T2DM	108 with and 208 without diabetic nephropathy	316	276	None	None	

SNP positions are according to the translation start site. Associations are stated using the homozygous of the major allele as the reference. ↑, increased levels; ↓, decreased levels; ⇆, no effect. CAD, coronary artery disease; T2DM, type 2 diabetes; LDL C, LDL cholesterol; SBP, systolic blood pressure. *Position according to authors.

strong and highly significant associations of *APM1* gene markers with circulating adiponectin, thus clarifying a primary association of the gene variants not just concomitant with disease and shedding some light on the two-block architecture and genealogy of the haplotypes.

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