Genetic Architecture of Plasma Adiponectin Overlaps With the Genetics of Metabolic Syndrome–Related Traits

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OBJECTIVE — Adiponectin, a hormone secreted by adipose tissue, is of particular interest in metabolic syndrome, because it is inversely correlated with obesity and insulin sensitivity. However, it is not known to what extent the genetics of plasma adiponectin and the genetics of obesity and insulin sensitivity are interrelated. We aimed to evaluate the heritability of plasma adiponectin and its genetic correlation with the metabolic syndrome and metabolic syndrome–related traits and the association between these traits and 10 *ADIPOQ* single nucleotide polymorphisms (SNPs).

RESEARCH DESIGN AND METHODS — We made use of a family-based population, the Erasmus Rucphen Family study (1,258 women and 967 men). Heritability analysis was performed using a polygenic model. Genetic correlations were estimated using bivariate heritability analyses. Genetic association analysis was performed using a mixed model.

RESULTS — Plasma adiponectin showed a heritability of 55.1%. Genetic correlations between plasma adiponectin HDL cholesterol and plasma insulin ranged from 15 to 24% but were not significant for fasting glucose, triglycerides, blood pressure, homeostasis model assessment of insulin resistance (HOMA-IR), and C-reactive protein. A significant association with plasma adiponectin was found for *ADIPOQ* variants rs17300539 and rs182052. A nominally significant association was found with plasma insulin and HOMA-IR and *ADIPOQ* variant rs17300539 after adjustment for plasma adiponectin.

CONCLUSIONS — The significant genetic correlation between plasma adiponectin and HDL cholesterol and plasma insulin should be taken into account in the interpretation of genome-wide association studies. Association of *ADIPOQ* SNPs with plasma adiponectin was replicated, and we showed association between one *ADIPOQ* SNP and plasma insulin and HOMA-IR.

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The dramatic increase in the prevalence of the metabolic syndrome in countries with a western lifestyle is precipitated by environmental variables. However, the individual susceptibility to the obesogenic environment is largely determined by genetic susceptibility (1). Central obesity, dyslipidemia, impaired glucose metabolism, and hypertension are the key elements determining the expression of the metabolic syndrome (2), which is associated with an increased risk for type 2 diabetes and cardiovascular disease (2).

Adipose tissue is an active endocrine tissue that can respond to changes in metabolic conditions by secreting biologically active substances (adipokines). The adipokine family can be divided into two overlapping sets of signaling molecules, namely those with metabolic/immunological function, which include interleukins 1β, 6, 8, 10, or 18, tumor necrosis factor-α and transforming growth factor-β, and those with endocrine function, which include leptin, retinol-binding protein 4, adiponectin, and resistin (3). Human adiponectin is a protein of 247 amino acids (30-kDa), encoded by a gene (*ADIPOQ*) located on chromosome 3q27 (4). Adiponectin is secreted and present in plasma in various multimeric forms, for which the biological significance remains to be determined. Rasmussen-Torvik et al. (5) showed that binding of adiponectin to adiponectin receptors (ADIPOR1 and ADIPOR2) in mice results in increased AMP-activated protein kinase activity and peroxisome proliferator–activated receptor-α activity. In humans, both receptors are expressed mainly in skeletal muscle and adiponectin could thus play a role in energy metabolism.

The role of adiponectin in energy metabolism is confirmed by its inverse correlation with body weight, metabolic syndrome, metabolic syndrome–related traits, and type 2 diabetes (6). In mouse models, adiponectin has been shown to play a role in energy homeostasis by regulating insulin sensitivity of the liver (7). In addition, adiponectin is suggested to exhibit anti-inflammatory properties (8). Thus, adiponectin could play a role in obesity–induced impairment of the metabolic state, systemic inflammation, and the corresponding risk for cardiovascular disease.

Limited data on the overall heritability of plasma adiponectin are available. Furthermore, it is not known whether the genetics of plasma adiponectin overlap with the genetics of body weight and insulin sensitivity/diabetes or other individual components of the metabolic syndrome. Several studies showed convincing association of genetic variants near and in the promoter region of the

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ADIPOQ gene with plasma adiponectin and type 2 diabetes or type 2 diabetes–related traits (5,9,10).

In the present study, we set out to evaluate the heritability of plasma adiponectin and its genetic correlation with the metabolic syndrome and metabolic syndrome–related traits (BMI, insulin, homeostasis model assessment of insulin resistance [HOMA-IR], and plasma C-reactive protein [CRP]).

RESEARCH DESIGN AND METHODS

— In the present study, we used data of the Erasmus Rucphen Family (ERF) study, which is embedded into a rural genetically isolated population (Genetic Research in Isolated Populations [GRIP]). This young, genetic isolate from the southwest Netherlands was initiated by <400 founders in the middle of 18th century. Minimal immigration occurred among the surrounding settlements for social and religious reasons. The population experienced a fast expansion and at the moment this region includes roughly 20,000 inhabitants. The ERF population is a cross-sectional cohort and includes 3,000 individuals who were not selected based on health information but rather comprise living descendants of 22 couples who had at least six children baptized in the community church in approximately 1850–1900. Details about the genealogy of the population have been published elsewhere (11,12). In the current study, we included 2,256 individuals of the ERF population for whom all study parameters were known. We did not exclude participants based on health status. The study protocol was approved by the medical ethics board of the Erasmus MC Rotterdam (Rotterdam, Netherlands). All investigations were carried out in accordance with the Declaration of Helsinki.

Data collection

Blood from participants was obtained in a fasted state. Total plasma insulin measurements were analyzed with an INSIRMA kit (BioSource), total plasma adiponectin with a human adiponectin RIA kit (Linco Research), and total plasma CRP with a CRP ELISA (Diagnostic Systems Laboratories). All measurements were performed to conform to the manufacturer's protocol. Insulin sensitivity was based on the HOMA-IR) (glucose × insulin/22.5). Plasma CRP showed kurtosis; therefore, upper plasma CRP levels exceeding three times the SD of the mean were removed from further analyses. Data on plasma adiponectin, insulin, HOMA-IR, and CRP were available for 2,256 individuals. Metabolic syndrome was assessed according to the criteria of the International Diabetes Federation (2006, Europids), which requires a minimum waist circumference and two of the following abnormalities: high fasting plasma glucose, low HDL cholesterol, high total plasma triglycerides, and high systolic (SBP) and/or diastolic blood pressure (DBP), described in detail in supplementary Table 1 (available in an online appendix at http://care.diabetesjournals.org/cgi/content/full/dc09-1385/DC1). Prevalence and heritability of the metabolic syndrome and related traits in the ERF have been reported previously (1,13).

Statistical analyses

Analysis of mean differences between groups was tested using ANOVA statistics (continuous variables) or χ^2 statistics (categorical variables). Correlations of plasma adiponectin with other traits were estimated in men and women separately with adjustment for age and BMI. Heritability estimations were obtained using SOLAR software (version 2.05; http://solar.sfbrgenetics.org) (1,11). Heritability and genetic association analyses were performed using normal log-transformed trait values and the "tdist" function in SOLAR. The polygenic model (covariates: sex and age) was applied. Heritability estimations included a second variance component, the sibship effect, which is an estimate of phenotypic similarity, due to effects of a shared (early) environment and genetically dominant effects (1). The effect of relevant medication use was assumed to be covered by the metabolic syndrome definition (model B). Inbreeding coefficients in heritability estimations were not significant and therefore were excluded from further analyses. To determine the genetic correlations of plasma adiponectin with other traits we used bivariate heritability analysis. We also estimated sex-specific heritability using bivariate analysis of traits stratified by sex. Because the latter analysis is confined to one quantitative trait, the environmental correlation component is forced to be zero. Bivariate analysis was applied to plasma adiponectin with the metabolic syndrome components and metabolic syndrome–related components using sex, age, and BMI as covariates (model A) or using sex, age, and metabolic syndrome as covariates (model B). The bivariate heritability analysis yields estimates of the total overlapping genetic and environmental component (correlation) of these traits.

The following *ADIPOQ* variants were selected after literature review (9) and confirmed for tag property using Haploview ($r^2 > 0.8$; minor allele frequency >10%) and CEU HapMap data: rs864265, rs822387, rs17300539 (−11391G/A), rs266729, rs182052, rs822396, rs2241766 (+45T/G), rs1501299 (+276G/T), rs3774262, and rs6773957. Selected variants were genotyped using a Sequenom iPLEX application (matrix-assisted laser desorption ionization/time of flight; Sequenom, San Diego, CA). Genotypes were screened for Mendelian errors using the pedigree structure (14). All 10 *ADIPOQ* variants achieved a call rate of >95% and all were in Hardy-Weinberg equilibrium ($P > 0.05$). For *ADIPOQ* genotype association, we assumed an additive model. Analysis of plasma adiponectin included the covariates sex, age, and BMI (model A) or covariates sex, age, and metabolic syndrome (model B). To investigate whether *ADIPOQ* single nucleotide polymorphisms (SNPs) were independently associated with plasma adiponectin, we applied a backward linear regression model containing all SNPs that were associated with plasma adiponectin at a false discovery rate <0.05. The following model was used: rs822387, rs17300539, rs182052, rs1501299, rs6773957, sex, age, and BMI as independent variables and plasma adiponectin as the dependent variable. The independence of the associated SNPs was confirmed in Haploview, and these SNPs were used in association analysis with the metabolic syndrome–related traits.

Association analyses of *ADIPOQ* variants with metabolic syndrome–related traits was performed using two models with different covariates: *1*) sex, age, and BMI and 2) sex, age, BMI, and plasma adiponectin. Adjustment for family structure of the association model was based on a pedigree matrix obtained using Illumina 6K linkage chip data. Analysis was performed using model residuals in a score test accounting for pedigree structure as implemented in GenABEL software (15) function "mmscore" (16). The Bayesian information criterion (BIC) for plasma adiponectin was implemented on associated *ADIPOQ* SNPs, sex, age, and BMI using R software. All other analyses were performed using SPSS 14.01 (September 2005; SSPS, Chicago, IL) software.

Table 1—*Characteristics of the ERF population*

	Women	Men
n	1,258	967
Age (years)	48.0 ± 14.3	49.4 ± 14.1*
Range (years)/minimum–maximum	68.0/18–86	68.5/18–86
Metabolic syndrome and its individual components		
Metabolic syndrome	369 (29.3)	359 (37.1)†
Waist circumference (cm)	81.9 ± 12.0	94.2 ± 11.5†
Glucose (mmol/l)	4.4 ± 0.9	4.8 ± 1.0†
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.1 ± 0.3†
Triglycerides (mmol/l)	1.2 ± 0.7	1.5 ± 0.9†
SBP (mmHg)	136.1 ± 21.2	143.4 ± 18.0†
DBP (mmHg)	78.6 ± 9.8	81.7 ± 9.8†
Metabolic syndrome–related traits		
BMI (kg/m^2)	26.6 ± 5.0	27.3 ± 4.1†
Insulin (μU/ml)	12.8 ± 5.8	13.9 ± 8.8*
HOMA-IR	2.6 ± 1.6	3.0 ± 2.4†
Adiponectin (mg/l)	12.4 ± 5.8	8.1 ± 4.1†
CRP (mg/l)	11.1 ± 22.2	10.0 ± 28.5†
Medication		
Glucose-lowering	26 (2.1)	27 (2.8)
Lipid-lowering	132 (10.5)	144 (14.9)*
Antihypertension	237 (18.8)	201 (20.8)

Data are means ± SD for continuous traits and *n* (%) for categorical traits. *Significantly different from women ($P < 0.05$). †$P < 0.01$, continuous traits based on ANOVA and noncategorical traits based on χ^2 test.

RESULTS — Characteristics of the ERF cohort are presented in Table 1. The prevalence of the metabolic syndrome, according to the International Diabetes Federation definition, was 29.3% in women and 37.1% in men. Virtually all mean values differed significantly ($P < 0.01$) between sexes.

To determine the association of plasma adiponectin with the metabolic syndrome and related traits, we calculated partial correlation coefficients of plasma adiponectin with each trait studied (supplementary Table 2, available in an online appendix) adjusted for age and BMI. Plasma adiponectin showed a high correlation with the metabolic syndrome ($\rho = -0.20$ and $\rho = -0.13$ in women and men, respectively) and with the lipid components HDL cholesterol and triglycerides (all $\rho \geq 0.15$, $P < 0.01$). The correlation of plasma adiponectin with BMI (adjustment only for age), fasting plasma insulin, HOMA-IR, and plasma CRP showed correlations coefficients ranging from 0.10 to 0.29 (all $P < 0.05$). In men, we observed, in general, lower correlation coefficients for all components, with the exception of DBP.

We next estimated the heritability of plasma adiponectin and metabolic syndrome–related traits. Heritability estimates of the metabolic syndrome and its individual components and BMI in the ERF were described earlier (1,13). All heritability estimates were highly significant and none of the metabolic syndrome–related traits showed a significant sibship effect estimate (S). The heritability estimate of plasma insulin was 21.4%, SEM = 5.2%, $P < 10^{-05}$ (S = 6.1%, SEM = 4.2%, $P = 0.07$), of HOMA-IR was 22.0%, SEM = 5.3%, $P < 10^{-05}$ (S = 5.8%, SEM = 4.3%, $P = 0.08$), and of plasma CRP level was 21.0%, SEM = 4.9%, $P < 10^{-05}$ (S = nil). The highest heritability estimate was found for plasma adiponectin: 55.1%, SEM = 4.7, $P < 10^{-6}$ (S = nil).

Next, we performed bivariate analysis of sex-stratified age-adjusted traits. We observed no significant difference in the heritability between the sexes and found a high correlation ($\rho = 100\%$) between the heritability of plasma adiponectin in men ($h^2 = 59.6\%$, SEM = 9.4) and women ($h^2 = 52.9\%$, SEM = 7.7). Details on sex-specific heritability of plasma insulin, HOMA-IR, and plasma CRP are presented detail in supplementary Table 3 (available in an online appendix). Sex-stratified bivariate heritability estimates of the metabolic syndrome and its individual components and BMI were described earlier (1,13).

Bivariate heritability analyses were performed using trait-by-trait analyses on plasma adiponectin combined with the metabolic syndrome and metabolic syndrome–related traits. To investigate whether the interrelation between the traits affected their genetic correlations, we used two statistical models: model A included BMI as the covariate and model B included metabolic syndrome as the covariate in addition to age and sex in the trait-by-trait analyses. The outcomes of the bivariate heritability analysis of plasma adiponectin and the metabolic syndrome and the metabolic syndrome related traits are presented in Table 2.

According to model A, metabolic syndrome, waist circumference, and HDL cholesterol demonstrated a significant ($P < 10^{-3}$) shared genetic component with plasma adiponectin (−42.7, −32.4, and +24.7%, respectively). Triglycerides, fasting plasma glucose, and both SBP and DBP showed a low and insignificant shared genetic component with plasma adiponectin. BMI and plasma insulin showed a significant ($P < 0.04$) shared genetic component with plasma adiponectin of, respectively, −32.4 and −20.0%, whereas our finding for HOMA-IR was borderline significant (genetic correlation [ρ_G] = −18.6%, $P = 0.06$). The genetic correlation of plasma adiponectin and plasma CRP was found to be not significant. The environmental correlations of plasma adiponectin with plasma glucose (−12.0%), HDL cholesterol (+35.6%), triglycerides (−26.0%), and plasma CRP (−13.4%) exceeded their shared genetic component value. In contrast, the genetic correlation of plasma adiponectin and metabolic syndrome was twice as high as their environmental correlation ($\rho_G = -42.7\%$ and environmental correlation [ρ_E] = −19.4%). According to model B (covariates sex, age, and metabolic syndrome), HDL cholesterol shared a significant (15.2%, $P < 0.05$) genetic component with plasma adiponectin. Plasma insulin shared a borderline significant (19.1%, $P = 0.056$) genetic component with plasma adiponectin. All observed environmental correlations obtained according to model B exceeded their genetic correlations (Table 2).

To investigate to what extent the high heritability of plasma adiponectin is due to variants located in the *ADIPOQ* gene, we performed genetic association analysis. Table 3 presents association of 10 *ADIPOQ* SNPs with plasma adiponectin, adjusted for sex, age, and BMI (model A)

Table 2—*Genetic and environmental correlation of plasma adiponectin with metabolic syndrome, metabolic syndrome components, and metabolic syndrome–related traits*

	ρ_G	SEM	*P*	ρ_E	SEM	*P**
Model A						
Metabolic syndrome and its individual components						
Metabolic syndrome	−42.7	11.3	$<10^{-04}$	−19.4	9.4	0.04
Waist circumference	−20.4	7.3	$<10^{-03}$	−18.8	5.8	$<10^{-04}$
Glucose	−2.6	8.7	0.76	−12.0	5.6	0.03
HDL cholesterol	24.7	7.2	$<10^{-04}$	35.6	5.5	$<10^{-9}$
Triglycerides	−12.2	8.9	0.17	−26.0	5.2	$<10^{-6}$
SBP	−10.3	9.1	0.26	−7.1	5.3	0.18
DBP	−9.5	9.2	0.31	6.0	5.4	0.27
Metabolic syndrome–related traits						
BMI†	−32.4	7.2	$<10^{-05}$	−20.7	5.9	$<10^{-04}$
Insulin	−20.0	9.9	0.04	−18.8	5.1	$<10^{-04}$
HOMA-IR	−18.6	9.9	0.06	−19.6	5.1	$<10^{-04}$
CRP	−11.6	12.3	0.34	−13.4	5.4	0.01
Model B						
Metabolic syndrome individual components						
Waist circumference	−15.0	8.9	0.092	−22.6	5.6	$<10^{-04}$
Glucose	0.5	8.9	0.955	−11.3	5.5	0.040
HDL cholesterol	15.2	7.7	0.048	37.2	5.4	$<10^{-11}$
Triglycerides	−11.9	9.8	0.225	−26.0	5.1	$<10^{-06}$
SBP	−6.3	9.3	0.498	8.5	5.3	0.100
DBP	−7.1	9.6	0.459	4.0	5.3	0.450
Metabolic syndrome–related traits						
BMI†	−12.9	7.9	0.103	−14.6	6.1	0.017
Insulin	−19.1	10	0.056	−19.1	5.1	$<10^{-03}$
HOMA-IR	−16.4	10	0.101	−20.4	5.1	$<10^{-04}$
CRP	−12.3	11.7	0.293	−14.4	5.5	0.009

Data are % unless indicated otherwise. Model A included covariates sex, age, and BMI; model B included covariates sex, age, and metabolic syndrome. **P* values were derived using a χ^2 test and covariates sex, age, and BMI. †Covariates sex and age only.

and adjusted for sex, age, and metabolic syndrome (model B). According to both models A and B, nominally significant associations with plasma adiponectin were found for two promoter SNPs (rs822387 and rs17300539), one SNP located in exon 1 (rs182052), one SNP locate in exon 2 (rs1501299), and one SNP (rs6773957) located in the 3′-untranslated region of the *ADIPOQ* gene. Four of these SNPs (except rs822396 and rs1501299) remained significant after conservative Bonferroni correction ($P < 0.005$). The effect size of the promoter SNPs was substantially higher than that of the other significantly associated SNPs.

To determine the best model explaining plasma adiponectin we used the BIC (17). The parameters used in the BIC analysis for plasma adiponectin were sex, age, BMI, and the *ADIPOQ* SNPs rs822387, rs17300539, rs182052, rs1501299, and rs6773957 ($n = 1{,}914$). BIC values are presented in supplementary Table 4 (available in an online appendix). The lowest BIC value was found for the model including four *ADIPOQ* SNPs (rs822387, rs17300539, rs182052, and rs6773957).

Backward linear regression analysis of the five associated SNPs indicated that rs17300539 and rs182052 were independently associated with plasma adiponectin ($P < 0.001$ and $P < 0.004$, respectively, in the joint model). Evaluation of r^2 between SNPs using Haploview confirmed the independence of the two *ADIPOQ* SNPs ($r^2 = 0.2$, plot not shown). Next, we analyzed whether the two significant SNPs, *ADIPOQ* rs17300539 and rs182052, were also associated with the metabolic syndrome and metabolic syndrome–related traits using two models. The first model included sex, age, and BMI as covariates. The second model included sex, age, BMI, and plasma adiponectin as covariates. The second model was used to investigate whether metabolic syndrome–related traits were associated independently of plasma adiponectin. No significant associations were found for rs182052. *ADIPOQ* rs17300539 showed a significant association with plasma insulin ($n = 1919$, $\beta = 0.072$, SEM = 0.031, $P = 0.022$) and with HOMA-IR ($n = 1{,}892$, $\beta = 0.084$, SEM = 0.036, $P = 0.021$), according to model 2.

CONCLUSIONS — Here we report a high heritability of plasma adiponectin (55.1%) and a similar genetic architecture between men and women. We also demonstrated that the genetic component of the HDL cholesterol and plasma insulin overlap significantly with that of plasma adiponectin. *ADIPOQ* rs17300539 and rs182052 were both found to contribute independently to the heritability of plasma adiponectin. Of these two SNPs, rs17300539 was also associated with plasma insulin and HOMA-IR, illustrating their genetic overlap with adiponectin.

Our estimate of the heritability of plasma adiponectin is similar to heritability (62%) reported by Patel et al. (18). Furthermore, our heritability estimates for the metabolic syndrome-related traits (plasma insulin, HOMA-IR, and plasma CRP) are in agreement with earlier reports (19–21). In our previous study (1) on the heritability of metabolic syndrome and its individual components, heritability varied from 10.6% (metabolic syndrome) to 42.9% (HDL cholesterol). The heritability of plasma adiponectin is higher than the heritability of metabolic syndrome or of its individual components, making it an attractive trait for genome-wide association (GWA) studies.

We did not find evidence for a sex-specific genetic component for plasma adiponectin (1,13). Patel et al. (18) studied the genetic correlation between plasma adiponectin and obesity traits in a large longitudinal family-based cohort and found that the genetic correlation (ρ_G) of plasma adiponectin with BMI and waist circumference was for both ~−40%. This result matches well with our estimate of the genetic correlation between adiponectin and BMI of −32.4% using sex, age, and BMI as covariates (model A). Furthermore, we found, using model A, a high and significant shared genetic component between plasma adiponectin and the metabolic syndrome (−42.7%). Moreover, this genetic correlation was twice as large as the environmental correlation. Furthermore, according to model A, waist circumference (−20.4%), HDL cholesterol (+24.7%), and plasma insulin (−20.0%) shared a significant genetic component

Table 3—*Association of variants in and around* ADIPOQ *with plasma adiponectin*

SNP	Position	*n*	Alleles (m/M)	Frequency (m)	Effect (m)	SEM	*P*
Model A							
rs864265	Pr	1,918	G/T	0.19	−0.012	−0.023	0.601
rs822387	Pr	1,917	C/T	0.95	0.146	0.040	**$3.0*10^{-4}$**
rs17300539	Pr	1,919	A/G	0.95	0.155	0.040	**$9.3*10^{-5}$**
rs266729	Pr	1,934	G/C	0.74	−0.025	−0.021	0.230
rs182052	Ex-1	1,916	A/G	0.68	−0.070	−0.020	**$3.0*10^{-4}$**
rs822396	Ex-1	1,925	A/G	0.22	0.043	0.021	0.049
rs2241766	In-1	1,939	G/T	0.93	0.037	0.036	0.303
rs1501299	Ex-2	1,921	T/G	0.73	0.057	0.021	0.006
rs3774262	Ex-2	1,937	A/G	0.93	0.035	0.036	0.330
rs6773957	3′-UTR	1,914	A/G	0.67	0.061	0.020	**0.002**
Model B							
rs864265	Pr	1,904	G/T	0.19	−0.006	−0.023	0.788
rs822387	Pr	1,904	C/T	0.95	0.143	0.040	**$3.6*10^{-4}$**
rs17300539	Pr	1,905	A/G	0.95	0.166	0.040	**$2.7*10^{-5}$**
rs266729	Pr	1,920	G/C	0.74	−0.017	−0.020	0.400
rs182052	Ex-1	1,902	A/G	0.68	−0.066	−0.019	**$6.7*10^{-4}$**
rs822396	Ex-1	1,911	A/G	0.22	0.043	0.022	**0.047**
rs2241766	In-1	1,924	G/T	0.93	0.046	0.036	0.202
rs1501299	Ex-2	1,907	T/G	0.73	0.055	0.021	0.007
rs3774262	Ex-2	1,923	A/G	0.93	0.046	0.036	0.200
rs6773957	3′-UTR	1,900	A/G	0.67	0.063	0.020	**0.001**

Model A included covariates sex, age, and BMI; model B included covariates sex, age, and metabolic syndrome. **P* values based on χ^2 test. *P* values in bold were selected for analysis in a backward linear regression model and were evaluated on linkage disequilibrium using Haploview (CEU HapMap). Ex, exon; IN, intron; m, effect allele; Pr, promoter; UTR, untranslated region.

with plasma adiponectin. Because many of the study parameters are strongly associated with each other, we also studied the genetic correlations, adjusting for metabolic syndrome (model B). Applying this conservative model, the genetic correlations found according to model A were consistent, again revealing the genetic correlations between plasma adiponectin and HDL cholesterol and plasma insulin. Our findings imply that genetic studies of plasma adiponectin might also lead to the identification of genes associated with HDL cholesterol and plasma insulin.

The *ADIPOQ* gene has been found to be consistently and significantly associated with plasma adiponectin in genetic association studies (9,22). To investigate the role of the *ADIPOQ* gene in the genetic overlap between plasma adiponectin and metabolic syndrome traits, we performed association analysis using 10 *ADIPOQ* SNPs. Hivert et al. (9) showed a significant association of the *ADIPOQ* SNP rs17300539 with plasma adiponectin. Our study of 10 *ADOPOQ* SNPs showed that plasma adiponectin was significantly and consistently associated with 6 *ADIPOQ* variants using adjustment for sex, age, and BMI (model A) or adjustment for sex, age, and metabolic syndrome (model B). Moreover, our study of 10 *ADOPOQ* SNPs showed that plasma adiponectin was significantly and independently associated with both *ADIPOQ* rs17300539 and rs182052 variants. *ADIPOQ* rs17300539 is located in the promoter region of the *ADIPOQ* gene, whereas *ADIPOQ* rs182052 is located in exon 1 of the *ADIPOQ* gene. Whether these two variants are actually causal remains to be determined. Because our heritability and association analyses on plasma adiponectin and associated traits are in concert with findings of other studies in general cohorts or other genetically isolated populations, it seems unlikely that our findings are specific for the genetically isolated ERF cohort.

Plasma adiponectin is strongly associated with metabolic syndrome, obesity, and, in particular, with plasma insulin and insulin sensitivity. There is evidence indicating that insulin directly affects plasma adiponectin (23–25). Thus, it is likely that the genetics of plasma adiponectin and insulin overlap. Hivert et al. (9) reported that rs173766743 was associated with the incidence of type 2 diabetes. Our analyses showed that rs17300539 was also associated with plasma insulin and HOMA, independently of plasma adiponectin. We did not observe any effect on this association using metabolic syndrome instead of BMI as a covariate (data not shown). Because HOMA-IR is a measure for insulin resistance that reflects a pre-diabetic state, we analyzed the r^2 between *ADIPOQ* rs17300539 and rs173766743 in Haploview using CEU HapMap data. This analysis did not show any evidence of linkage disequilibrium between these variants ($r^2 = 0$). An explanation for this apparent discrepancy may be the adjustment for plasma adiponectin levels in our association analyses. Whether the association of rs173766743 with type 2 diabetes is independent of plasma adiponectin was not reported. The association of rs17300539 with plasma insulin and HOMA-IR is independent of plasma adiponectin in our analyses, which implies a direct effect of this SNP on plasma insulin and insulin sensitivity. One possible explanation is that *ADIPOQ* rs17300539 is associated with a functional variation of the adiponectin protein affecting insulin sensitivity independent of plasma levels of the protein. However, this association would have to be replicated in independent analyses before further investigation.

In summary, the present study confirms and extends the correlation of plasma adiponectin with HDL cholesterol and plasma insulin. The high heritability of plasma adiponectin is promising for GWAs. The genetics of plasma adiponectin is similar between sexes. The genetics of plasma adiponectin showed a significant and consistent overlap with the genetics of HDL cholesterol and plasma insulin, which implies that GWAs of plasma adiponectin might also result in the detection of genetic variation associated with HDL cholesterol and plasma insulin.

Genetic association analyses indicated that *ADIPOQ* variation is strongly associated with plasma adiponectin and also that *ADIPOQ* rs17300539 is associated with plasma insulin and HOMA-IR independently of plasma adiponectin. These genetic association data are thus in line with the observed genetic overlap between plasma adiponectin and plasma insulin.

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