# Genetics of metabolic syndrome and related traits

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Chapter 7

# The genetic architecture of plasma adiponectin overlaps with the genetics of metabolic syndrome related traits

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**ABSTRACT**

**Objective:** Adiponectin, a hormone secreted by adipose tissue is of particular interest to metabolic syndrome (MetS), since it is inversely correlated with obesity and insulin sensitivity. However, it is unknown 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 MetS and MetS related traits and the association between these traits and ten *ADIPOQ* SNPs.

**Research Design and Methods:** We made use of a family-based population, the Erasmus Rucphen family (ERF) study (1258 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 correlation between plasma adiponectin HDL-C and plasma insulin ranged from 15% to 24% but were not significant for fasting glucose, TG, blood pressure, HOMA-IR and CRP. Significant association with plasma adiponectin was found for *ADIPOQ* variants rs17300539 and rs182052. Nominally significant association was found with plasma insulin and HOMA-IR and *ADIPOQ* variant rs17300539 after adjusting for plasma adiponectin.

**Conclusions:** The significant genetic correlation between plasma adiponectin and HDL-C, 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.

The dramatic increase in the prevalence of the metabolic syndrome (MetS) 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 MetS[2] which is associated with an increased risk for type 2 diabetes (T2D) and cardiovascular disease (CVD)[2].

Adipose tissue is an active endocrine tissue which can respond to changes in metabolic conditions by secreting biologically active substances (adipokines). The adipokine family can be divided in two overlapping sets of signaling molecules, namely those with metabolic / immunologic function, which include interleukins 1ß, 6, 8, 10 or 18, tumor necrosis factor alpha and tumor growth factor beta, 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 of which the biological significance remains to be determined. Yamauchi *et al* showed that binding of adiponectin to adiponectin receptors (ADIPOR1 and ADIPOR2) in mice results in increased AMPK activity and PPAR-$\alpha$ activity. In humans, both receptors are mainly expressed in skeletal muscle and adiponectin could thus play a role in energy metabolism[5].

Limited data on the overall heritability of plasma adiponectin is 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 MetS. Several studies showed convincing association of genetic variants near and in the promoter region of the *ADIPOQ* gene with plasma adiponectin and T2D or T2D related traits[9,5,10].

In the present study, we set out to evaluate the heritability of plasma adiponectin and its genetic

correlation with the MetS and the MetS related traits BMI, insulin, HOMA-IR and plasma CRP.

## RESEARCH DESIGN AND METHODS

### STUDY POPULATION

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 of the Netherlands was initiated by <400 founders in the middle of 18th century. Minimal immigration occurred between the surrounding settlements due to social and religious reasons. The population experienced a fast expansion and at the moment this region counts 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 6 children baptized in the community church around 1850-1900. Details about the genealogy of the population have been described elsewhere[11,12]. In the current study, we included 2256 individuals of the ERF population from 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, the 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 the INS-Irma kit of Biosource (cat.#: KIP1254), total plasma adiponectin with the Human adiponectin RIA kit (cat#: HADP-61HK) of Linco Research and total plasma CRP with the US C-reactive protein ELISA (cat.# DSL-10-42100) of Diagnostic Systems Laboratories, Inc. All measurements were performed conform the manufactures protocol. Insulin sensitivity was based on the homeostasis model assessment insulin resistance (HOMA-IR; glucose * insulin divided by 22.5). Plasma CRP showed kurtosis, therefore, upper plasma CRP levels exceeding three times the standard deviation of the mean were removed from further analyses. Data on plasma adiponectin, insulin, HOMA-IR and CRP was available for 2256 individuals. MetS was assessed according to the criteria of the International Diabetes Federation (IDF- 2006, Europids), which requires a minimum waist circumference (WC,) and two of the following abnormalities: high fasting plasma glucose (glucose), low HDL-cholesterol (HDL-C), high total plasma triglycerides (TG), and high systolic and/or diastolic blood pressure (SBP/DBP), described in detail in supplemental table 1. Prevalence and heritability of MetS and related traits in ERF have been reported previously[1,13].

### STATISTICAL ANALYSES

Analysis of mean differences between groups was tested using analysis of variance statistics (ANOVA, continuous variables) or chi squared statistics ($Chi^2$, categorical variables). Correlations of plasma adiponectin with other traits were estimated in men and women separately adjusting for age and BMI. Heritability estimations were obtained using SOLAR software (version 2.05, http://solar.sfbrgenetics.org[11,1]. Heritability and genetic association analyses were performed using normal-log

Table 1: Characteristics of the ERF population

| | Women (n=1258) | Men (n=967) |
|---|---|---|
| Age (yr) | 48.0 ± 14.3 | 49.4 ± 14.1 * |
| range (yr) / min-max | 68.0 / 18-86 | 68.5 / 18-86 |
| **Metabolic syndrome and its individual components** | | |
| Metabolic Syndrome n(%) | 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 (mm Hg) | 136.1 ± 21.2 | 143.4 ± 18.0 ** |
| DBP (mm Hg) | 78.6 ± 9.8 | 81.7 ± 9.8 ** |
| **Metabolic syndrome related traits** | | |
| Body mass index (kg/m2) | 26.6 ± 5.0 | 27.3 ± 4.1 ** |
| Insulin (_U/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 med. n (%) | 26 (2.1) | 27 (2.8) |
| Lipid lowering med. n (%) | 132 (10.5) | 144 (14.9) * |
| Anti-hypertension med. n (%) | 237 (18.8) | 201 (20.8) |

SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance; CRP: C-reactive protein; med.: medication. Values presented are mean ± SD for continuous traits and number (percent) for categorical traits.

* Significant different from women (P-value < 0.05), ** (P-value < 0.01), continuous traits based on ANOVA, non- categorical traits based on Chi2 test.

transformed trait values and SOLAR's "tdist" function. The polygenic model (covariates: gender, age) was applied. Heritability estimations included a second variance component, the sibship effect (S), 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 MetS definition (Models B). Inbreeding coefficients in heritability estimations were not significant and therefore excluded from further analyses. To determine the genetic correlations of plasma adiponectin with other traits we used bivariate heritability analysis. We also estimated gender-specific heritability using bivariate analysis of traits stratified by gender. 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 MetS components and MetS related components using gender, age and BMI as covariates (Model A) or using gender, age and MetS 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; MAF >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 Sequenom iPLEX (MALDITOF, Sequenom Inc. San Diego, USA). Genotypes were screened for Mendelian errors using the pedigree structure (14). All ten *ADIPOQ* variants achieved a call rate of >95% and all were in HWE (*P*>0.05). For *ADIPOQ* genotype-association we assumed an additive model. Analysis of plasma adiponectin included covariates gender, age and BMI (Model A) or covariates gender, age and MetS (Model B). To investigate whether *ADIPOQ* SNPs were independently associated with plasma adiponectin, we applied a backward linear regression model containing all SNPs which were associated with plasma adiponectin at FDR < 0.05. The following model was used: rs822387, rs17300539, rs182052, rs1501299, rs6773957, gender, age and BMI as independent variables and plasma adiponectin as dependent variable. The independence of the associated SNPs was confirmed in HAPLOVIEW and these SNPs were used in association analysis with the MetS related traits.

Association analyses of ADIPOQ variants with MetS related traits was performed using two models with different covariates: (1) gender, age, BMI, (2) gender, 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 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, gender, age and BMI using R software. All other analyses were performed using SPSS 14.01 (September 2005) software.

Table 2: Genetic and environmental correlation of plasma adiponectin with MetS, MetS components and MetS related traits

| **Model A** | **_G** | **SE** | **P** | **_E** | **SE** | **P** |
|---|---|---|---|---|---|---|
| **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** | | | | | | |
| Body mass index ¥ | -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** | **_G** | **SE** | **P** | **_E** | **SE** | **P** |
| **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** | | | | | | |
| Body mass index ¥ | -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 |

Model A: included covariates – gender, age and body mass index; Model B: covariates – gender, age and metabolic syndrome. _G: genetic correlation (%); _E: environmental correlation (%). SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance; CRP: C-reactive protein. P: P-values were derived using Chi2 test, covariates gender, age and body mass index ¥ covariate gender and age only.

## RESULTS

Characteristics of the ERF cohort are presented in table 1. The prevalence of the MetS, according to the IDF definition, was 29.3% in women and 37.1% in men. Virtually all mean values differed significantly ($P<0.01$) between genders.

To determine the association of plasma adiponectin with the MetS and related traits, we calculated the partial correlation coefficients of plasma adiponectin with each studied trait (supplemental table 2) adjusted for age and BMI. Plasma adiponectin showed a high correlation with the MetS ($\rho = -0.20$ and $\rho = -0.13$ in, respectively, women and men) and with the lipid components HDL-C and TG (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 for all components lower correlation coefficients, with the exception

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

| Model A | | | Alleles | Frequency | Effect | | |
|---|---|---|---|---|---|---|---|
| SNP | Position | n | M/m | (m) | (m) | SE | P |
| Prs864265 | Pr | 1918 | G/T | 0.19 | -0.012 | -0.023 | 0.601 |
| rs822387 | Pr | 1917 | C/T | 0.95 | 0.146 | 0.040 | **$3.0\ 10^{-4}$** |
| rs17300539 | Pr | 1919 | A/G | 0.95 | 0.155 | 0.040 | **$9.3\ 10^{-5}$** |
| rs266729 | Pr | 1934 | G/C | 0.74 | -0.025 | -0.021 | 0.230 |
| rs182052 | Ex-1 | 1916 | A/G | 0.68 | -0.070 | -0.020 | **$3.0\ 10^{-4}$** |
| rs822396 | Ex-1 | 1925 | A/G | 0.22 | 0.043 | 0.021 | 0.049 |
| rs2241766 | In-1 | 1939 | G/T | 0.93 | 0.037 | 0.036 | 0.303 |
| rs1501299 | Ex-2 | 1921 | T/G | 0.73 | 0.057 | 0.021 | **0.006** |
| rs3774262 | Ex-2 | 1937 | A/G | 0.93 | 0.035 | 0.036 | 0.330 |
| rs6773957 | 3'-UTR | 1914 | A/G | 0.67 | 0.061 | 0.020 | **0.002** |
| **Model B** | | | **Alleles** | **Frequency** | **Effect** | | |
| **SNP** | **Position** | **n** | **M/m** | **(m)** | **(m)** | **SE** | **P** |
| rs864265 | Pr | 1904 | G/T | 0.19 | -0.006 | -0.023 | 0.788 |
| rs822387 | Pr | 1904 | C/T | 0.95 | 0.143 | 0.040 | **$3.6\ 10^{-4}$** |
| rs17300539 | Pr | 1905 | A/G | 0.95 | 0.166 | 0.040 | **$2.7\ 10^{-5}$** |
| rs266729 | Pr | 1920 | G/C | 0.74 | -0.017 | -0.020 | 0.400 |
| rs182052 | Ex-1 | 1902 | A/G | 0.68 | -0.066 | -0.019 | **$6.7\ 10^{-4}$** |
| rs822396 | Ex-1 | 1911 | A/G | 0.22 | 0.043 | 0.022 | **0.047** |
| rs2241766 | In-1 | 1924 | G/T | 0.93 | 0.046 | 0.036 | 0.202 |
| rs1501299 | Ex-2 | 1907 | T/G | 0.73 | 0.055 | 0.021 | **0.007** |
| rs3774262 | Ex-2 | 1923 | A/G | 0.93 | 0.046 | 0.036 | 0.200 |
| rs6773957 | 3'-UTR | 1900 | A/G | 0.67 | 0.063 | 0.020 | **0.001** |

Model A: included covariates – gender, age and body mass index; Model B: covariates – gender, age and metabolic syndrome. m; effect allele. Pr; promoter, Ex; exon, In; intron, UTR; untranslated region. *P*: *P*-values based on Chi2 test. *P*-values printed in bold were selected for analysis in a backward linear regression model and were evaluated on linkage disequilibrium using Haploview (HAPMAP CEU).

of diastolic blood pressure.

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

Next, we performed bivariate analysis of gender-stratified age-adjusted traits. We observed no significant difference in the heritability between the genders and found a high correlation ($\rho$ =100%) between the heritability of plasma adiponectin in men ($h^2$ =59.6%, SE = 9.4) and women ($h^2$ =52.9%, SE = 7.7). Details on gender specific heritability of plasma insulin, HOMA-IR and plasma CRP are presented detail in supplemental table 3. Gender-stratified bivariate heritability estimates of the MetS and its individual components and BMI have been described earlier[1,13].

Bivariate heritability analyses were performed using trait by trait analyses on plasma adiponectin combined with the MetS and the MetS related traits. To investigate whether the interrelation between

the traits affected their genetic correlations, we used two statistical models: Model A included BMI as covariate and Model B included MetS as covariate in addition to age and gender in the trait by trait analyses. The outcomes of the bivariate heritability analysis of plasma adiponectin and the MetS and the MetS related traits are presented in table 2.

According to Model A, MetS, WC and HDL-C demonstrated a significant ($P<10^{-3}$) shared genetic component with plasma adiponectin (-42.7%, -32.4% and +24.7% respectively). TG, FPG 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%, -20.0%, while our finding for HOMA-IR was borderline significant ($\rho$ 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-C (+35.6%), TG ( -26.0%) and plasma CRP (-13.4%) exceeded their shared genetic component value. In contrast, the genetic correlation of plasma adiponectin and MetS was twice as high as their environmental correlation ($\rho$ G = -42.7% and $\rho$ E = -19.4%). According to Model B (covariates gender, age and MetS), HDL-C shares 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 extend 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 ten *ADIPOQ* SNPs with plasma adiponectin, adjusted for gender, age and BMI (Model A) and adjusted for gender, age and MetS (Model B) . According to both Model 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 the other significantly associated SNPs.

To determine the best model explaining plasma adiponectin we used the Bayesian information criterion (BIC)[17]. The parameters used in the BIC analysis for plasma adiponectin were gender, age, BMI and the *ADIPOQ* SNPs: rs822387, rs17300539, rs182052, rs1501299 and rs6773957 (N=1914). BIC values are presented in supplemental table 4. 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 the SNPs using Haploview, confirmed the independency 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 MetS and MetS related traits using two models. The first model included gender, age and BMI as covariates. The second model included gender, age BMI and plasma adiponectin as covariates. The second model was used to investigate whether MetS related traits were associated independently of plasma adiponectin. No significant associations were found for rs182052. *ADIPOQ* rs17300539 showed an significant association with plasma insulin (N= 1919, ß=0.072, SE=0.031, $P= 0.022$) and with HOMA-IR (N=1892, ß=0.084, SE=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-C and plasma insulin overlap significantly with that of plasma adiponectin. The *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 reported by Patel *et al*. (62%)[18]. Furthermore, our heritability estimates of the MetS related traits (plasma insulin, HOMA-IR and plasma CRP) are in agreement with earlier reports[19,20,21]. In our previous study[1] on the heritability of MetS and its individual components, heritability varied from 10.6% (MetS) to 42.9% (HDL-C). The heritability of plasma adiponectin is higher than the heritability of MetS or of its individual components, making it an attractive trait for genome wide association studies.

We did not find evidence for a gender-specific genetic component for plasma adiponectin[1,13]. Patel *et al*[18] studied in a large longitudinal family based cohort, the genetic correlation between plasma adiponectin and obesity traits and found that the genetic correlation (ρ G) of plasma adiponectin with BMI and WC was for both approximately -40%. This matches well with our estimate of the genetic correlation between adiponectin and BMI of -32.4% using gender, 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 MetS (-42.7%). Moreover, this genetic correlation was twice as large as the environmental correlation. Furthermore, according to Model A, WC (-20.4%), HDL-C (+24.7%) and plasma insulin (-20.0%) shared a significant genetic component with plasma adiponectin. Since many of the study parameters are strongly associated with each other, we also studied the genetic correlations adjusting for MetS (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-C and plasma insulin. Our findings imply that genetic studies of plasma adiponectin might also lead to the identification of genes associated with HDL-C and plasma insulin.

The *ADIPOQ* gene has been found to be consistently and significantly associated with plasma adiponectin in genetic association studies or GWA[22,9]. To investigate the role of the *ADIPOQ* gene in the genetic overlap between plasma adiponectin and MetS traits, we performed association analysis using 10 *ADIPOQ* SNPs. Hivert *et al*[9] showed 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 six *ADIPOQ* variants using adjustment for gender, age and BMI (Model A) or adjustment for gender, age and MetS (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. Since 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 MetS, obesity, and in particular with plasma insulin and insulin sensitivity. There is evidence indicating that insulin directly affects plasma

adiponectin[23,24,25]. Thus it is likely that the genetics of plasma adiponectin and insulin overlap. Hivert *et al* reported that rs173766743 was associated with the incidence of T2D[9]. Our analyses showed that rs17300539 was also associated with plasma insulin and HOMA, independently of plasma adiponectin. We did not observed any effect on this association using MetS instead of BMI as covariate (data not shown). Since HOMA-IR is a measure for insulin resistance which reflects a pre-diabetic state, we analyzed the $r^2$ between *ADIPOQ* rs17300539 and rs173766743 in Haploview using HAPMAP CEU 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 T2D is independent of plasma adiponectin was not reported. Since the association of rs17300539 with plasma insulin and HOMA-IR is independent of plasma adiponectin in our analyses, this implies a direct effect of this SNP on plasma insulin and insulin sensitivity. One explanation could be 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 conclusion, the present study confirms and extends the correlation of plasma adiponectin with HDL-C and plasma insulin. The high heritability of plasma adiponectin is promising for GWAS. The genetics of plasma adiponectin is similar between genders. The genetics of plasma adiponectin showed a significant and consistent overlap with the genetics of HDL-C and plasma insulin, which implies that GWAS of plasma adiponectin might also result in the detection of genetic variation associated with HDL-C , plasma insulin.

Genetic association analyses indicated that *ADIPOQ* variation is strongly associated with plasma adiponectin and indicated 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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## SUPPLEMENTARY MATERIAL

Supplemental table 1: Metabolic syndrome definition according to the International Diabetes Federation (IDF, 2006)[a]

| | |
|---|---|
| _ Waist circumference (cm) | ≥ 94 |
| _ Waist circumference (cm) | ≥ 80 |
| Fasting plasma glucose (mmol/L) | ≥ 5.6[b,c] |
| Systolic bloodpressure (mm Hg) | ≥ 130[c] |
| Diastolic blood pressure (mm Hg) | ≥ 85[c] |
| _ HDL-Cholesterol (mmol/L) | < 1.03[c] |
| _ HDL-Cholesterol (mmol/L) | < 1.29[c] |
| Triglycerides (mmol/L) | ≥ 1.7[c] |

a Europids
b included previously diagnosed type 2 diabetes patients
c included treatment patients.

Supplemental table 2: Partial correlation coefficients of plasma adiponectin with the MetS, its individual components and MetS related traits.

| | **Women** (n=1258) | **Men** (n=967) |
|---|---|---|
| **Metabolic syndrome and its individual components** | | |
| Metabolic Syndrome | -0.20 ** | -0.13 ** |
| Waist circumference | -0.23 ** | -0.12 * |
| Glucose | -0.10 ** | -0.04 |
| HDL-cholesterol | 0.35 ** | 0.27 ** |
| Triglycerides | -0.21 ** | -0.15 ** |
| SBP | -0.03 | 0.01 |
| DBP | 0.03 | -0.04 |
| **Metabolic syndrome related traits** | | |
| Body mass index ¥ | -0.29 ** | -0.20 ** |
| Insulin | -0.17 ** | -0.10 ** |
| HOMA-IR | -0.18 ** | -0.10 ** |
| CRP | -0.12 ** | -0.08 * |

SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance; CRP: C-reactive protein. Partial correlation coefficient: * P-value < 0.05; ** P-value < 0.01, covariates age and body mass index, ¥ covariate age only.

Supplemental table 3: Gender specific heritability of the MetS related traits

| | **Women** | | | **Men** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **$h^2$ (%)** | **SE** | ***P*** | **$h^2$ (%)** | **SE** | ***P*** | **$_G$** | **SE** | ***P*** |
| Adiponectin | 52.9 | 6.5 | <10-15 | 59.6 | 7.5 | <10-14 | 100.0 | - | - |
| Insulin | 28.2 | 7.4 | <10-03 | 21.6 | 7.3 | <10-03 | 100.0 | - | - |
| HOMA-IR | 28.1 | 7.7 | <10-03 | 24.5 | 9.4 | <10-03 | 100.0 | - | - |
| CRP | 32.2 | 8.1 | <10-04 | 17.1 | 9.8 | 0.08 | 91.7 | 28.6 | 0.77 |

$_G$: genetic correlation (%); $_E$ : environmental correlation (%). HOMA: Homeostatic Model Assessment-Insulin Resistance; CRP: C-reactive protein. *P*: P-values were derived using the Chi2 test.

Supplemental table 4: Bayesian information criterion for 5 ADIPOQ SNPS with plasma adiponectin

| nr | rs822387 | rs17300539 | rs182052 | rs1501299 | rs6773957 | BIC/1000 |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 2.567 |
| 2 | 1 | 0 | 0 | 0 | 0 | 2.491 |
| 3 | 0 | 1 | 0 | 0 | 0 | 2.498 |
| 4 | 0 | 0 | 1 | 0 | 0 | 2.505 |
| 5 | 0 | 0 | 0 | 1 | 0 | 2.510 |
| 6 | 0 | 0 | 0 | 0 | 1 | 2.498 |
| 7 | 1 | 1 | 0 | 0 | 0 | 2.463 |
| 8 | 0 | 0 | 0 | 1 | 1 | 2.452 |
| 9 | 1 | 0 | 1 | 0 | 0 | 2.460 |
| 10 | 1 | 0 | 0 | 1 | 0 | 2.446 |
| 11 | 1 | 0 | 0 | 0 | 1 | 2.453 |
| 12 | 0 | 1 | 1 | 0 | 0 | 2.463 |
| 13 | 0 | 1 | 0 | 1 | 0 | 2.454 |
| 14 | 0 | 1 | 0 | 0 | 1 | 2.493 |
| 15 | 0 | 0 | 1 | 1 | 0 | 2.482 |
| 16 | 0 | 0 | 1 | 0 | 1 | 2.501 |
| 17 | 1 | 1 | 1 | 0 | 0 | 2.424 |
| 18 | 1 | 0 | 1 | 1 | 0 | 2.433 |
| 19 | 1 | 1 | 0 | 1 | 0 | 2.422 |
| 20 | 1 | 1 | 0 | 0 | 1 | 2.449 |
| 21 | 0 | 1 | 1 | 1 | 0 | 2.439 |
| 22 | 0 | 1 | 1 | 0 | 1 | 2.485 |
| 23 | 0 | 0 | 1 | 1 | 1 | 2.457 |
| 24 | 1 | 0 | 0 | 1 | 1 | 2.450 |
| 25 | 0 | 1 | 0 | 1 | 1 | 2.435 |
| 26 | 1 | 0 | 1 | 0 | 1 | 2.448 |
| 27 | 1 | 1 | 1 | 1 | 0 | 2.420 |
| **28** | **1** | **1** | **1** | **0** | **1** | **2.410** |
| 29 | 0 | 1 | 1 | 1 | 1 | 2.441 |
| 30 | 1 | 1 | 0 | 1 | 1 | 2.426 |
| 31 | 1 | 0 | 1 | 1 | 1 | 2.439 |
| 32 | 1 | 1 | 1 | 1 | 1 | 2.413 |

Thirty two Bayesian information criterion (BIC) models based on the log-likelihood estimates, calculated using the parameters gender, age, BMI and SNP(s). N=1914. BIC presented as BIC/1000.