Genetics of metabolic syndrome and related traits

Henneman, P.

Chapter 8

Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome-wide association analyses including 4659 European individuals

Iris M. Heid[1,2]*, Peter Henneman[3]*, Andrew Hicks[4]*, Stefan Coassin[5], Thomas Winkler[1], Yurii S. Aulchenko[6], Christian Fuchsberger[4], Kijoung Song[7], Marie-France Hivert[8], Dawn M. Waterworth[7], Nicholas J. Timpson[9], J. Brent Richards[10,11], John R.B. Perry[12], Toshiko Tanaka[13,14], Najaf Amin[3], Barbara Kollerits[5], Irene Pichler[4], Ben A. Oostra[6], Barbara Thorand[2], Rune R. Frants[3], Thomas Illig[2], Josée Dupuis[15], Beate Glaser[9], Tim Spector[11], Jack Guralnik[16], Josephine M. Egan[17], Jose C. Florez[18,19,20], David M. Evans[9], Nicole Soranzo[11,21], Stefania Bandinelli[22], Olga D. Carlson[17], Timothy M. Frayling[12], Keith Burling[23], George Davey Smith[9], Vincent Mooser[7], Luigi Ferrucci[14], James B. Meigs[18,24], Peter Vollenweider[25], Ko Willems van Dijk[3,26], Peter Pramstaller[4,27,28]*, Florian Kronenberg[5]#*, Cornelia M. v. Dujn[3]* (* authors contributed equally)

[1] Department of Epidemiology and Preventive Medicine, Regensburg University Medical Center, Regensburg, Germany

[2] Institute of Epidemiology, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany

[3] Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

[4] Institute of Genetics Medicine, European Academy Bozen/Bolzano (EURAC), Bolzano, Italy. Affiliated Institute of the University of Luebeck, Germany.

[5] Division of Genetic Epidemiology; Department of Medical Genetics, Molecular and Clinical Pharmacology; Innsbruck Medical University, Innsbruck, Austria

[6] Department of Epidemiology, Erasmus MC Rotterdam, The Netherlands

[7] Genetics Division, GlaxoSmithKline, King of Prussia, Pennsylvania, USA

[8] Service d'Endocrinologie, Département de Médecine, Université de Sherbrooke, Quebec, Canada

[9] MRC Centre for Causal Analyses in Translational Epidemiology, Department of Social Medicine, University of Bristol, Bristol BS8 2BN, UK

[10] Departments of Medicine and Human Genetics, McGill University, Montréal, H3T 1E2, Canada

[11] Twin Research and Genetic Epidemiology, King's College London, London, SE1 7EH, UK

[12] Genetics of Complex Traits, Peninsula Medical School, Magdalen Road, Exeter, UK

[13] Medstar Research Institute, Baltimore, MD, USA

[14] Clinical Research Branch, National Institute of Aging, Baltimore, MD, USA

[15] Department of Biostatistics, Boston University School of Public Health, Boston, MA, and National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, USA

[16] Laboratory of Epidemiology, Demography and Biometry, National Institute on Aging, Bethesda, Maryland, USA

[17] Laboratory of Clinical Investigation, National Institute of Aging, Baltimore, MD, USA

[18] Department of Medicine, Harvard Medical School, Boston, MA, USA

[19] Center for Human Genetic Research and Diabetes Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA

[20] Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, USA

[21] Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK
[22] Geriatric Rehabilitation Unit, Azienda Sanitaria Firenze (ASF), Florence, Italy
[23] University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrookes' Hospital, University of Cambridge, Cambridge CB2 0QQ, UK
[24] General Medicine Division, Massachusetts General Hospital, Boston, MA, USA
[25] Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
[26] Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands
[27] Department of Neurology, General Central Hospital, Bolzano, Italy
[28] Department of Neurology, University of Lübeck, Lübeck, Germany

Atheroscerosis. 2009

ABSTRACT

Objective. Plasma adiponectin is strongly associated with various components of metabolic syndrome, type 2 diabetes and cardiovascular outcomes. Concentrations are highly heritable and differ between men and women. We therefore aimed to investigate the genetics of plasma adiponectin in men and women.
Methods. We combined genome-wide association scans of three population-based studies including 4659 persons. For the replication stage in 13795 subjects, we selected the 20 top signals of the combined analysis, as well as the 10 top signals with p-values less than $1.0*10^{-4}$ for each the men- and the women-specific analyses. We further selected 73 SNPs that were consistently associated with metabolic syndrome parameters in previous genome-wide association studies to check for their association with plasma adiponectin.
Results. The *ADIPOQ* locus showed genome-wide significant p-values in the combined ($p=4.3*10^{-24}$) as well as in both women- and men-specific analyses ($p=8.7*10^{-17}$ and $p=2.5*10^{-11}$, respectively). None of the other 39 top signal SNPs showed evidence for association in the replication analysis. None of 73 SNPs from metabolic syndrome loci exhibited association with plasma adiponectin ($p>0.01$).
Conclusions. We demonstrated the *ADIPOQ* gene as the only major gene for plasma adiponectin, which explains 8.7% of the phenotypic variance. We further found that neither this gene nor any of the metabolic syndrome loci explained the sex differences observed for plasma adiponectin. Larger studies are needed to identify more moderate genetic determinants of plasma adiponectin.

INTRODUCTION

Plasma adiponectin is a quantitative parameter, which has a strong role in modulating insulin sensitivity and glucose homeostasis. It has been found to be decreased in humans with type 2 diabetes and cardiovascular disease (CVD)[1,2] and decreased plasma adiponectin was found to be associated with deteriorated levels of virtually all parameters of the metabolic syndrome[3-5]. Experiments in mice transgenic or deficient for the adiponectin gene have underscored the functional role of adiponectin on various components of the metabolic syndrome and diabetes mellitus[3,6,7].

Concerning CVD outcomes the observations on adiponectin are heterogeneous as recently reviewed extensively[8]: experimental data demonstrate that adiponectin stimulates the production of nitric oxide, positively affects inflammatory mechanisms, has anti-apoptotic properties and is involved in vascular remodeling. Clinical data are diverse depending mainly on the disease stage when investigated. Low levels seem to be associated with worse outcomes when measured in healthy conditions. However, there is accumulating data that in diseased states such as chronic heart failure or existing CVD high rather than low levels predict CVD and non-CVD mortality. Knowing the genes which affect plasma adiponectin might be helpful to disentangle adiponectin as cause or consequence of disease states using a Mendelian randomization approach[9].

Plasma adiponectin shows pronounced differences between men and women with about 1.5 times higher concentrations in women[10]. An explanation for these differences is lacking as plasma adiponectin is only moderately influenced by nutritional behavior, physical activity or other environmental components[5,8,11]. However, there is clear evidence for a high heritability of about 50%[4,12-14] which one study even suggested to be sex-dependent[14]. In line with lower plasma adiponectin in men, higher prevalences of type 2 diabetes and impaired fasting glucose were also reported in men[15].

Recent genome-wide association (GWA) scans have highlighted the potential of genetic factors with differential sex effects on concentrations of uric acid[16-18] and lipids[19], waist circumference[20] or schizophrenia[21]. Many of these phenotypes show pronounced sex-specific differences in plasma concentrations or prevalence. A sex-differential SNP association with a quantitative phenotype can even mask a real association if data are analyzed without stratification. One example is a SNP near the *LYPLAL1* gene which recently showed a strong association with waist-hip-ratio in women but not in men and would have been missed in the sex-combined analysis[20]. To our knowledge, sex-specific differences for genetic effects on plasma adiponectin have not been investigated so far.

In the study at hand, we aimed to identify not only novel genes modulating plasma adiponectin but also whether genetic effects are differential between men and women. We combined this meta-analysis with a candidate gene approach considering all genes which have recently been associated with singular components of the metabolic syndrome in GWA studies.

METHODS

STUDY COHORTS AND GENOTYPING

Our gene discovery included 4659 subjects (women=2562, men=2097) derived from three population-based studies, the Erasmus Rucphen Family Study (ERF, n=1820)[22], the follow-up of the third survey from the "Kooperative Gesundheitsforschung in der Region Augsburg" Study (KORA-F3, n=1644)[23], and the MICROS Study (n=1195)[24]. The replication contained 13795 subjects (women=7673, men=6122

from the study cohorts CoLaus (n=5381), Framingham (n=2228), GEMS (n=1780), ALSPAC (n=1415), TWINS UK (n=1399), InChianti (n=1027) and BLSA (n=565).

All studies had genotypes available from genome-wide SNPs imputed based on the HAPMAP CEU r22 reference sample after quality control. Measurement of adiponectin was made by ELISAs (from Mercodia, BioVendor and R&D Systems) or RIA (Linco). Details on study cohorts including the phenotyping for adiponectin measurements, genotyping methods, statistical analysis, and descriptive statistics are provided in the Supplementary Material and Supplementary Table S1.

STUDY DESIGN AND STATISTICAL ANALYSIS

The study design is summarized in Figure 1. GWA analyses (stage 1): GWA analyses were conducted using a standardized protocol in each of the three stage 1 studies. For each of the 2,585,854 SNPs, linear regression using an additive genetic model was performed for log-transformed adiponectin values adjusting for age, sex, and BMI and accounting for the uncertainty in the inferred genotype from the imputation by utilizing the estimated genotype probabilities (implemented in MACH2QTL and GenABEL/ProABEL, respectively). All analyses were repeated for men and women separately. Relatedness between study participants was accounted for where appropriate (ERF, MICROS). Genomic control was applied when appropriate with study-specific lambda factors being 1.05, 1.05, and 0.99 for ERF, KORA, and MICROS, respectively. The beta-estimates of the three cohorts were combined using a fixed effect model. Also, a scaling-invariant p-value pooling meta-analysis using a weighted Z-score method was applied. For each SNP, we tested for significant differences between pooled men-specific beta-estimates across the three GWA studies as well as women-specific beta-estimates (see Supplementary Material for details).

GWA SNP selection: We selected three types of interesting regions to identify potentially novel signals for plasma adiponectin: (1) from the sex-combined sample (20 loci), (2) from the analysis in women (10 loci) and (3) in men (10 loci). Loci were considered as interesting and one SNP per locus was selected, if the combined p-values were less than 1*10-4 and if study-specific MAF was greater than 5% and imputation quality r^2 greater than 0.2.

Replication analysis (stage 2): For the selected 40 SNPs, we attempted replication based on 7 studies with the same study-specific SNP analysis as for stage 1 studies. A stage 2 only and a joint analysis of stage 1 and 2 (n=18454) was performed using the scaling-invariant weighted Z-score method.

Further statistical issues: In stage 1, we had 92% power to detect a variant that explains 1% of the variance of plasma adiponectin with genome-wide significance (alpha=5*10-8). In the stage 1 and stage 2 combined analysis, we had 99% power to yield genome-wide significant evidence for the 40 selected SNPs if they explained 1% of more of the variance in plasma adiponectin.

Candidate gene approach: From the literature, we identified loci associated with metabolic syndrome parameters in large GWA studies to obtain a list of candidate gene SNPs for adiponectin levels. We examined the association of these SNPs with plasma adiponectin from our stage 1 sex-combined and sex-stratified meta-analyses. For this candidate gene approach, we had 92% power to detect a SNP association that explains 0.5% of the variance accounting for the 73 SNPs tested (alpha=0.0007).

Percentage of variance explained: The general population design of KORA enabled computation of the proportion of the adiponectin variance explained by all analyzable ADIPOQ SNPs (i.e. SNPs

available in all three GWA studies in the 50kb region of the ADIPOQ locus with MAF>5%), by an independent SNP set of these (i.e. selecting the SNP with the lowest p-value in the meta-analysis for each bin of SNPs with pairwise r^2>0.2; r^2 information was taken from HAPMAP), or by the top SNP alone. Computations were performed by linear regression on the standardized residuals (log of adiponectin concentrations adjusted for age, BMI and – if appropriate – for sex) and computing the R^2 measure of the model adjusting for the SNP(s) using PROC REG by SAS.

Heritability: The family-based design of MICROS allowed us to compute heritability of plasma adiponectin using a polygenic model for standardized residuals of plasma adiponectin (adjusted for age and BMI - and sex if applicable). Heritability was also computed with additional adjustment of the top ADIPOQ SNP, with the independent SNP set as described above (see above). Computations were performed using the R library GenABEL[25].

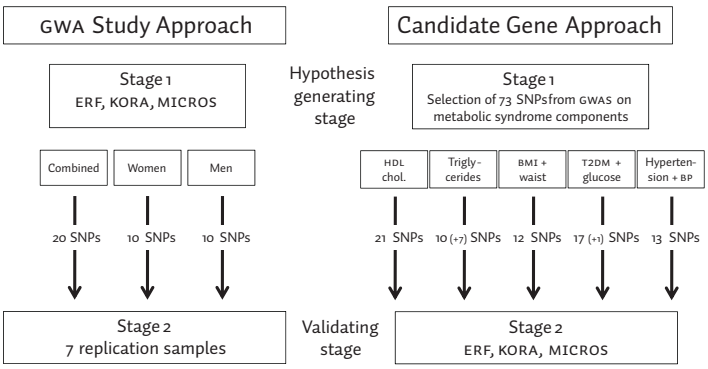


Fig.1. Study design illustrating the genome-wide association (GWA) study approach and the candidate gene approach.

Bioinformatic analysis: Bioinformatic analysis for potential functional SNPs was done in two stages, using bioinformatic tools outlined in the GenEpi Toolbox[26] (Supplementary Material).

RESULTS

GWA ANALYSIS (STAGE 1)

Figure 2 shows the p-value, *ADIPOQ*-region and q-q-plots from the meta-analysis results of plasma adiponectin of the three GWA studies, ERF, KORA and MICROS cohorts. Results are presented for the sex-combined (n=4659) analysis as well as stratified for women (n=2562) and men (n=2097). The combined analysis yielded one genome-wide significant locus (Figure 2A), the *ADIPOQ* locus ($p=4.3*10^{-24}$), which was consistent in women ($p=8.7*10^{-17}$) and men ($p=2.5*10^{-11}$) (Figure 2B). The q-q plot did not show evidence for bias due to population stratification in any of the analyses (Figure 2C). The top *ADIPOQ* SNP rs17366568 (Table 1) exhibited low imputation quality in ERF and MICROS that was genotyped using the Illumina platform in contrast to high imputation quality in KORA genotyped using the Affymetrix platform. However, other SNPs in this region such as rs3774261 reached genome-wide significance in the combined analysis ($p=3.0*10^{-16}$) and had good imputation quality in all three stage 1 samples ($0.82<r^2<0.97$).

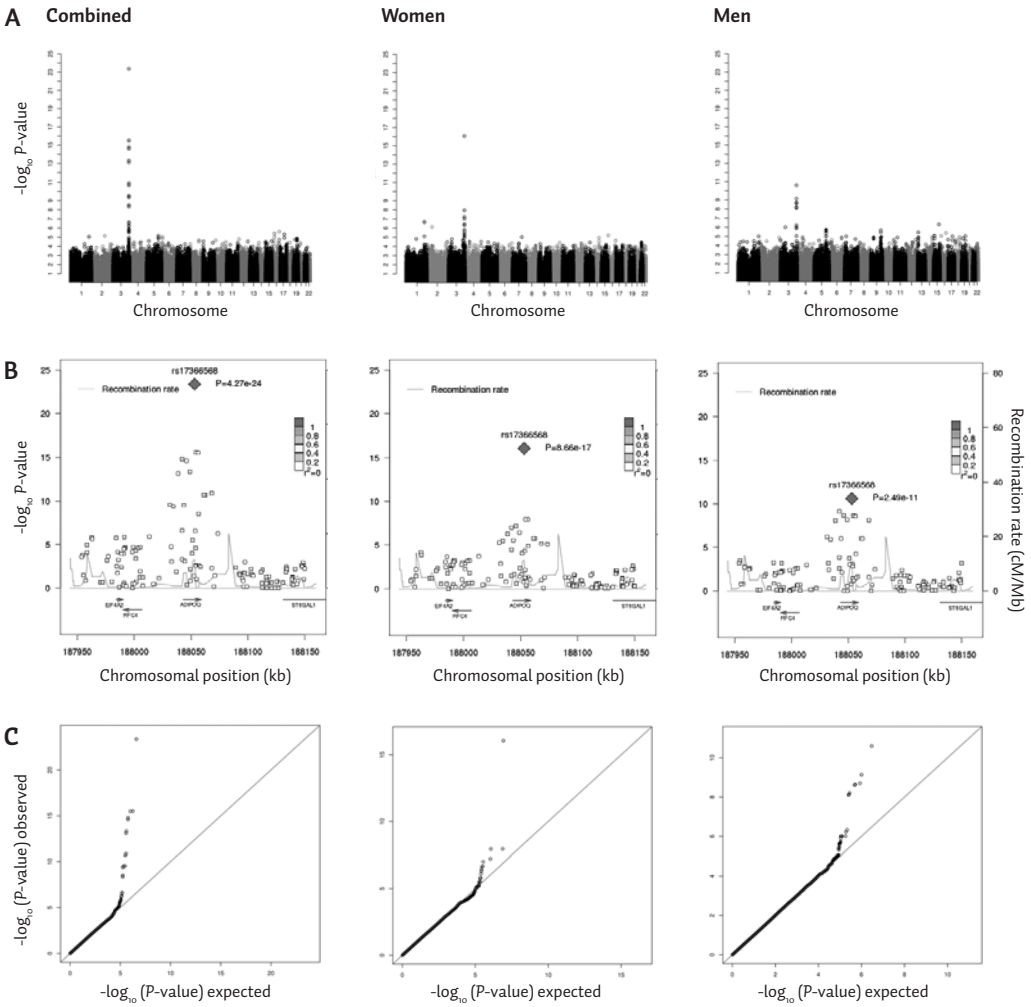


Fig.2. The analyses in panel A-C are provided for the combined sex analysis as well as the analysis stratified for women and men. A. Manhattan plots showing p-values of association of each SNPs in the meta-analysis with plasma adiponectin levels. SNPs are plotted on the X-axis to their position on each chromosome against association with plasma adiponectin on the Y-axis (shown as –log10 P-value). B. Regional Manhattan plots showing significance of association of all SNPs in the ADIPOQ region (3q27). SNPs are plotted on the X-axis to their position on chromosome 3 against association with plasma adiponectin on the Y-axis (shown as –log10 P-value). In each panel, the top-SNP rs17366568 is shown as red diamond. The SNPs surrounding this top-SNP are color-coded (see inset) to reflect their LD with the top-SNP using pair-wise r2 values from the KORA study. Estimated recombination rates from HAPMAP-CEU are plotted in blue to illustrate the local LD structure on a secondary Y-axis. Genes and their direction of transcription are provided below the plots using data from the UCSC genome browser. C. Quantile-quantile (QQ) plots of SNPs. Expected p-values are plotted on X-axis against the observed p-values plotted on the Y-axis.

REPLICATION ANALYSIS (STAGE 2)

Characteristics of the 40 SNPs taken forward for replication are provided in Supplementary Table S2. From the combined, women-, and men-specific GWA-analyses (n=13795, 7673, and 6122, respectively), only the *ADIPOQ* SNP remained significant in the combined analyses (Supplementary Table S3). *P*-values for rs17366568 were $1.09*10^{-41}$, $2.8*10^{-22}$ and $7.8*10^{-23}$ for the combined and the analysis stratified for women and men, respectively (Table 1).

SEX-SPECIFIC ANALYSES

In line with previous reports, plasma adiponectin in women was approximately 1.5 times higher than in men in each of the three stage 1 studies (Supplementary Table S1). Heritability computations in the family-based MICROS study showed slightly higher estimates of 65.1% for women and 54.0% for men (Table 2).

For each SNP, we evaluated whether the sex-specific beta-estimates combined across the three stage 1 studies were significantly different between men and women pointing towards a gender-SNP interaction. The q-q plot for the p-values of sex differences indicated some observed sex difference of genetic effects beyond that expected by chance (Supplementary Figure S1A), but not due to differences in the *ADIPOQ* region. For none of the SNPs in the GWA studies, the sex-specific beta-estimates were significantly different between men and women on a genome-wide level (Supplementary Figure S1B). For the *ADIPOQ* top SNP rs17366568 the p-value for sex difference was 0.62.

ASSOCIATION OF METABOLIC SYNDROME CANDIDATE GENE SNPS WITH ADIPONECTIN

From the literature, we identified loci associated with metabolic syndrome parameters in large GWA studies to obtain a list of candidate gene SNPs for adiponectin levels (Figure 1). These were partially overlapping for the various metabolic syndrome components and included 21 SNPs for HDL cholesterol, 17 for triglycerides (7 of them were also found for HDL cholesterol and were therefore only

Table 1: Genome-wide significant association of the rs17366568 (G>A) SNP in the ADIPOQ locus

				Combined			Women			Men	
Population	EAF*	Rsqr†	n	Beta‡	*P*	n	Beta‡	*P*	n	Beta‡	*P*
Stage 1											
ERF	0.91	0.37	1817	0.103	2.7E-07	1052	0.115	1.0E-05	765	0.088	0.004
KORA	0.89	0.91	1643	0.173	1.7E-15	830	0.204	1.9E-11	813	0.142	5.8E-06
MICROS	0.90	0.27	1195	0.114	3.0E-06	678	0.102	4.1E-04	517	0.182	1.6E-05
Combined**	0.90	-	4655		4.3E-24	2560		8.7E-17	2095		2.5E-11
Stage											
Colaus	0.88	1.00	5261	0.132	3.0E-13	2759	0.119	1.1E-06	2502	0.146	5.1E-08
Framingham	0.88	1.00	2220	0.072	0.003	1213	0.050	0.108	1007	0.094	0.012
GEMS	0.87	1.00	1780	0.149	2.9E-06	732	0.084	0.095	1048	0.194	2.1E-06
ALSPAC	0.92	0.37	1415	0.395	2.9E-14	691	0.453	9.4E-09	724	0.351	3.5E-07
TWINS UK	0.998	NA	1399	0.154	0.078	1399	0.154	0.078	-	-	-
InChianti	0.94	NA	1027	-0.056	0.481	562	-0.130	0.268	465	0.007	0.95
BLSA	0.92	0.61	565	0.263	0.004	266	-0.028	0.822	299	0.488	2.5E-04
Combined**	0.89	-	13667	-	5.2E-22	7622	-	2.7E-10	6045	-	8.1E-14
Stage 1 + 2											
Combined**	0.89	-	18322		1.1E-41	10182		2.8E-22	8140		7.8E-23

* EAF = effect allele frequency (i.e. frequency of G) for sex-combined analysis

† Rsqr = imputation certainty

‡ Beta estimate from linear regression adjusted for age, BMI, and (if appropriate) for sex per unit change [log(µg/mL)] for the risk allele G

** Results are provided for a beta-pooling meta-analysis using the fixed effect model weighting for the inverse variance. When a scaling-invariant p-value pooling meta-analysis using the sample size weighted z-score method was applied for sensitivity analysis, we found no major differences between both methods.

counted once), 12 for BMI and/or waist circumference, 18 for type 2 diabetes and/or glucose levels (one of them was already mentioned for BMI and is therefore only counted once), and 13 for hypertension and blood pressure. Details on these SNPs are given in Supplementary Table S4.

Only 3 out of the 73 SNPs showed p-values between 0.01 and 0.05 for example for the gender-combined analysis (with 3.65 expected under the assumption of no association). No p-value was below the Bonferroni-adjusted significance level of 0.007. Thus, our data indicated no association of these metabolic syndrome parameter SNPs with plasma adiponectin.

SENSITIVITY ANALYSES

Sensitivity analyses repeating all analyses without the adjustment for BMI showed the same results regarding the *ADIPOQ* genome-wide significant results, the lack of sex difference, the lack of other SNPs in the replication stage to show replication, and the lack of metabolic syndrome SNPs to show association with plasma adiponectin.

Table 2: Heritability and percentage of variance explained by the ADIPOQ locus SNPs: Heritability of plasma adiponectin in the family-based study MICROS and percentage of plasma adiponectin variance (KORA) explained by the ADIPOQ locus SNPs in KORA (region on chr 3, position 188.030 – 188.080kb).

	Combined	Women	Men
Heritability (%) in MICROS			
no SNP adjustment	59.6	65.1	54.0
adjusted for top hit rs17366568	58.4	64.6	51.5
adjusted for "independent "SNPs (n=9)[a]	52.9	55.1	48.1
% of variance of plasma adiponectin in KORA explained by			
top hit rs17366568	3.8	5.3	2.4
for "independent" SNPs (n=9)[a]	5.9	6.3	5.1
all SNPs with MAF >5% (n=33)[b]	6.7	6.4	5.5

Computations were based on standardized sex-combined or sex-specific residuals of plasma adiponectin adjusted for age (and sex if applicable) and BMI without and with additional SNP adjustment; includes only SNPs with MAF>5% available in all three studies.

[a] Among the SNPs of the ADIPOQ region with MAF > 5% and available in all three GWA studies: selecting the SNP with the smallest p-value from each bin of SNPs with pairwise r_>0.2: rs1063539, rs16861194, rs17300539, rs17366568, rs17366743, rs3774261, rs6810075, rs7615090, rs822394

[b] All SNPs of the ADIPOQ region with MAF > 5% and available in all three GWA studies: rs6810075, rs10937273, rs12637534, rs1648707, rs864265, rs822387, rs16861194, rs17300539, rs266729, rs182052, rs16861205, rs16861209, rs822391, rs16861210, rs822394, rs822396, rs12495941, rs7649121, rs17366568, rs2241767, rs3821799, rs3774261, rs3774262, rs17366743, rs6773957, rs1063537, rs2082940, rs1063539, rs7639352, rs6444175, rs7628649, rs17373414, rs9860747, rs1501296, rs7615090

ADIPOQ REGION

A closer look at the *ADIPOQ* region revealed that the top SNP rs17366568 was completely independent of all other SNPs in that region. A linkage disequilibrium (LD) plot depicting D' and r^2 measures (Figure 3) revealed that for many SNPs in the *ADIPOQ* region the r^2 was weak even if they were located in the same LD block (as defined by D'). At least nine SNP groups were significantly and independently associated with plasma adiponectin. The percentage of plasma adiponectin variance explained by the top hit was 3.8% and increased to 5.9% when including an independent SNP set (selecting the SNP with the smallest p-value in each bin of pairwise r^2<0.2) and peaked at 6.7% when including all SNPs with MAF>5% in the 50 kb region covering the three LD blocks (Table 2).

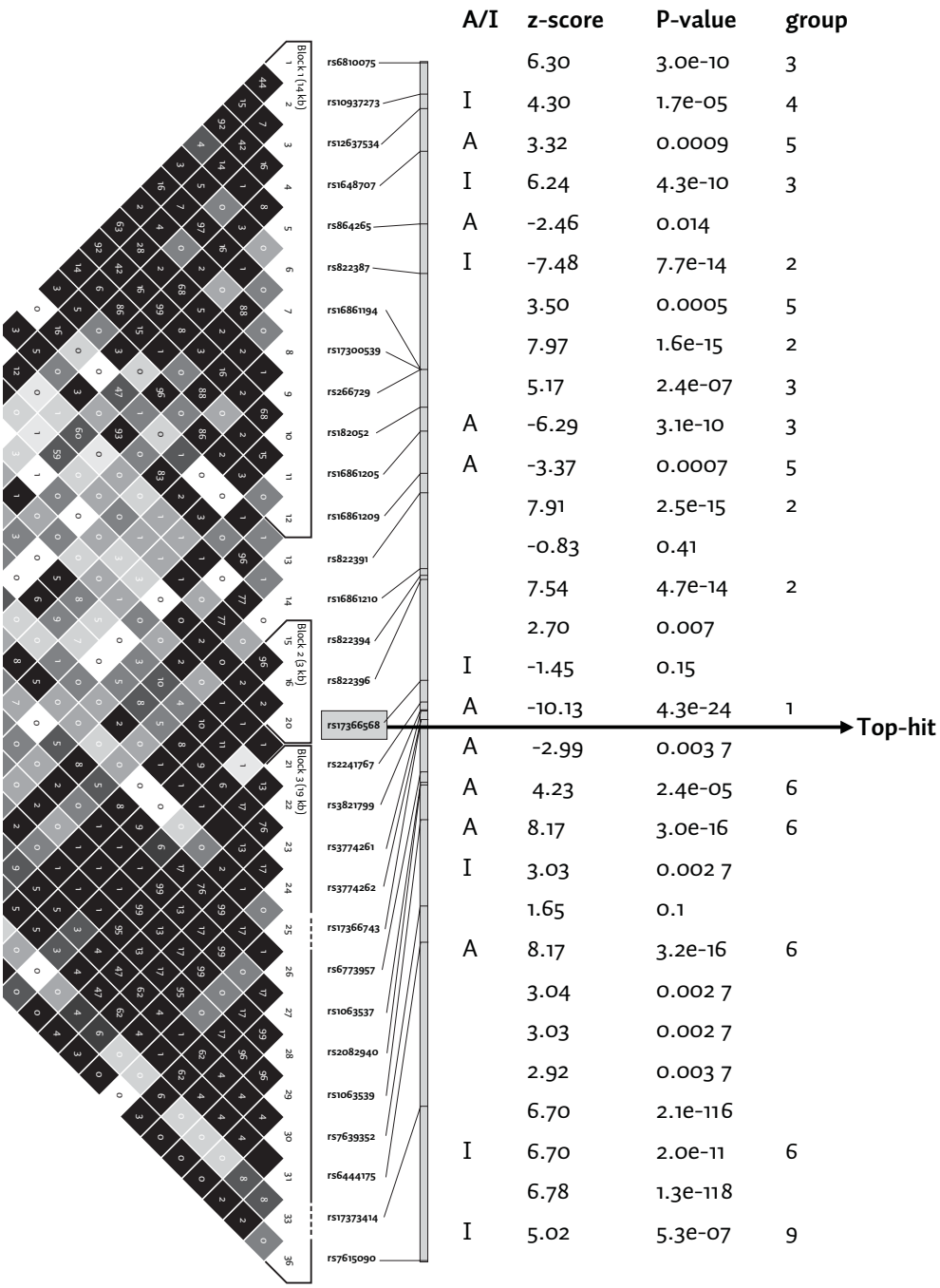


Fig.3. Linkage disequilibrium (LD) plot of SNPs in the ADIPOQ region spanning 50kb (positions 188030-188080kb). The grey shading of the diamonds represent the pair-wise D' and the numbers in the diamonds represent the pair-wise r2 between the two SNPs defined by the top left and the top right sides of the diamond. The figure clearly shows that the top-hit rs17366568 is located within an own LD block and shows virtually no correlations with any other SNP in the entire 50kb region. The columns on the right side of the Figure show i) whether a particular SNP is genotyped by the Affymetrix 500K chip (A) or the Illumina HumanHap300 chip (I); all other SNPs are imputed; ii) the z-scores and iii) the p-values for each SNP-adiponection association for the combined analysis of the cohorts ERF, KORA and MICROS; iv) SNPs that are correlated with an r2>0.60 are grouped in groups 1-9.

Bioinformatic analysis revealed two main putative functional elements located in the second and the third LD block as depicted in Figure 3. Three SNPs located immediately up- and downstream of rs17366568 (for details see Supplementary Table S5) are predicted to affect 10, 6 or 4, respectively, transcription factor binding sites (using adipose tissue-specific analysis). No transcription factor binding sites or splicing regulation elements were detected for rs17366568 itself. Therefore, it is likely that rs17366568 is not the functional variant, but relates to a functional element located in the immediate vicinity (although regulatory potential was very low throughout the region).

Analysis of LD block 3 (encompassing exon 3 and a large intergenic region downstream of the *ADIPOQ* locus) revealed three putative regulatory promoter regions located approximately 5.1 kb, 6.3 kb and 15.8 kb downstream of the *ADIPOQ* locus. Interestingly, especially the proximal two regulatory regions are known to be affected by several copy number regions (see Figure 4 and Supplementary Table S6). However, no SNP in our data set was located directly in these CNVs, whose functional relevance may therefore require further investigation. More generally, the whole genomic region of ADIPOQ seems to be highly affected by copy number variations (Figure 4).

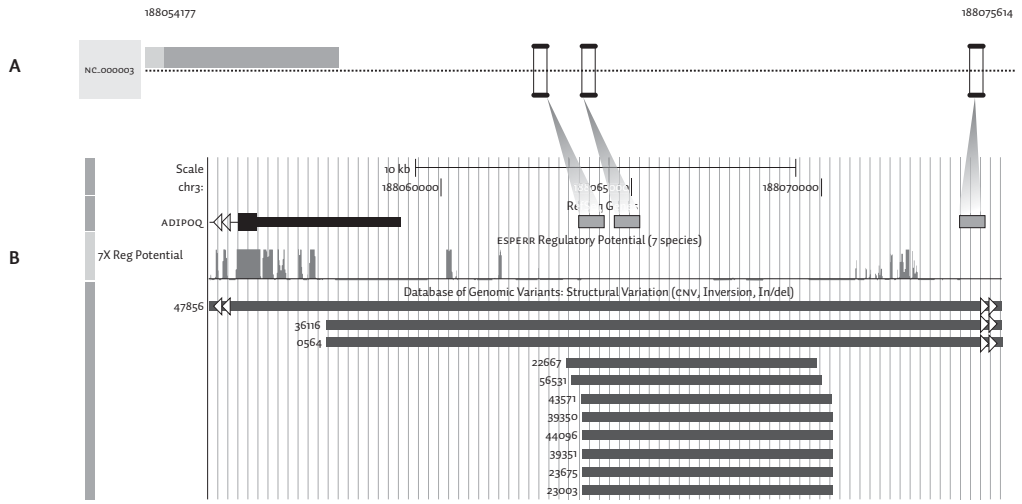


Fig.4. Predicted regulatory regions in the ADIPOQ downstream region and their affection by copy number variations. Panel A: Genomatix Software Suite: Position of the regulatory promoter regions predicted by PromoterInspector (red boxes) and their position relative the ADIPOQ gene region (green box). Panel B: UCSC Browser: Position of the predicted regulatory regions (red boxes) relative to known copy number variations in the ADIPOQ gene region (represented by bold blue lines). The numbers on the left side correspond to the accession number of the respective copy number variation in the Database of Genomic Variants.

DISCUSSION

In the meta-analysis of genome-wide SNP association with plasma adiponectin in three population-based studies including a total of 4655 subjects, we found genome-wide significant evidence for the association with the *ADIPOQ* locus, which is a known locus for plasma adiponectin[10,27]. Furthermore, we did not identify any genome-wide significant evidence for association in any other locus when replicating the other 39 most strongly associated loci in 13795 independent samples. Despite the clear sex difference in plasma adiponectin, there was no sex difference observed for the *ADIPOQ* SNP associations. Finally, we found, despite the strong association between plasma adiponectin and

the metabolic syndrome, no significant association with adiponectin for any of the chosen variants within reported loci for metabolic syndrome parameters.

Our GWA study identified only one major locus for plasma adiponectin, the *ADIPOQ* gene region. The only other GWA study on adiponectin was performed in 1845 individuals of the GEMS Study and identified also only the *ADIPOQ* locus with genome-wide significance[28]. The other top seven hits from that study could not be replicated in our GWA study, neither in the combined (all p-values >0.28) nor in the sex-specific analysis (p>0.16). In our GWA discovery stage, the power was more than 90% to detect novel loci which explain 1% of the adiponectin variance, and, including the replication stage, over 99% to show genome-wide significant evidence of the 40 SNPs in the 18454 subjects. Therefore, our data suggests a lack of a major gene locus other than *ADIPOQ*.

ADIPOQ was studied earlier as a candidate gene and the relationship to plasma levels has long been recognized. The SNP rs17366568 showing the strongest association in our GWA study explained 3.8% of the variance and this number increased to 6.7% if all analyzable SNPs in the *ADIPOQ* region were included into the model. This pronounced difference of the explained variance between the two models can be explained by a large number of SNPs independently contributing to adiponectin levels. The SNPs contributing most to the explained variance are not only located in the three different LD blocks but also several genetic variants within each of at least two of the three blocks contribute to the explained variance. In total, the explained variance was very similar to the 8% reported earlier [10]. Functional studies within the promoter of the *ADIPOQ* gene revealed a pronounced influence of three SNPs also investigated in our study and the corresponding haplotypes on the promoter activity which was accompanied by changes in the DNA binding activity interfering with transcription factor bindings sites[29]. Other studies showed that histone acetylation might influence the transcriptional regulation of the *ADIPOQ* gene[30] and that pioglitazone increases plasma adiponectin by posttranscriptional regulation[31]. Finally, an extensive bioinformatic analysis revealed that the *ADIPOQ* region might be a highly copy number variable region. It remains to be determined how strong the effect of these CNVs on plasma adiponectin is.

Since adiponectin has been viewed as a marker for the metabolic syndrome, we have also studied 73 SNPs that have been associated with any of the major determinants of metabolic syndrome in previous GWA studies. This candidate gene-based analysis did not yield any convincing associations with plasma adiponectin. This was surprising due to the strong link between plasma adiponectin and the metabolic syndrome or any of its components[3-5], but in-line with previous reports on a lack of association of the *ADIPOQ* SNPs with metabolic syndrome parameters[10]. Whether plasma adiponectin affects metabolic syndrome parameters or metabolic syndrome parameters modulate adiponectin is highly debated as illustrated in Supplementary Figure S2. If the association of any of these 73 SNPs had been very strong with adiponectin - stronger than with the metabolic syndrome parameters - this would have pointed towards a gene locus primarily affecting plasma adiponectin and consecutively modulating the metabolic syndrome parameters. This is not suggested by our data (panel A of Supplementary Figure S2). Our data on these 73 metabolic syndrome SNPs lacks association with adiponectin beyond that expected by chance. This would rather support the idea that genetic pathways for plasma adiponectin are different from the pathways depicted by these 73 loci (panel B), or, alternatively, that pathways depicted by these 73 loci affect plasma adiponectin via the metabolic syndrome parameter and the lack of association was due to loss of power for a parameter further down the road (panel C). Both ideas (panel B and C) would point towards the hypothesis that genetically determined adiponectin does not modulate metabolic syndrome parameters directly.

The present data suggests that the sex differences in plasma adiponectin can not be explained by any major gene. The GWA approach yielded no genome-wide significant difference between men and women for any SNP, not even the *ADIPOQ* locus. In fact, none of the variants studied in the replication or in our candidate gene approach based on metabolic syndrome loci showed a significant sex difference. Therefore, the sex-difference in plasma adiponectin might rather be explained by sex hormones[5] or sex-specific epigenetic programming that could be transmitted to subsequent generations in a sex-specific manner leading to transgenerational effects as recently suggested[32].

The heritability estimates of plasma adiponectin are high with roughly 50-60%[4,12-14]. The *ADIPOQ* locus accounts for 6.7% of the variance in our populations-based KORA Study, which is in-line with previous reports[10]. This is also in-line with 6.6% of the heritability accounted for by this locus in our family-based MICROS Study. While the *ADIPOQ* locus association with plasma adiponectin is thus among the strongest associations for quantitative phenotypes in genetic epidemiology, it explains only a small proportion of the overall heritability, a puzzle observed for many other phenotypes (e.g. lipids or obesity measures)[19,20,33]. Potential explanations of this gap between explained and estimated heritability are unknown rare variants with strong effects on adiponectin[34], unknown common loci influencing adiponectin with small effects, or deflation of association estimates due to heterogeneity between studies, uncertainties in the genotypes from imputation or uncertainties in the phenotype assessment. Our study suggests that these other genetic variants influencing plasma adiponectin are variants that explain less than 1% of the phenotypic variance. To localize these loci and to build up gene networks which identify even trans-acting quantitative trait loci, will require substantially larger data sets in combination with gene expression analysis.

STRENGTHS AND LIMITATIONS OF THE STUDY

A limitation of our study is the limited sample size for gene discovery for small genetic effects, in particular when conducting stratified analyses. Furthermore, our top hit in the *ADIPOQ* locus had limited imputation quality in two of the included GWA studies, which can be explained by the fact that KORA used a different SNP-panel (Affymetrix 500K chip) for GWAS genotyping than ERF and MICROS (Illumina HumanHap300). For most of the other SNPs followed in replication samples, the imputation quality was quite high. The relatively low imputation quality of our top-hit in two of the studies explains the lower (but still genome-wide significant) p-values in these two studies compared to KORA. This is entirely in-line with measurement error theory: a "measurement error" (like the uncertainty induced by the imputation) that does not depend on the phenotype (as the case here assuming that genotyping does not depend on adiponectin in the plasma) is expected to attenuate the precision of an underlying association yielding larger p-values. Therefore, the association in ERF and MICROS was rather underestimated than false positive. Finally, it can be considered a limitation of most GWAS studies that gonsomes are not analyzed due to technical issues not yet solved concerning the imputation of SNPs which, however, is a prerequisite to allow meta-analysis of data over various genotyping platforms used.

The strong point of our study is the population-based design, in which the participants have not been ascertained based on the presence of pathology. Hypothesizing a genetic basis of sex differences in plasma adiponectin, a further advantage is the sex-stratified analysis since a sex-combined analysis would otherwise mask an association. Further, the family-based MICROS study enables us to estimate heritability.

CONCLUSIONS

We present a genome-wide association study on adiponectin which the first time attempts to explain adiponectin sex difference by the underlying genetics. We conclude that there is no major gene involved in modulating plasma adiponectin other than the known *ADIPOQ* locus and that there is no major gene explaining the differences of plasma adiponectin between men and women.

Acknowledgements

We thank all staff members involved in the MONICA/KORA Augsburg Studies as well as the general practitioner and other clinicians for compiling the Genetic Research in Isolated Populations, Erasmus Rucphen Family (ERF) study. The technical assistance of Barbara Luhan for measurement of adiponectin in the KORA Study is highly appreciated. We also thank Julia Müller for help in table management. For the MICROS study, we thank the primary care practitioners Raffaela Stocker, Stefan Waldner, Toni Pizzecco, Josef Plangger, Ugo Marcadent and the personnel of the Hospital of Silandro (Department of Laboratory Medicine) for their participation and collaboration in the research project. Finally, we express our appreciation to all study participants.

The acknowledgements of financial and other support for each study is provided in the Supplementary Material.

REFERENCES

1. Li S, Shin HJ, Ding EL, van Dam RM: Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2009;302:179-188.

2. Tilg H, Moschen AR: Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat.Rev.Immunol. 2006;6:772-783.

3. Kadowaki T, Yamauchi T, Kubota N, et al: Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J.Clin.Invest 2006;116:1784-1792.

4. Hivert MF, Manning AK, McAteer JB, et al: Common variants in the adiponectin gene (ADIPOQ) associated with plasma adiponectin levels, type 2 diabetes, and diabetes-related quantitative traits: the Framingham Offspring Study. Diabetes 2008;57:3353-3359.

5. Henneman P, Janssens ACJW, Zillikens MC, et al: Menopause impacts the relation of plasma adiponectin levels with the metabolic syndrome. J.Intern.Med. 2009.

6. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat.Med. 2001;7:947-953.

7. Yamauchi T, Kamon J, Waki H, et al: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat.Med. 2001;7:941-946.

8. Antoniades C, Antonopoulos AS, Tousoulis D, Stefanadis C: Adiponectin: from obesity to cardiovascular disease. Obes.Rev. 2009;10:269-279.

9. Davey SG, Ebrahim S: 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int.J.Epidemiol. 2003;32:1-22.

10. Heid IM, Wagner SA, Gohlke H, et al: Genetic architecture of the *APM1* gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes 2006;55:375-384.

11. Mantzoros CS, Williams CJ, Manson JE, Meigs JB, Hu FB: Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. Am.J.Clin.Nutr. 2006;84:328-335.

12. Comuzzie AG, Funahashi T, Sonnenberg G, et al: The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. J.Clin.Endocrinol.Metab 2001;86:4321-4325.
13. Patel SR, Larkin EK, Redline S: Shared genetic basis for obstructive sleep apnea and adiposity measures. Int.J.Obes.(Lond) 2008;32:795-800.
14. Miljkovic-Gacic I, Wang X, Kammerer CM, et al: Genetic determination of adiponectin and its relationship with body fat topography in multigenerational families of African heritage. Metabolism 2007;56:234-238.
15. Rathmann W, Haastert B, Icks A, et al: High prevalence of undiagnosed diabetes mellitus in Southern Germany: target populations for efficient screening. The KORA survey 2000. Diabetologia 2003;46:182-189.
16. Döring A, Gieger C, Mehta D, et al: SLC2A9 influences uric acid concentrations with pronounced sex-specific effects. Nat. Genet. 2008;40:430-436.
17. Vitart V, Rudan I, Hayward C, et al: SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. Nat.Genet. 2008;40:437-442.
18. Dehghan A, Kottgen A, Yang Q, et al: Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. Lancet 2008;372(9654):1953-1961.
19. Aulchenko YS, Ripatti S, Lindquist I, et al: Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat.Genet. 2009;41:47-55.
20. Lindgren CM, Heid IM, Randall JC, et al: Genome-Wide Association Scan Meta-Analysis Identifies Three Loci Influencing Adiposity and Fat Distribution. PLoS Genet 2009;5:e1000508.
21. Shifman S, Johannesson M, Bronstein M, et al: Genome-wide association identifies a common variant in the reelin gene that increases the risk of schizophrenia only in women. PLoS Genet 2008;4:e28.
22. Henneman P, Aulchenko YS, Frants RR, et al: Prevalence and heritability of the metabolic syndrome and its individual components in a Dutch isolate: the Erasmus Rucphen Family study. J.Med.Genet 2008;45:572-577.
23. Wichmann HE, Gieger C, Illig T, for the MONIKA/KORA Study Group: KORA-gen: Resource for Population Genetics, Controls and a Broad Spectrum of Disease Phenotypes. Gesundheitswesen 2005;67:S26-S30.
24. Pattaro C, Aulchenko YS, Isaacs A, et al: Genome-wide linkage analysis of serum creatinine in three isolated European populations. Kidney Int. 2009.
25. Aulchenko YS, Ripke S, Isaacs A, Van Duijn CM: GenABEL: an R library for genome-wide association analysis. Bioinformatics 2007;23:1294-1296.
26. Coassin S, Brandstätter A, Kronenberg F: Lost in the space of bioinformatic tools: a constantly updated survival guide for genetic epidemiology. The GenEpi Toolbox. Atherosclerosis (in press).
27. Vasseur F, Helbecque N, Dina C, et al: Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum.Mol.Genet. 2002;11:2607-2614.
28. Ling H, Waterworth DM, Stirnadel HA, et al: Genome-wide Linkage and Association Analyses to Identify Genes Influencing Adiponectin Levels: The GEMS Study. Obesity.(Silver.Spring). 2009;17(4):737-744.
29. Laumen H, Saningong AD, Heid IM, et al: Functional characterization of promoter variants of the adiponectin gene complemented by epidemiological data. Diabetes 2009;58:984-991.
30. Musri MM, Corominola H, Casamitjana R, Gomis R, Parrizas M: Histone H3 lysine 4 dimethylation signals the transcriptional competence of the adiponectin promoter in preadipocytes. J.Biol.Chem. 2006;281:17180-17188.
31. Rasouli N, Yao-Borengasser A, Miles LM, Elbein SC, Kern PA: Increased plasma adiponectin in response to pioglitazone does not result from increased gene expression. Am.J.Physiol Endocrinol.Metab 2006;290:E42-E46.
32. Gabory A, Attig L, Junien C: Sexual dimorphism in environmental epigenetic programming. Mol.Cell Endocrinol. 2009;304:8-18.
33. Willer CJ, Speliotes EK, Loos RJ, et al: Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat.Genet. 2009;41(1):25-34.

34. Cohen JC, Kiss RS, Pertsemlidis A, et al: Multiple rare alleles contribute to low plasma levels of HDL cholesterol. Science 2004;305:869-872.

SUPPLEMENTARY MATERIAL

STUDY COHORTS

All participants in all studies gave informed consent and each study was approved by the appropriate Research Ethics Committees. Body-mass-index (BMI, weight divided by height^2) was assessed measuring weight and height in the study-center or by self-report. Details on adiponectin assays used for phenotyping and descriptive statistics are provided in Supplementary Table S1.

GENOME-WIDE ASSOCIATION STUDY COHORTS

Erasmus Rucphen Family study (ERF): The Erasmus Rucphen Family (ERF) study is comprised of a family-based cohort embedded in the Genetic Research in Isolated Populations (GRIP) program in the Southwest of the Netherlands. Descriptions of ERF's design have been previously published (Aulchenko et al., 2004). Briefly, twenty-two families that had a minimum of five children baptized in the community church between 1850 and 1900 were identified with the help of detailed genealogical records. All living descendants of these couples, and their spouses, were invited to take part in the study. Participants included in the current study total 2079 individuals for whom complete phenotypic and genotypic information was available. Covariates were obtained during the baseline examination.

KORA studies: The KORA cohorts (Cooperative Health Research in the Region of Augsburg, KOoperative Gesundheitsforschung in der Region Augsburg) are several cohorts representative of the general population in Augsburg und two surrounding counties that were initiated as part of the WHO MONICA Study. The KORA S3 is a survey examined in 1994/95 with standardized examinations described in detail elsewhere (Löwel et al., 2005). Ten years age-sex strata have been sampled from the 25 to 74 year old population with a stratum size of 640 subjects. 3,006 individuals participated in a follow-up examination of S3 in 2004/05 which is called KORA F3. All study participants underwent a standardized face-to-face interview by certified medical staff and a standardized medical examination including blood draw and anthropometric measurements. The 1644 subjects for the KORA GWA analysis (the KORA S3/F3 500K study) were chosen from KORA F3.

Microisolates in South Tyrol Study (MICROS): The MICROS study is part of the genomic health care program 'GenNova' and was carried out in three villages of the Val Venosta, South Tyrol (Italy), in 2001-03. It comprised members of the populations of Stelvio, Vallelunga and Martello. A detailed description of the MICROS study is available elsewhere (Pattaro et al., 2007). Information on the participant's health status was collected through a standardized questionnaire. Laboratory data were obtained from standard blood analyses. Covariates were obtained during the interview phase.

Genome-wide genotyping had been performed using the Illumina 300K array of the HumanHap300 (ERF, MICROS) or the Affymetrix 500k array (KORA-F3).

REPLICATION STUDY COHORTS

CoLaus (Caucasian Cohorte Lausannoise) Study: The CoLaus study investigates the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. The 3251 females and 2937 males Caucasian participants, aged between 35 and 75 years, were selected using a simple

non-stratified random sample of the population registry of the city of Lausanne, Switzerland, as previously described (Firmann et al., 2008). Participation rate was 41%. Recruitment began in June 2003 and ended in May 2006. All participants attended the outpatient clinic of the University Hospital of Lausanne in the morning after an overnight fast. From 5435 participants genotypes are available from genotyping with Affymetrix chips (GeneChip Human Mapping 500K array and the BRLMM calling algorithm). Adiponectin levels were available for 5381 study participants and were measured by ELISA assay (R&D systems, Minneapolis, MN). Statistical analyses was conducted using Quicktest v0.94.

Framingham: The Framingham Heart Study has investigated risk factor determinants of CVD over decades in a general population (Dawber et al., 1966). It began in 1948 with the recruitment of 5209 residents aged 28-62 years in about two-thirds of the households in the town of Framingham, Massachusetts. Participants have undergone biennial examinations since the study began. In 1971, the Framingham Offspring Study (Kannel et al., 1979) was started, in part, to evaluate the role of genetic components in CVD etiology. In total, there were 5124 subjects aged 5-70 years at entry including the children of the original cohort and their spouses. The Framingham Heart Study consists almost entirely of subjects of European descent from England, Ireland, France, and Italy. Genotyping was performed using Affymetrix 500K array supplemented by the MIPS 50K array. Total adiponectin levels were available for 2228 genotyped study participants from the Offspring cohort and were measured by ELISA (R&D Systems, Minneapolis, MN) (Hivert et al., 2008). Statistical GWA analysis was performed using linear mixed effect models implemented in the function lmekin from the R kinship package (www.r-project.org), where the SNP is incorporated in the model as a fixed covariate while a familial random effect component is included to account for familial correlation.

GEMS *(Genetic Epidemiology of Metabolic Syndrome):* The study population of the Genetic Epidemiology of Metabolic Syndrome (GEMS) study consisted of dyslipidaemic cases (age 20-65 years, n=1025) matched with normolipidaemic controls (n=1008) by sex and recruitment site. Detailed information on the GEMS study design, sampling frame, and recruitment procedures has been published (Stirnadel et al., 2008). Genotyping was performed using Affymetrix GeneChip Human Mapping 500K array and the BRLMM calling algorithm. Adiponectin levels were available for 1780 study participants and were measured by ELISA assay (R&D systems, Minneapolis, MN). Statistical analyses was performed using Quicktest v0.94.

ALSPAC*:* The Avon Longitudinal Study of Parents and their Children (ALSPAC) is a population-based birth cohort study consisting initially of over 13000 women and their children recruited in the county of Avon, U.K in the early 1990s (http://www.bristol.ac.uk/alspac/). Both mothers and children have been extensively followed from the 8th gestational week onwards using a combination of self-reported questionnaires, medical records and physical examinations. Biological samples including DNA have been collected for ~10,500 of the children from this cohort. Ethical approval was obtained from the ALSPAC Law and Ethics committee and relevant local ethics committees, and written informed consent provided by all parents (Golding et al., 2001). 1518 ALSPAC individuals were genotyped using the Illumina HumanHap317K SNP chip. This chip contains 317504 SNPs and provides approximately 75% genomic coverage of the Utah CEPH (CEU) HAPMAP samples for common SNPs at r^2 >0.8. Markers with minor allele frequency <1%, SNPs with >5% missing genotypes and, any marker that failed an exact test of Hardy-Weinberg equilibrium ($p < 10^{-7}$) were excluded from further analyses and before imputation. After data cleaning, 315807 SNPs were left in the ALSPAC genome-wide association analysis (Timpson et al., 2009). Plasma adiponectin concentrations were determined in samples from 1415 individuals using ELISA (R&D Systems) with inter-assay CV being 7%. Analyses were performed using STATA and PLINK.

TwinsUK: The TwinsUK cohort (www.twinsuk.ac.uk) is an adult twin registry shown to be representative of the UK singleton population (Andrew et al., 2001). A total of 1399 (women were included in the analysis, Genotyping was performed using the Illumina HumanHap 300 Illumina HumanCNV370 Duo chips (Richards et al., 2008). Adiponectin levels were available for 1399 study participants and were measured with an in-house two-site ELISA assay using antibodies and standards from R&D Systems Europe (Abingdon, Oxford UK). The day-to-day coefficients of variation for adiponectin were 5.4% at a concentration of 3.6 μg/ml, 5.2% at 9.2 μg/ml and 5.8% at 15.5 μg/ml. Statistical analysis was conducted applying Merlin software package (Abecasis et al., 2002).

InCHIANTI: INCHIANTI is an epidemiological study of risk factors contributing to the decline in physical functioning in late life (Ferrucci et al., 2000). Individuals were selected from the population registries of two small towns in Tuscany, Italy. Participants, all of white European origin, were invited to a clinic visit for evaluation of health status as described in detail previously (Bartali et al., 2002). SNPs were genotyped on the Illumina 550k array (Melzer et al., 2008), with missing SNPs imputed using IMPUTE software. Adiponectin levels were available for 1027 study participants and were measured by RIA assay (Human Adiponectin RIA Kit, Linco Research, Inc, Missouri, USA) Statistical analyses were conducted using SNPTEST.

Baltimore Longitudinal Study of Aging (BLSA): The Baltimore Longitudinal Study of Aging (BLSA) is an observational study that began in 1958 to investigate normative aging in community dwelling adults who were healthy at study entry (Shock et al., 1984). Participants are examined every one to four years depending on their age. Currently there are approximately 1100 active participants enrolled in the study. The analysis was restricted to subjects with European ancestry. Genotyping was performed using Illumina HumanHap 550K. Adiponectin levels were available for 565 study participants and were measured by RIA (LINCO) having intra-assay and inter-assay variation of 1.8-6.2% and 6.9-9.3% respectively. Each analysis was further adjusted for the top two principal components derived from an EIGENSTRAT analysis utilizing ~10,000 randomly selected SNPs from the 550K SNP panel.

ADDITIONAL INFORMATION ON STATISTICAL METHODS

Metal-software: All combined analysis were performed using the METAL software (Abecasis and Willer, 2007, http://www.sph.umich.edu/csg/abecasis/metal). We used the METAL implemented study-wise genomic control correction as well as genomic control correction of the METAL results.

To combine the three GWA studies (stage 1), we performed a beta-pooling meta-analysis using the fixed effect model (inverse variance weighted) and a scaling-invariant p-value pooling meta-analysis (using the weighted z-score method). We found no major difference between both methods in this GWA stage. For the replication stage (stage 2) and stage 1 and stage 2 combined, we conducted the scaling-invariant p-value pooling as there were greater differences between adiponectin assays in the full set of studies. We present the weighted Z-score method results throughout the manuscript.

Test to compare gender-stratified beta-estimates from GWA analyses: Each study has provided SNP-association results for men and women separately. For each SNP, we pooled the men-specific beta-estimates across all studies (beta_men and its standard error se_beta_men) as well as the women-specific beta-estimates (beta_women and se_beta_women) using the fixed effect model. For each SNP, significant difference between gender-specific pooled beta estimates, beta_men and beta_women, was obtained by using the approximately normally distributed test statistics of beta_men – beta_women divided by the sum of their variance estimates minus the covariance of the beta-estimates

(i.e. se_beta_men² + se_beta_women² + 2 x corr (beta_men, beta_women) x se_beta_men x se_beta_women). The correlation of the beta_men and beta_women was obtained by using the empirical distribution of the beta-estimates across all SNPs under the assumption that the abundance of these SNP-associations are under the null hypothesis of no association.

BIOINFORMATIC ANALYSIS

Bioinformatic analysis for potential functional SNPs was done in two stages, using bioinformatic tools outlined in (Coassin et al., 2009). Firstly, all SNPs of the imputed data set in the *ADIPOQ* gene region have been analyzed for potential functional effects using SNPseek (http://snp.wustl.edu/cgi-bin/SNPseek/index.cgi) and SNPnexus (http://www.snp-nexus.org/) as well as FASTSNP (http://fastsnp.ibms.sinica.edu.tw/). In the second stage attempting to find potential functional variants not included in HAPMAP, all SNPs reported by Ensembl Variation v.56 in the region between rs6810075 and rs7615090 (see Supplementary Figure S2) were submitted to FASTSNP. SNPs which were predicted to affect any kind of functional element were then further investigated using the Genomatix Software Suite (Genomatix Software GmbH, Munich, Germany) and the PupaSuite for transcription factor binding site analysis as well as F-SNP (http://compbio.cs.queensu.ca/F-SNP/) for further refinement of splicing regulation effects and other kinds of functional elements. Since FASTSNP recognizes only SNPs in gene regions, all intergenic SNP both up- and downstream of the *ADIPOQ* locus were analyzed for transcription factor binding sites using the Genomatix Software Suite. All analyses in the Genomatix Software Suite were done using only transcription factors specifically expressed in the adipose tissue as well as ubiquitous ones. Additionally, the presence of general functional elements and regulatory potential (ESPERR) in the intergenic region was investigated in the UCSC Genome browser and intergenic regions were scanned for regulatory promoter elements using PromoterInspector from Genomatix. Known copy number variations were retrieved from the Database of Genomic Variants (http://projects.tcag.ca/variation/).

ACKNOWLEDGEMENTS AND FUNDING

The ERF Study was funded by the Centre for Medical Systems Biology (CMSB, www.cmsb.nl) and the NutriGenomics Consortium (www.nutrigenomicsconsortium.nl) in the framework of the Netherlands Genomics Initiatives (NGI) and by the European Network of Genomic And Genetic Epidemiology (ENGAGE) consortium (www.euengage.org).

KORA: This analysis on adiponectin was partially funded by the „Tiroler Wissenschaftsfonds“ (Project UNI-0407/29) and by the „Genomics of Lipid-associated Disorders – GOLD“ of the „Austrian Genome Research Programme GEN-AU“ to F. Kronenberg. The MONICA/KORA Augsburg cohort study was financed by the Helmholtz Zentrum München. It was further funded by the NIH subcontract from the Children's Hospital, Boston, US, (H.-E. Wichmann and I.M. Heid, prime grant 1 R01 DK075787-01A1 to J.N.Hirschhorn) and the German National Genome Research Net NGFN2 and NGFNplus (H.-E. Wichmann 01GS0823).
The MICROS Study was supported by the Ministry of Health and Department of Educational Assistance, University and Research of the Autonomous Province of Bolzano and the South Tyrolean Sparkasse Foundation.

The CoLaus Study was supported by research grants from GlaxoSmithKline, from the Swiss National Science Foundation (Grant number 33CSCO-122661) and from the Faculty of Biology and Medicine of Lausanne, Switzerland. We thank Yolande Barreau, Mathieu Firmann, Vladimir Mayor, Anne-Lise Bastian, Binasa Ramic, Martine Moranville, Martine Baumer, Marcy Sagette, Jeanne Ecoffey and Sylvie Mermoud for data collection. Finally we would like to express our gratitude to all the participants.

The Framingham Heart Study is supported by the National Heart, Lung, and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195), its contract with Affymetrix, Inc for genotyping services (Contract No.N02-HL-6-4278) and the resources of the Framingham Heart Study SNP Health Association Resource (SHARe) project, the National Institutes of Health, National Center for Research Resources, General Clinical Research Centers Program (Grant Number M01-RR-01066), an American Diabetes Association Career Development Award (J.B.M), a research grant from sanofi-aventis (J.B.M.), the Boston University Linux Cluster for Genetic Analysis (LinGA) funded by the NIH NCRR Shared Instrumentation grant (1S10RR163736-01A1) and the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center, the by the National Heart, Lung, and Blood Institute's Framingham Heart Study (Contract No. N01-HC-25195), National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK) R01 DK078616 to J.B.M, J.D., and J.C.F., NIDDK K24 DK080140 to J.B.M., NIDDK Research Career Award K23 DK65978, a Massachusetts General Hospital Physician Scientist Development Award and a Doris Duke Charitable Foundation Clinical Scientist Development Award to J.C.F., and the Boston University Linux Cluster for Genetic Analysis (LinGA) funded by the NIH NCRR Shared Instrumentation grant (1S10RR163736-01A1). M.F.H. was supported by the Centre de Recherche Medicale de l'Universite de Sherbrooke (CRMUS).

ALSPAC: We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. The UK Medical Research Council, the Wellcome Trust and the University of Bristol provide core support for ALSPAC. This work was supported by the Wellcome Trust.

TwinsUK: The study was funded by the Wellcome Trust; European Community's Seventh Framework Programme (FP7/2007-2013)/grant agreement HEALTH-F2-2008-201865-GEFOS and (FP7/2007-2013), ENGAGE project grant agreement HEALTH-F4-2007-201413 and the FP-5 GenomEUtwin Project (QLG2-CT-2002-01254). The study also receives support from the Dept of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas'. NHS Foundation Trust in partnership with King's College London. TDS is an NIHR senior Investigator. The project also received support from a Biotechnology and Biological Sciences Research Council (BBSRC) project grant. (G20234) .The authors acknowledge the funding and support of the National Eye Institute via an NIH/CIDR genotyping project (PI: Terri Young). Brent Richards receives salary support from the Canadian Institutes of Health Research. We thank the staff from the Genotyping Facilities at the Wellcome Trust Sanger Institute for sample preparation, Quality Control and Genotyping led by Leena Peltonen and Panos Deloukas; Le Centre National de Génotypage, France, led by Mark Lathrop, for genotyping; Duke University, North Carolina, USA, led by David

Goldstein, for genotyping; and the Finnish Institute of Molecular Medicine, Finnish Genome Center, University of Helsinki, led by Aarno Palotie. Genotyping was also performed by CIDR as part of an NEI/NIH project grant.

INCHIANTI: The INCHIANTI study baseline (1998-2000) was supported as a „targeted project" (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (Contracts: 263 MD 9164 and 263 MD 821336).
BLSA: The BLSA Study was supported in part by the Intramural Research Program of the NIH, National Institute on Aging. A portion of that support was through a R&D contract with MedStar Research Institute.

Supplementary Table S1: Characteristics of Study Samples

	# of subjects	Age (yrs)			BMI (kg/m_)			Adiponectin (μg/ml)			Adiponectin
Study	(% Women)	Combined	Women	Men	Combined	Women	Men	Combined	Women	Men	Assay
Genomewide association study samples											
KORA	1644 (50.5)	62.5±10.1	62.1±10.1	63.0±10.1	28.1±4.5	28.0±5.1	28.2±3.8	10.6±4.7	12.3±4.9	9.9±3.7	ELISA [a]
ERF	1820 (57.9)	48.7± 4.4	48.0±14.4	49.6±14.4	26.9±4.6	26.5±4.9	27.4±4.2	10.7±5.7	12.5±5.9	8.1±4.2	RIA [b]
MICROS	1195 (56.7)	44.8 ± 16.7	44.8±17.1	44.9±16.1	25.4±4.8	25.0±5.3	25.8±3.9	13.5±8.4	15.6±9.6	10.7±5.3	ELISA [c]
Replication study samples											
CoLaus	5381 (52.2)	53.2±10.8	53.6±10.7	52.7±10.8	25.8±4.6	25.1±4.9	26.6±4.2	10.1±8.1	12.4±9.4	7.4±5.4	ELISA [d]
Framingham	2228 (54.6)	60.4±9.5	60.4±9.5	60.3±9.5	27.8±5.0	27.2±5.4	28.4±4.3	10.5±6.4	13.0±6.8	7.6±4.5	ELISA [d]
GEMS	1780 (41.1)	52.5±9.5	52.4±9.6	52.7±9.4	28.5±3.6	28.7±4.0	28.3±3.4	6.8±4.8	8.3±5.5	5.8±4.0	ELISA [d]
ALSPAC	1415 (48.8)	9.9 ±0.3	9.8 ±0.2	9.9 ±0.3	17.2±3.5	17.2±4.1	17.2±2.7	13.0±5.3	13.3±5.5	12.8±5.1	ELISA [d]
TwinsUK	1399 (100)	48.5±13.1	48.5±13.1	-	25.1±4.7	25.1±4.7	-	8.1±3.9	8.1±3.9	-	ELISA[d]
InCHIANTI	1027 (54.7)	67.6±15.3	68.5±15.4	66.6±15.1	27.1±4.1	27.1±4.6	27.1±3.4	13.5±9.8	15.9±10.8	10.5±7.6	RIA [b]
BLSA	565 (47.1)	67.9±13.8	65.5+14.6	69.9+12.7	26.8±4.5	26.0+4.9	27.6+4.1	13.4±8.5	15.4±8.9	11.5±7.7	RIA [b]

Values stated are n (%) or mean ± SD

Assays used for measurement of adiponectin: [a] Mercodia; [b] Linco; [c] BioVendor; [d] R&D Systems

Supplementary Table S2: SNP characteristics for the SNPs selected for replication. Numbers stated are the minor allele frequencies and the imputation certainties (R_) for each of the three stage 1 studies KORA (n=1817), ERF (n=1195), or MICROS (n=1643).

			Allele		Minor allele frequency			Rsqr		
SNP	Chr	Pos (bp)	Major	Minor	KORA	ERF	MICROS	KORA	ERF	MICROS
Combined										
rs17366568	3	188053155	G	A	0.11	0.09	0.10	0.91*	0.37	0.27
rs8058648	16	25736408	G	C	0.38	0.37	0.36	0.64	0.74	0.73
rs7735993	5	140659543	A	G	0.21	0.32	0.16	0.98	0.98*	1.00*
rs6433017	2	151554095	C	T	0.15	0.15	0.13	0.29	0.44	0.41
rs936524	19	43924986	G	A	0.25	0.25	0.20	0.98	0.96	0.95
rs17554694	19	22108988	G	A	0.21	0.17	0.14	1.00*	0.91	0.85
rs1426438	12	114024489	A	G	0.18	0.22	0.20	0.98	0.98*	1.00*
rs2804441	10	128306965	T	C	0.48	0.50	0.46	0.88	0.90	0.92
rs1272041	9	114935061	G	C	0.38	0.43	0.44	1.00*	0.99	1.00
rs13201655	6	6295138	G	T	0.38	0.41	0.42	0.96	0.91	0.83
rs7235989	18	8726346	T	G	0.45	0.50	0.37	0.61	0.85	0.89
rs2460620	15	44085750	C	T	0.26	0.23	0.20	0.96*	0.80*	0.97*
rs6448895	4	12122006	C	G	0.10	0.09	0.08	0.64	0.58	0.75
rs7221927	17	76479722	T	C	0.33	0.34	0.30	0.96*	0.68	0.62
rs476546	11	132593133	A	G	0.33	0.30	0.26	0.96	0.99	0.99
rs10041164	5	103071961	C	T	0.40	0.41	0.49	0.96*	0.99*	0.99*
rs17810558	4	147342325	G	A	0.10	0.10	0.07	0.93	0.97	0.99
rs418410	5	31722785	T	G	0.48	0.45	0.46	0.75	0.96*	0.93*
rs7128545	11	32488991	G	A	0.09	0.12	0.13	0.76	0.78	0.81
rs7921500	10	10372106	C	T	0.34	0.39	0.31	0.73	0.87	0.87
Women										
rs7544470	1	212468592	T	A	0.38	0.42	0.35	0.90	0.99	1.00
rs12617829	2	55812968	C	T	0.10	0.13	0.10	0.85	0.91	0.83
rs9928327	16	2190234	G	T	0.12	0.09	0.03	0.17	0.94*	1.00*
rs1868521	3	42268266	G	A	0.06	0.06	0.06	0.38	0.46	0.34
rs2272439	16	87474262	G	A	0.17	0.12	0.15	0.40	0.91*	0.99*
rs2271265	4	48138173	T	A	0.05	0.07	0.07	0.98	0.98	1.00
rs12206888	6	167297384	G	A	0.08	0.08	0.08	0.67	0.45	0.36
rs11599120	10	132866667	T	G	0.25	0.32	0.24	0.65	0.97	0.99
rs17332108	5	60167641	T	C	0.26	0.20	0.25	1.00	0.99	0.98
rs2005029	17	2201888	A	C	0.39	0.49	0.38	0.80	0.95	0.97
Men										
rs2169877	15	83886806	A	G	0.25	0.29	0.18	0.93*	0.75	0.68
rs13073708	3	59443394	A	G	0.19	0.17	0.28	0.83	0.84	0.79
rs6495001	15	31339385	T	G	0.12	0.20	0.17	0.67	0.97*	0.97*
rs12598394	16	9945733	T	A	0.12	0.11	0.11	0.84	0.90	0.82
rs11767869	7	22823528	T	C	0.19	0.20	0.10	0.98*	0.97	1.00
rs17310106	14	96200749	C	T	0.34	0.36	0.31	0.91	0.90	0.94
rs8096456	18	13986751	G	A	0.23	0.18	0.21	0.99*	0.80	0.91
rs2328878	6	25405664	A	G	0.50	0.45	0.47	0.84	0.98*	1.00*
rs10879888	12	73877027	A	G	0.30	0.31	0.29	0.99*	0.97	0.97
rs6811805	4	184856851	A	G	0.07	0.07	0.07	0.22	0.59	0.67

* SNPs marked were genotyped, all other SNPs were imputed as described.

Supplementary Table S3: Association results for SNPs entering replication stage selected from gender-combined or gender-stratified analyses. For the top 20 SNPs representing independent loci from the gender-combined GWA analyses and the top 10 SNPs from the men only as well as 10 SNPs from the women only GWA analysis, replication stage data was obtained. Zscores and p-values are stated from meta-analyses of the three stage 1 studies, KORA, ERF, and MICROS (n=4659, men=2097, women=2562) as well as stage 1 and stage 2 studies combined (n=18425, men=8190, women=10235). Z scores are given into the direction of effect allele A1. Study-specific results were combined using the weighted Z-score method. Results are ordered by p-values from stage 1 in the three strata of analysis (combined, women and men).

						Combined				**Women**				**Men**			
			Nearest	Allele		Stage 1		Stage 1+2		Stage 1		Stage 1+2		Stage 1		Stage 1+2	
SNP	Chr	Position (bp)	gene	A1	A2	Zscore	P	Zscore	P	Zscore	P	Zscore	P	Zscore	P	Zscore	P
Combined																	
rs17366568	3	188053155	ADIPOQ	a	g	-10.125	4.3E-24	-13.527	1.1E-41	-8.322	8.7E-17	-9.709	2.8E-22	-6.674	2.5E-11	-9.838	7.8E-23
rs8058648	16	25736408	HS3ST4	c	g	4.613	4.0E-06	2.624	0.009	3.050	0.002	0.854	0.393	3.407	0.001	2.910	0.004
rs7735993	5	140659543	SLC25A2	a	g	-4.491	7.1E-06	-0.305	0.761	-2.142	0.032	-0.126	0.900	-4.408	1.0E-05	-0.262	0.793
rs6433017	2	151554095	RBM43	t	c	-4.487	7.2E-06	-1.377	0.168	-3.181	0.001	-1.558	0.119	-3.196	0.001	-0.213	0.831
rs936524	19	43924986	CAPN12	a	g	-4.338	1.4E-05	-2.592	0.010	-3.111	0.002	-1.619	0.106	-2.631	0.009	-1.973	0.049
rs17554694	19	22108988	ZNF257	a	g	4.285	1.8E-05	2.653	0.008	3.557	3.8E-04	2.412	0.016	2.841	0.004	1.529	0.126
rs1426438	12	114024489	TBX3	a	g	-4.188	2.8E-05	-2.176	0.030	-3.717	2.0E-04	-1.452	0.146	-1.917	0.055	-1.452	0.147
rs2804441	10	128306965	C10orf90	t	c	4.138	3.5E-05	2.443	0.015	2.057	0.040	0.867	0.386	3.895	9.8E-05	2.683	0.007
rs1272041	9	114935061	SLC31A2	c	g	4.101	4.1E-05	2.568	0.010	1.525	0.127	1.442	0.149	4.316	1.6E-05	2.216	0.027
rs13201655	6	6295138	F13A1	t	g	4.098	4.2E-05	2.173	0.030	3.806	1.4E-04	2.370	0.018	1.917	0.055	0.729	0.466
rs7235989	18	8726346	KIAA0802	t	g	-4.068	4.7E-05	-2.580	0.010	-2.561	0.010	-1.316	0.188	-4.225	2.4E-05	-2.907	0.004
rs2460620	15	44085750	SQRDL	t	c	4.060	4.9E-05	2.092	0.036	2.111	0.035	1.291	0.197	3.318	0.001	1.252	0.211
rs6448895	4	12122006	RAB28	c	g	-3.999	6.4E-05	-1.383	0.167	-3.061	0.002	-0.881	0.378	-2.550	0.011	-1.038	0.299
rs7221927	17	76479722	KIAA1303	t	c	3.983	6.8E-05	2.909	0.004	3.980	6.9E-05	1.737	0.082	1.969	0.049	2.629	0.009
rs476546	11	132593133	OPCML	a	g	-3.944	8.0E-05	-1.889	0.059	-3.546	3.9E-04	-1.858	0.063	-1.575	0.115	-0.434	0.664
rs10041164	5	103071961	NUDT12	t	c	-3.788	1.5E-04	-2.008	0.045	-2.416	0.016	-0.547	0.584	-2.608	0.009	-2.145	0.032
rs17810558	4	147342325	LSM6	a	g	-3.728	1.9E-04	-1.839	0.066	-2.602	0.009	-1.339	0.181	-2.572	0.010	-1.216	0.224
rs418410	5	31722785	PDZD2	t	g	-3.723	2.0E-04	-3.406	0.001	-2.081	0.037	-1.005	0.315	-3.620	2.9E-04	-4.007	6.2E-05
rs7128545	11	32488991	EIF3M	a	g	3.646	2.7E-04	1.625	0.104	1.822	0.068	0.350	0.726	3.413	0.001	2.032	0.042
rs7921500	10	10372106	CUGBP2	t	c	-3.578	3.5E-04	-3.479	0.001	-3.250	0.001	-2.042	0.041	-1.874	0.061	-2.885	0.004

						Combined				Women				Men			
			Nearest	Allele		Stage 1		Stage 1+2		Stage 1		Stage 1+2		Stage 1		Stage 1+2	
SNP	Chr	Position (bp)	gene	A1	A2	Zscore	P	Zscore	P	Zscore	P	Zscore	P	Zscore	P	Zscore	P
Men																	
rs2169877	15	83886806	AKAP13	a	g	3.548	3.9E-04	0.970	0.332	0.611	0.541	0.115	0.909	5.038	4.7E-07	1.509	0.131
rs13073708	3	59443394	FHIT	a	g	-3.344	0.001	-3.577	3.5E-04	-1.073	0.283	-1.796	0.073	-4.892	1.0E-06	-3.896	9.8E-05
rs6495001	15	31339385	RYR3	t	g	2.533	0.011	1.072	0.284	-0.727	0.467	-0.526	0.599	4.446	8.8E-06	2.069	0.039
rs12598394	16	9945733	GRIN2A	a	t	-3.530	4.2E-04	-1.569	0.117	-1.310	0.190	-1.165	0.244	-4.441	9.0E-06	-1.319	0.187
rs11767869	7	22823528	TOMM7	t	c	-2.247	0.025	-1.955	0.051	0.863	0.388	0.509	0.610	-4.418	9.9E-06	-3.446	5.7E-04
rs17310106	14	96200749	PAPOLA	t	c	3.323	0.001	1.322	0.186	1.060	0.289	0.772	0.440	4.384	1.2E-05	1.422	0.155
rs8096456	18	13986751	MC2R	a	g	2.317	0.020	0.525	0.599	-0.372	0.710	-0.348	0.728	4.121	3.8E-05	1.306	0.192
rs2328878	6	25405664	LRRC16A	a	g	2.269	0.023	0.784	0.433	-0.110	0.913	-1.026	0.305	4.103	4.1E-05	2.548	0.011
rs10879888	12	73877027	KCNC2	a	g	-3.596	3.2E-04	-2.480	0.013	-1.700	0.089	-0.245	0.807	-4.102	4.1E-05	-3.819	1.3E-04
rs6811805	4	184856851	C4orf41	a	g	2.008	0.045	1.960	0.050	-1.236	0.216	0.135	0.893	3.990	6.6E-05	2.816	0.005
Women																	
rs7544470	1	212468592	SMYD2	a	t	4.440	9.0E-06	2.116	0.034	5.188	2.1E-07	3.134	0.002	1.654	0.098	0.314	0.753
rs12617829	2	55812968	PNPT1	t	c	-3.814	1.4E-04	-2.052	0.040	-4.942	7.7E-07	-2.773	0.006	-0.558	0.577	-0.066	0.947
rs9928327	16	2190234	CASKIN1	t	g	-2.960	0.003	-4.063	4.9E-05	-4.520	6.2E-06	-3.941	8.1E-05	0.980	0.327	-1.337	0.181
rs1868521	3	42268266	CCK	a	g	-2.736	0.006	-1.806	0.071	-4.313	1.6E-05	-2.463	0.014	0.148	0.883	-0.166	0.868
rs2272439	16	87474262	CBFA2T3	a	g	2.944	0.003	1.253	0.210	4.227	2.4E-05	1.739	0.082	0.613	0.540	0.280	0.779
rs2271265	4	48138173	SLAIN2	a	t	4.045	5.2E-05	2.243	0.025	4.174	3.0E-05	2.404	0.016	2.219	0.026	1.235	0.217
rs12206888	6	167297384	RNASET2	a	g	-2.935	0.003	-1.659	0.097	-4.084	4.4E-05	-2.212	0.027	-0.658	0.511	-0.542	0.588
rs11599120	10	132866667	TCERG1L	t	g	-2.147	0.032	-1.096	0.273	-3.931	8.4E-05	-2.573	0.010	0.599	0.549	0.870	0.384
rs17332108	5	60167641	ELOVL7	t	c	2.835	0.005	1.121	0.262	3.880	1.0E-04	2.020	0.043	0.109	0.913	-0.413	0.679
rs2005029	17	2201888	SGSM2	a	c	-2.767	0.006	0.437	0.662	-3.753	1.8E-04	-0.395	0.693	-0.359	0.720	1.146	0.252

Supplementary Table S4: Metabolic syndrome parameter SNPs and their association with adiponectin. SNPs were selected as the most strongly associated SNP (according to p-value) in published genome-wide association studies (GWAS) for HDL cholesterol, triglycerides, waist circumference or BMI, type 2 diabetes mellitus or glucose concentrations, and hypertension. Stated are number of subjects, the p-value and the effect estimate (if available) from the published GWAS (stage 1 and stage 2 results combined if not stated otherwise) analysis for the respective trait. The p-value of these SNPs with adiponectin in the present study is computed from linear regression on log-transformed adiponectin concentration adjusted for age, sex, and BMI in the three meta-analyzed stage 1 studies (KORA, ERF, MICROS) (n=4655, women=2560, men=2095).

							Results from literature	**p-values for adiponectin**		
Gene	Chr	Position	rsnumber	Alleles	f (%)	Reference	n / p / effect	combined	women	men
HDL cholesterol										
LIPG	18	45421212	rs4939883	T (C)	17	(Kathiresan et al., 2009)	19,785 / 7.0x10-15 / -0.14 SD	0.72	0.73	0.80
CETP	16	55562980	rs1532624	C (A)	57	(Aulchenko et al., 2009)	19,674 / 9.4x10-94 / 8.24 z-sc	0.23	0.041	0.60
PLTP	20	44009909	rs7679	C (T)	19	(Kathiresan et al., 2009)	40,248 / 4.0x10-9 / -0.07 SD	0.49	0.02	0.11
NR1H3	11	47242866	rs7120118	C (T)	42	(Sabatti et al., 2009)	4,525 / 3.6x10-8 / 0.04 mmol/l	0.10	0.03	0.67
LPL	8	19888502	rs12678919	G (A)	10	(Kathiresan et al., 2009)	19,794 / 2.0x10-34 / 0.23 SD	0.53	0.78	0.50
LIPC	15	56470658	rs1532085	G (A)	59	(Aulchenko et al., 2009)	19,736 / 9.7x10-36 / 5.03 z-sc	0.70	0.99	0.56
ABCA1	9	106696891	rs3905000	G (A)	86	(Aulchenko et al., 2009)	17,913 / 8.6x10-13 / -4.37 z-sc	0.33	0.82	0.05
LCAT	16	66459571	rs2271293	A (G)	11	(Kathiresan et al., 2009)	31,946 / 9.0x10-13 / 0.07 SD	0.44	0.72	0.14
APOA1C3A4A5	11	116154127	rs964184	G (C)	14	(Kathiresan et al., 2009)	19,794 / 1.0x10-12 / -0.17 SD	0.55	0.80	0.16
APOB	2	21059688	rs6754295	C (A)	25	(Aulchenko et al., 2009)	17,915 / 4.4x10-8 / 2.63 z-sc	0.38	0.44	0.61
CTCF-PRMT8	16	66459571	rs2271293	G (A)	87	(Aulchenko et al., 2009)	17,910 / 8.3x10-16 / 4.99 z-sc	0.44	0.72	0.14
MADD-FOLH1	11	48475469	rs7395662	G (A)	61	(Aulchenko et al., 2009)	17,917 / 6.0x10-11 / 2.82 z-sc	0.69	0.11	0.35
GALNT2	1	228362314	rs4846914	G (A)	40	(Kathiresan et al., 2009)	19,794 / 4.0x10-8 / -0.05 SD	0.44	0.76	0.07
MVK/MMAB	12	108379551	rs2338104	C (G)	45	(Kathiresan et al., 2009)	19,793 / 1.0x10-10 / -0.07 SD	0.55	0.24	0.57
CLPTM1	19	50169221	rs16979595	A (G)	16	(Wallace et al., 2008)	1,636 / 6.1x10-3 / NA	0.74	0.79	0.21
FADS1-2-3	11	61327359	rs174547	C (T)	33	(Kathiresan et al., 2009)	40,330 / 2.0x10-12 / -0.09 SD	0.75	0.19	0.41
TTC39B	9	15279578	rs471364	C (T)	33	(Kathiresan et al., 2009)	40,414 / 3.0x10-10 / -0.08 SD	0.88	0.41	0.68
HNF4A	20	42475778	rs1800961	T (C)	3	(Kathiresan et al., 2009)	30,714 / 8.0x10-10 / -0.19 SD	0.98	0.57	0.38
ANGPTL4	19	8375738	rs2967605	T (C)	16	(Kathiresan et al., 2009)	35,151 / 1.0x10-8 / -0.12 SD	0.54	0.71	0.61
no name	17	2375258	rs9891572	T (C)	16	(Sabatti et al., 2009)	4,525 / 2.3x10-7 / 0.05 mmol/l	0.70	0.45	0.92
GRIN3A	9	103402758	rs1323432	A (G)	88	(Willer et al., 2008)	8,656 / 2.5x10-8 / 1.93 mg/dL	0.45	0.44	0.80

							Results from literature	p-values for adiponectin		
Gene	Chr	Position	rsnumber	Alleles	f (%)	Reference	n / p / effect	combined	women	men
Triglycerides [e]										
TOMM40-APOE	19	50106291	rs439401	G (A)	32	(Aulchenko et al., 2009)	17,913 / 1.8x10-09 / NA	0.15	0.04	0.97
NCAN, CILP2, PBX4	19	19523220	rs17216525	T (C)	7	(Kathiresan et al., 2009)	19,840 / 4.0x10-11 / -0.11 SD [a]	0.61	0.22	0.87
XKR6-AMAC1L2	8	11082571	rs7819412	G (A)	48	(Kathiresan et al., 2009)	33,336 / 3.0x10-08 / -0.04 SD [a]	0.86	0.89	0.97
LOC440069	11	116112647	rs1558861	T (C)	18	(Kooner et al., 2008)	≈12,000 / 1.6x10-23 / 0.08 SD	0.64	0.81	0.64
TRIB1	8	126560154	rs2954029	T (A)	44	(Kathiresan et al., 2009)	19,840 / 3.0x10-19 / -0.11 SD [a]	0.04	0.01	0.86
GCKR	2	27584444	rs1260326	T (C)	45	(Kathiresan et al., 2009)	19,840 / 2.0x10-31 / 0.12 SD [a]	0.34	0.92	0.19
NR1H3	11	47242866	rs7120118	A (G)	42	(Sabatti et al., 2009)	4,525 / 3.6x10-08 / 0.04 mmol/l	0.10	0.03	0.67
no name	15	36935941	rs2624265	C (T)	42	(Sabatti et al., 2009)	4,526 / 4.3x10-07 / 0.07 mmol/l	0.44	0.85	0.44
MLXIPL	7	72502805	rs714052	G (A)	12	(Kathiresan et al., 2009)	19,840 / 3.0x10-15 / -0.16 SD [a]	0.49	0.34	0.82
ANGPTL3, DOCK7	1	62704280	rs1167998	C (A)	32	(Aulchenko et al., 2009)	17,913 / 2.0x10-12 / NA	0.19	0.34	0.27
Body mass index										
FTO	16	52378028	rs9939609	A (T)	41	(Willer et al., 2009)	113,204/ 4.9x10-74/ 0.33 kg/m_ [b]	0.17	0.37	0.36
MC4R	18	56002077	rs17782313	C (T)	21	(Willer et al., 2009)	110,567 / 1.1x10-20 / 0.20 kg/m_ [b]	0.87	0.39	0.59
TMEM18	2	624905	rs6548238	C (T)	84	(Willer et al., 2009)	114,643 / 3.2x10-26 / 0.26 kg/m_ [b]	0.76	0.80	0.63
KCTD15	19	39013977	rs11084753	G (A)	45	(Willer et al., 2009)	101,526 / 4.5x10-12 / 0.06 kg/m_ [b]	0.98	0.86	0.91
GNPDA2	4	45023455	rs10938397	G (A)	41	(Willer et al., 2009)	81,758 / 3.4x10-16 / 0.19 kg/m_ [b]	0.98	0.78	0.62
SH2B1	16	28790742	rs7498665	G (A)	34	(Willer et al., 2009)	116,497 / 2.2x10-14 / 0.15 kg/m_ [b]	0.85	0.90	0.73
MTCH2	11	47619625	rs10838738	G (A)	67	(Willer et al., 2009)	110,737 / 1.9x10-11 / 0.07 kg/m_ [b]	0.38	0.47	0.93
NEGR1	1	72524461	rs2815752	A (G)	62	(Willer et al., 2009)	113,319 / 6.0x10-8 / 0.10 kg/m_ [b]	0.32	0.08	0.80
Waist circumference [f]										
TFAP2B	6	50911009	rs987237	G (A)	16	(Lindgren et al., 2009)	118,691/ 1.9x10-11/ 6.72 z-sc [c]	0.21	0.11	0.72
MSRA	8	9897480	rs7826222	G (C)	18	(Lindgren et al., 2009)	80,210/ 8.9x10-9/ 5.75 z-sc [c]	0.03	0.39	0.03
LYPLAL1	1	217710,837	rs2605100	G (A)	69	(Lindgren et al., 2009)	47,633/ 2.6x10-8 / 5.57 z-sc	0.16	0.40	0.17
NRXN3	14	79014915	rs10146997	G (A)	21	(Heard-Costa et al., 2009)	70,014 / 5.3x10-8 / 0.0498 z-sc	0.82	0.87	0.67

to be continued on page 98-99

Supplementary Table S4 Continue

							Results from literature	p-values for adiponectin		
Gene	Chr	Position	rsnumber	Alleles	f (%)	Reference	n / p / effect	combined	women	men
Type 2 diabetes mellitus [g]										
PPARG	3	12368125	rs1801282	C (G)	90	(Zeggini et al., 2007)	14,586+17,968 [d] / 1.7_10-6 / OR: 1.14	0.91	0.85	0.64
KCNJ11	11	17365206	rs5215	C (T)	40	(Zeggini et al., 2007)	14,586+17,968 [d] / 5.0_10-11 / OR: 1.14	0.08	0.06	0.63
TCF7L2	10	114744078	rs7901695	C (T)	28	(Zeggini et al., 2007)	14,586+17,968 [d] / 1.0_10-48 / OR: 1.37	0.64	0.83	0.75
IGF2BP2	3	186994389	rs4402960	T (G)	30	(Zeggini et al., 2007)	14,586+17,968 [d] / 8.6_10-14 / OR: 1.14	0.07	0.88	0.02
CDKN2(A)/2B	9	22124094	rs10811661	T (C)	80	(Zeggini et al., 2007)	14,586+17,968 [d] / 7.8_10-15 / OR: 1.20	0.20	0.49	0.29
CDKAL1	6	20769013	rs10946398	A (C)	66	(Zeggini et al., 2007)	14,586+17,968 [d] / 4.1_10-11 / OR: 1.12	0.24	0.10	0.96
SLC30A8	8	118253964	rs13266634	C (T)	76	(Zeggini et al., 2007)	14,586+17,968 [d] / 5.3_10-8 / OR: 1.12	0.46	0.19	0.96
HHEX/IDE	10	94455539	rs5015480	C (T)	58	(Zeggini et al., 2007)	14,586+17,968 [d] / 5.7_10-10 / OR:1.13	0.42	0.80	0.08
NOTCH2	1	120230001	rs10923931	T (G)	11	(Zeggini et al., 2008)	58,667 / 4.0x10-08 / OR: 1.13	0.17	0.14	0.85
ADAMTS9	3	64686944	rs4607103	C (T)	76	(Zeggini et al., 2008)	62,387 / 1.2x10-08 / OR: 1.09	0.68	0.66	0.09
THADA	2	43644474	rs7578597	T (C)	90	(Zeggini et al., 2008)	60,832 / 1.1x10-09 / OR: 1.15	0.29	0.09	0.63
TSPAN8 / LGR5	12	69949369	rs7961581	C (T)	27	(Zeggini et al., 2008)	62,301 / 1.1x10-09 / OR: 1.09	0.03	0.01	0.78
CDC123, CAMKID	10	12368016	rs12779790	G (A)	18	(Zeggini et al., 2008)	62,366 / 1.2x10-10 / OR: 1.11	0.72	0.44	0.72
JAZF1	7	27953796	rs864745	T (C)	50	(Zeggini et al., 2008)	59,617 / 5.0x10-14 / OR: 1.10	0.44	0.98	0.28
KCNQ1	11	2796327	rs2237892	C (T)	92	(Yasuda et al., 2008)	1,612+1,424 [d] / 6.7x10-13 / OR: 1.49	0.45	0.62	0.28
Glucose [h]										
G6PC2	2	169471394	rs560887	A (G)	30	(Bouatia-Naji et al., 2008)	9,353 / 4.0x10-23 / -0.06 mmol/l	0.39	0.38	0.67
MTNR1B	11	92348358	rs10830963	G (C)	30	(Prokopenko et al., 2009)	36,610 / 3.2x10-50 / 0.072 mmol/l	0.27	0.05	0.83
Hypertension and blood pressure										
MTHFR	1	11797044	rs17367504	G (A)	14	(Newton-Cheh et al., 2009)	82,973 / 2.0x10-13 / -0.85 mmHg SBP	0.19	0.16	0.38
CYP17A1	10	104836168	rs11191548	T (C)	91	(Newton-Cheh et al., 2009)	132,552 / 7.0x10-24 / 1.16 mmHg SBP	0.62	0.66	0.87
PLCD3	17	40563647	rs12946454	T (A)	28	(Newton-Cheh et al., 2009)	77,690 / 1.0x10-08 / 0.57 mmHg SBP	0.73	0.32	0.54
MDS1	3	170648590	rs1918974	T (C)	54	(Newton-Cheh et al., 2009)	87,891 / 8.0x10-08 / -0.27 mmHg DBP	0.85	0.80	0.97
PRDM8 / FGF5	4	81541520	rs16998073	T (A)	19	(Newton-Cheh et al., 2009)	101,623 / 1.0x10-21 / 0.50 mmHg DBP	0.27	0.54	0.18
C10orf107	10	63194597	rs1530440	T (C)	19	(Newton-Cheh et al., 2009)	87,273 / 1.0x10-09/ -0.39 mmHg DBP	0.10	0.30	0.15
SH2B3 / ATXN2	12	110470476	rs653178	T (C)	53	(Newton-Cheh et al., 2009)	79,661 / 3.0x10-18 / -0.46 mmHg DBP	0.67	0.20	0.55

							Results from literature	p-values for adiponectin		
Gene	Chr	Position	rsnumber	Alleles	f (%)	Reference	n / p / effect	combined	women	men
CYP1A1	15	72864420	rs1378942	C (A)	37	(Newton-Cheh et al., 2009)	134,258 / 1.0x10-23 / 0.43 mmHg DBP	0.11	0.79	0.11
ZNF652	17	44795465	rs16948048	G (A)	39	(Newton-Cheh et al., 2009)	82,441 / 5.0x10-09 / 0.31 mmHg DBP	0.72	0.29	0.18
ATP2B1	12	88533090	rs2681472	NA	NA	(Levy et al., 2009)	29,136 / 1.7x10-08 / -0.16 mmHg	0.24	0.79	0.07
ITGA9	3	37571809	rs7640747	NA	NA	(Levy et al., 2009)	29,136 / 4.8x10-07/ 0.12 mmHg	0.98	0.41	0.41
CACNB2	10	18748804	rs11014166	NA	NA	(Levy et al., 2009)	29,136 / 7.8x10-07/ -0.11 mmHg	0.26	0.94	0.07
CDH13	16	81200160	rs11646213	A (T)	41	(Org et al., 2009)	3557 / 5.3x10-08 / OR: 0.67	0.10	0.34	0.15

Alleles: Effect allele (Non-effect allele)

f (%), Frequency of effect allele (%)

Abbreviations: z-sc, z-score units; SD, standard deviation; NA, not applicable; SBP, systolic blood pressure; DBP, diastolic blood pressure; OR, odds ratio; Chr, chromosome

[a] Sample size and p value are provided for the combined stage 1 and stage 2 samples.

[b] Sample size and p value are provided for the combined stage 1, stage 2 and DECODE sample, estimate is taken from the stage 2 population-based cohorts

[c] Sample size and p value are provided for the combined stage 1, stage 2 and CHARGE sample.

[d] Number of cases + controls

[e] Genes already mentioned for HDL cholesterol are no longer mentioned for triglycerides (e.g. PLTP, LPL, LIPC, APOA1C3A4A5, APOB, GALNT2, FADS1-FADS2-FADS3)

[f] Genes already mentioned for BMI are no longer mentioned for waist circumference (e.g. FTO, MC4R)

[g] FTO was already mentioned for BMI and is no longer mentioned for type 2 diabetes

[h] GCKR was already mentioned for triglycerides and is no longer mentioned for glucose

Supplementary Table S5: Bioinformatic analysis of all SNPs in the proximity of rs17366568 (between rs822396and rs2241767).

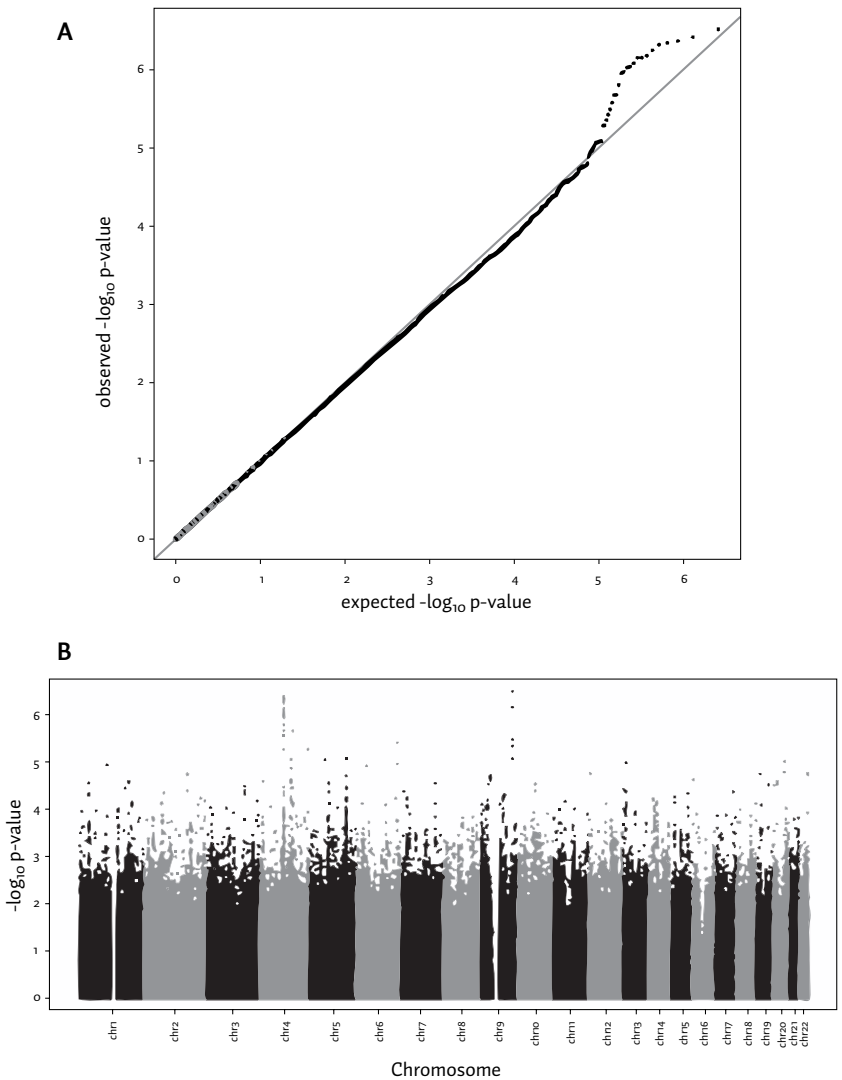
SNP	Distance to rs17366568	FASTSNP Prediction	FASTSNP Risk Score [min. 1 - max. 5]	Region	Genomatix Adipose-TFBS	PupaSuite	F-SNP	Visual SNP	HAPMAP
rs34265972	-2751	Intronic enhancer	1-2	intronic	0	Conserved	n.a.	n.a.	no
rs13066093	-2410	Intronic enhancer	1-2	intronic	0	No effect	n.a.	n.a.	no
rs12495941	-2273	Intronic enhancer	1-2	intronic	0	No effect	n.a.	n.a.	yes
rs36219755	-1675	Intronic enhancer	1-2	intronic	1	No effect	n.a.	n.a.	no
rs7649121	-1668	Intronic enhancer	1-2	intronic	0	No effect	n.a.	n.a.	yes
rs7627128	-1654	Intronic enhancer	1-2	intronic	1	No effect	n.a.	n.a.	yes
rs36219760	-1261	Intronic enhancer	1-2	intronic	0	No effect	n.a.	n.a.	no
rs9877202	-846	Intronic enhancer	1-2	intronic	0	No effect	n.a.	n.a.	yes
rs36219762	-791	Intronic enhancer	1-2	intronic	10	No effect	n.a.	n.a.	no
rs17366568	**0**	**Intronic enhancer**	**1-2**	**intronic**	**0**	**No effect**	**n.a.**	**n.a.**	**yes**
rs34046054	41	Intronic enhancer	1-2	intronic	6	No effect	n.a.	n.a.	no
rs34513325	178	Intronic enhancer	1-2	intronic	4	No effect	n.a.	n.a.	no
rs2241766	439	Sense/synonymous; Splicing regulation	2-3	coding	0	No effect	Splicing regulation	No effect	no
rs62622816	490	Sense/synonymous; Splicing regulation	2-3	coding	0	No effect	n.a.	splicing regulation	no
rs13061862	555	Missense (non-conservative); Splicing regulation	3-4	coding	0	Splicing regulation	splicing, protein damaging	protein damaging	yes
rs1501299	670	Intronic enhancer	1-2	intronic	0	No effect	No effect	No effect	yes

n.a.; not analyzed

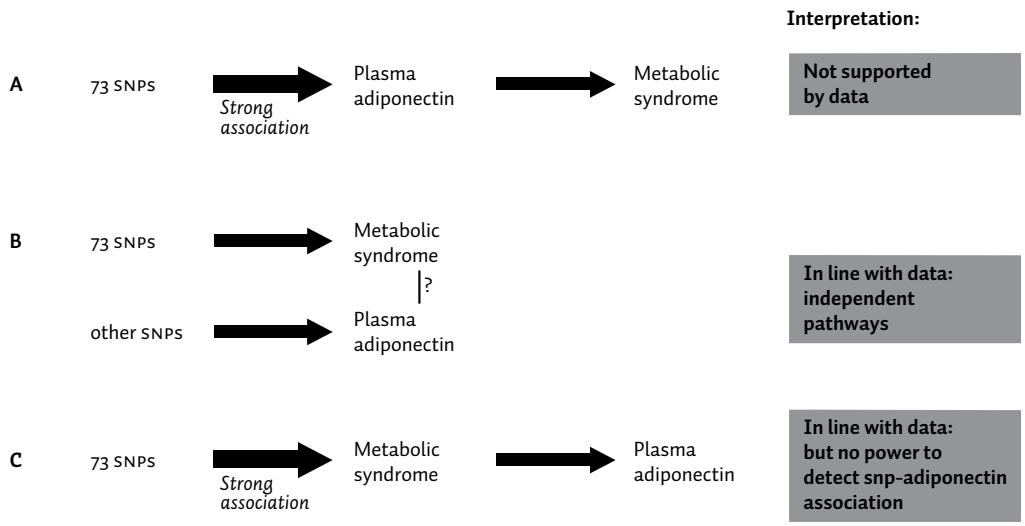
A description of the programs used and mentioned in the column headers of this table is provided in the chapter "Bioinformatic Analysis" on page 6 of the Supplementary Material.

Supplementary Table S6: Position of the predicted promoter regions downstream of ADIPOQ gene region.

		Position		
	Size [bp]	Start	End	Distance from ADIPOQ
Region 1	331	188.063.725	188.064.056	4.779 bp
Region 2	379	188.064.893	188.065.272	5.947 bp
Region 3	319	188.074.441	188.074.760	15.495 bp



Supplementary Figure S1: Differences between the gender-specific beta-estimates. Panel A: the quantile-quantile (QQ) plot of SNPs for the respective p-values shows some observed gender difference of SNP effects beyond the expected by chance. Expected p-values are plotted on X-axis against the observed p-values plotted on the Y-axis. P-values derived from the 200 kB region around ADIPOQ (position ranging from 187950 to 188150 Kb) are depicted as red dots. Panel B: Manhattan plot showing p-values for the difference between men and women of association of each SNPs in the meta-analysis with plasma adiponectin levels. SNPs are plotted on the X-axis to their position on each chromosome against p-values for the gender difference in the SNP association with plasma adiponectin on the Y-axis (shown as –log10 P-value).



Supplementary Figure S2: Illustration on the debate whether plasma adiponectin affects metabolic syndrome parameters or metabolic syndrome parameters modulate adiponectin (for explanation, see Discussion section of the main paper). The 73 SNPs refer to the SNPs selected from previous genome-wide association studies on metabolic syndrome parameter loci (see Supplementary Table S4).

REFERENCES

1. Abecasis, G.R., Cherny, S.S., Cookson, W.O., Cardon, L.R., 2002. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nat.Genet 30, 97-101.

2. Andrew, T., Hart, D.J., Snieder, H., de, L.M., Spector, T.D., MacGregor, A.J., 2001. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. Twin.Res. 4, 464-477.

3. Aulchenko, Y.S., Heutink, P., Mackay, I., Bertoli-Avella, A.M., Pullen, J., Vaessen, N. et al., 2004. Linkage disequilibrium in young genetically isolated Dutch population. Eur.J.Hum.Genet 12, 527-534.

4. Aulchenko, Y.S., Ripatti, S., Lindquist, I., Boomsma, D., Heid, I.M., Pramstaller, P.P. et al., 2009. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. Nat.Genet. 41, 47-55.

5. Bartali, B., Benvenuti, E., Corsi, A.M., Bandinelli, S., Russo, C.R., Di, I.A. et al., 2002. Changes in anthropometric measures in men and women across the life-span: findings from the INCHIANTI study. Soz.Praventivmed. 47, 336-348.

6. Bouatia-Naji, N., Rocheleau, G., Van, L.L., Lemaire, K., Schuit, F., Cavalcanti-Proenca, C. et al., 2008. A polymorphism within the G6PC2 gene is associated with fasting plasma glucose levels. Science. 320(5879), 1085-1088.

7. Coassin, S., Brandstätter, A., Kronenberg, F., 2009. Lost in the space of bioinformatic tools: a constantly updated survival guide for genetic epidemiology. The GenEpi Toolbox. Atherosclerosis

8. Dawber, T.R., Kannel, W.B., 1966. The Framingham study. An epidemiological approach to coronary heart disease. Circulation 34, 553-555.

9. Ferrucci, L., Bandinelli, S., Benvenuti, E., Di, I.A., Macchi, C., Harris, T.B. et al., 2000. Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the INCHIANTI study. J.Am.Geriatr.Soc. 48, 1618-1625.

10. Firmann, M., Mayor, V., Vidal, P.M., Bochud, M., Pecoud, A., Hayoz, D. et al., 2008. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. BMC Cardiovasc.Disord. 8, 6.

11. Golding, J., Pembrey, M., Jones, R., 2001. ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr.Perinat.Epidemiol. 15, 74-87.

12. Heard-Costa, N.L., Zillikens, M.C., Monda, K.L., Johansson, A., Harris, T.B., Fu, M. et al., 2009. NRXN3 is a novel locus for waist circumference: a genome-wide association study from the CHARGE Consortium. PLoS Genet 5, e1000539.

13. Hivert, M.F., Manning, A.K., McAteer, J.B., Florez, J.C., Dupuis, J., Fox, C.S. et al., 2008. Common variants in the adiponectin gene (ADIPOQ) associated with plasma adiponectin levels, type 2 diabetes, and diabetes-related quantitative traits: the Framingham Offspring Study. Diabetes 57, 3353-3359.

14. Kannel, W.B., Feinleib, M., McNamara, P.M., Garrison, R.J., Castelli, W.P., 1979. An investigation of coronary heart disease in families. The Framingham offspring study. Am J Epidemiol. 110, 281-290.

15. Kathiresan, S., Willer, C.J., Peloso, G.M., Demissie, S., Musunuru, K., Schadt, E.E. et al., 2009. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat.Genet. 41, 56-65.

16. Kooner, J.S., Chambers, J.C., guilar-Salinas, C.A., Hinds, D.A., Hyde, C.L., Warnes, G.R. et al., 2008. Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. Nat.Genet. 40, 149-151.

17. Levy, D., Ehret, G.B., Rice, K., Verwoert, G.C., Launer, L.J., Dehghan, A. et al., 2009. Genome-wide association study of blood pressure and hypertension. Nat.Genet , 677-687.

18. Lindgren, C.M., Heid, I.M., Randall, J.C., Lamina, C., Steinthorsdottir, V., Qi, L. et al., 2009. Genome-Wide Association Scan Meta-Analysis Identifies Three Loci Influencing Adiposity and Fat Distribution. PLoS Genet 5, e1000508.

19. Löwel, H., Döring, A., Schneider, A., Heier, M., Thorand, B., Meisinger, C. et al., 2005. The MONICA Augsburg surveys - Basis for prospective cohort studies. Gesundheitswesen 67 Suppl 1, S13-S18.

20. Melzer, D., Perry, J.R., Hernandez, D., Corsi, A.M., Stevens, K., Rafferty, I. et al., 2008. A genome-wide association study identifies protein quantitative trait loci (pQTLs). PLoS.Genet. 4, e1000072.

21. Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M.D., Bochud, M., Coin, L. et al., 2009. Genome-wide association study identifies eight loci associated with blood pressure. Nat.Genet

22. Org, E., Eyheramendy, S., Juhanson, P., Gieger, C., Lichtner, P., Klopp, N. et al., 2009. Genome-wide scan identifies CDH13 as a novel susceptibility locus contributing to blood pressure determination in two European populations. Hum.Mol.Genet. 18, 2288-2296.

23. Pattaro, C., Marroni, F., Riegler, A., Mascalzoni, D., Pichler, I., Volpato, C.B. et al., 2007. The genetic study of three population microisolates in South Tyrol (MICROS): study design and epidemiological perspectives. BMC Med.Genet 8, 29.

24. Prokopenko, I., Langenberg, C., Florez, J.C., Saxena, R., Soranzo, N., Thorleifsson, G. et al., 2009. Variants in MTNR1B influence fasting glucose levels. Nat.Genet. 41(1), 77-81.

25. Richards, J.B., Rivadeneira, F., Inouye, M., Pastinen, T.M., Soranzo, N., Wilson, S.G. et al., 2008. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet 371, 1505-1512.

26. Sabatti, C., Service, S.K., Hartikainen, A.L., Pouta, A., Ripatti, S., Brodsky, J. et al., 2009. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. Nat.Genet. 41, 35-46.

27. Shock, N.W., Greulick, R.C., Andres, R., Arenberg, D., Costa, P., Lakatta, E. et al., 1984. Normal Human Aging: The Baltimore Study of Aging. NIH Publication N.84-2450

28. Stirnadel, H., Lin, X., Ling, H., Song, K., Barter, P., Kesaniemi, Y.A. et al., 2008. Genetic and phenotypic architecture of metabolic syndrome-associated components in dyslipidemic and normolipidemic subjects: the GEMS Study. Atherosclerosis 197, 868-876.

29. Timpson, N.J., Tobias, J.H., Richards, J.B., Soranzo, N., Duncan, E.L., Sims, A.M. et al., 2009. Common variants in the region around Osterix are associated with bone mineral density and growth in childhood. Hum.Mol.Genet 18, 1510-1517.

30. Wallace, C., Newhouse, S.J., Braund, P., Zhang, F., Tobin, M., Falchi, M. et al., 2008. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. American Journal of Human Genetics 82, 139-149.

31. Willer, C.J., Sanna, S., Jackson, A.U., Scuteri, A., Bonnycastle, L.L., Clarke, R. et al., 2008. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat.Genet. 40, 161-169.

32. Willer, C.J., Speliotes, E.K., Loos, R.J., Li, S., Lindgren, C.M., Heid, I.M. et al., 2009. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat.Genet. 41(1), 25-34.
33. Yasuda, K., Miyake, K., Horikawa, Y., Hara, K., Osawa, H., Furuta, H. et al., 2008. Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nat.Genet. 40(9), 1092-1097.
34. Zeggini, E., Scott, L.J., Saxena, R., Voight, B.F., Marchini, J.L., Hu, T. et al., 2008. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat.Genet. 40, 638-645.
35. Zeggini, E., Weedon, M.N., Lindgren, C.M., Frayling, T.M., Elliott, K.S., Lango, H. et al., 2007. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 316, 1336-1341.