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Henneman, P.

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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 Coassin⁵, Thomas Winkler¹, Yurii S. Aulchenko⁶, Christian Fuchsberger⁴, Kijoung Song⁷, Marie-France Hivert⁸, Dawn M. Waterworth⁷, Nicholas J. Timpson⁹, J. Brent Richards^{10,11}, John R.B. Perry¹², Toshiko Tanaka^{13,14}, Najaf Amin³, Barbara Kollerits⁵, Irene Pichler⁴, Ben A. Oostra⁶, Barbara Thorand², Rune R. Frants³, Thomas Illig², Josée Dupuis¹⁵, Beate Glaser⁹, Tim Spector¹¹, Jack Guralnik¹⁶, Josephine M. Egan¹⁷, Jose C. Florez^{18,19,20}, David M. Evans⁹, Nicole Soranzo^{11,21}, Stefania Bandinelli²², Olga D. Carlson¹⁷, Timothy M. Frayling¹², Keith Burling²³, George Davey Smith⁹, Vincent Mooser⁷, Luigi Ferrucci¹⁴, James B. Meigs^{18,24}, Peter Vollenweider²⁵, Ko Willems van Dijk^{3,26}, Peter Pramstaller^{4,27,28*}, Florian Kronenberg^{5,29*}, Cornelia M. v. Duijn^{3*} (* authors contributed equally)

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

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

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

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

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

⁶Department of Epidemiology, Erasmus MC Rotterdam, The Netherlands

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

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

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

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

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

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

¹³Medstar Research Institute, Baltimore, MD, USA

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

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

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

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

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

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

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

²¹Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK

²²Geriatric Rehabilitation Unit, Azienda Sanitaria Firenze (ASF), Florence, Italy

²³University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrookes' Hospital, University of Cambridge, Cambridge CB2 0QQ, UK

²⁴General Medicine Division, Massachusetts General Hospital, Boston, MA, USA

²⁵Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

²⁶Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands

²⁷Department of Neurology, General Central Hospital, Bolzano, Italy

²⁸Department of Neurology, University of Lübeck, Lübeck, Germany

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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 ($p=4.3 \times 10^{-24}$) as well as in both women- and men-specific analyses ($p=8.7 \times 10^{-17}$ and $p=2.5 \times 10^{-11}$, 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 syndrome³⁻⁵. 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 extensively⁸: 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 approach⁹.

Plasma adiponectin shows pronounced differences between men and women with about 1.5 times higher concentrations in women¹⁰. 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-dependent¹⁴. In line with lower plasma adiponectin in men, higher prevalences of type 2 diabetes and impaired fasting glucose were also reported in men¹⁵.

Recent genome-wide association (GWA) scans have highlighted the potential of genetic factors with differential sex effects on concentrations of uric acid¹⁶⁻¹⁸ and lipids¹⁹, waist circumference²⁰ or schizophrenia²¹. 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 analysis²⁰. 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 ($\alpha=5 \times 10^{-8}$). 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% or 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 GenABEL²⁵.

Bioinformatic analysis: Bioinformatic analysis for potential functional SNPs was done in two stages, using bioinformatic tools outlined in the GenEpi Toolbox²⁶ (Supplementary Material).

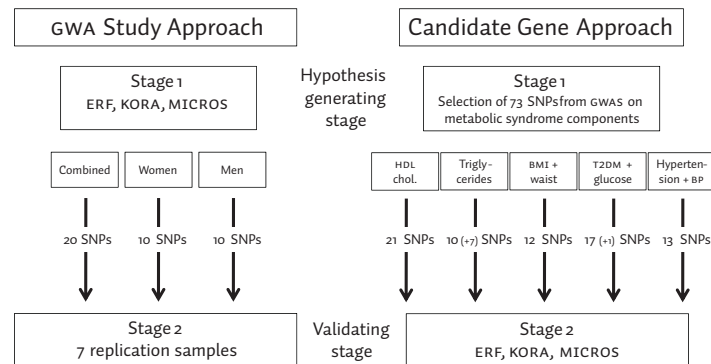


Fig.1. Study design illustrating the genome-wide association (GWA) study approach and the candidate gene approach.

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 ($p=4.3 \times 10^{-24}$), which was consistent in women ($p=8.7 \times 10^{-17}$) and men ($p=2.5 \times 10^{-11}$) (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 ($p=3.0 \times 10^{-16}$) and had good imputation quality in all three stage 1 samples ($0.82 < r^2 < 0.97$).

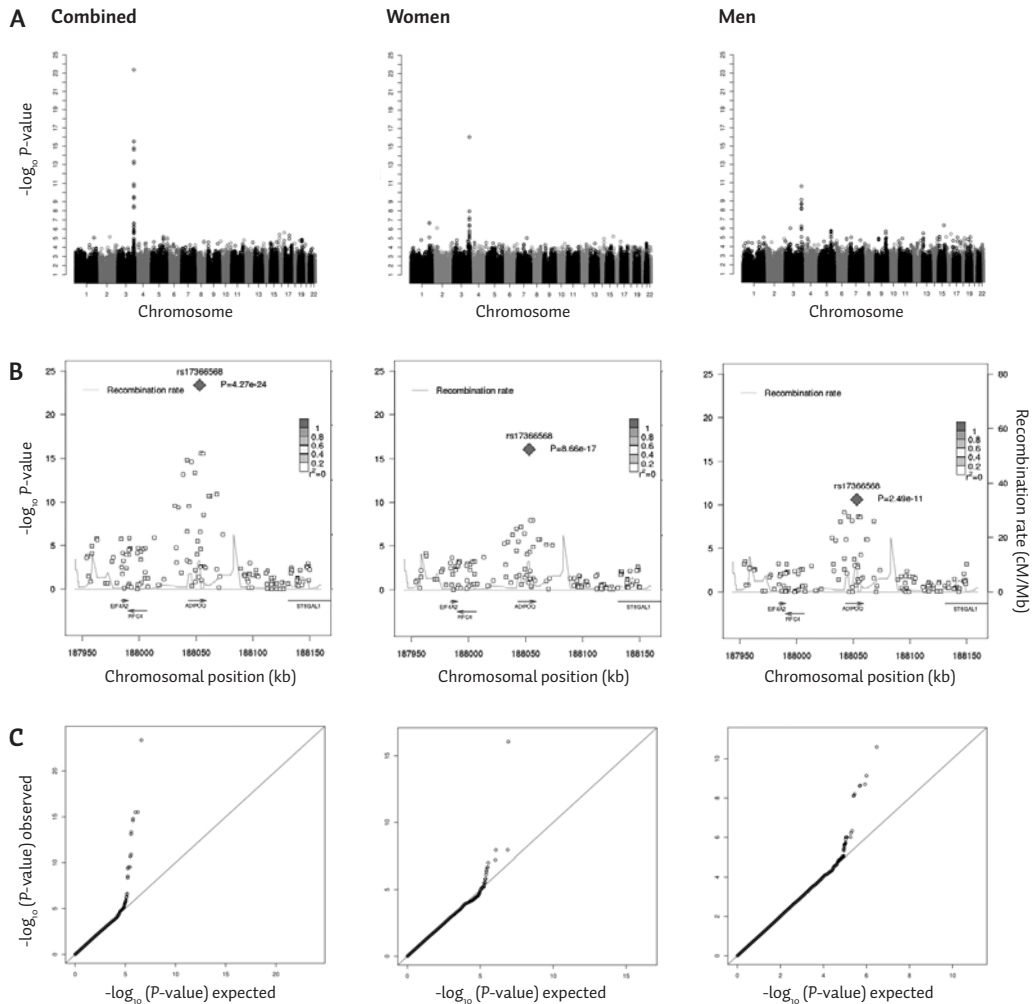


Fig. 2. The analyses in panel A-c are provided for the combined sex analysis as well as the analysis stratified for women and men. A. Manhattan plots showing p-values of association of each SNPs in the meta-analysis with plasma adiponectin levels. SNPs are plotted on the X-axis to their position on each chromosome against association with plasma adiponectin on the Y-axis (shown as $-\log_{10} P$ -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 $-\log_{10} P$ -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 r -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

| Population | EAF* | Rsqr [†] | n | Combined | | Women | | | Men | | |
|--------------------|-------|-------------------|-------|-------------------|---------|-------|-------------------|---------|------|-------------------|---------|
| | | | | 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(μ g/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 r² measures (Figure 3) revealed that for many SNPs in the ADIPOQ region the r² 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).

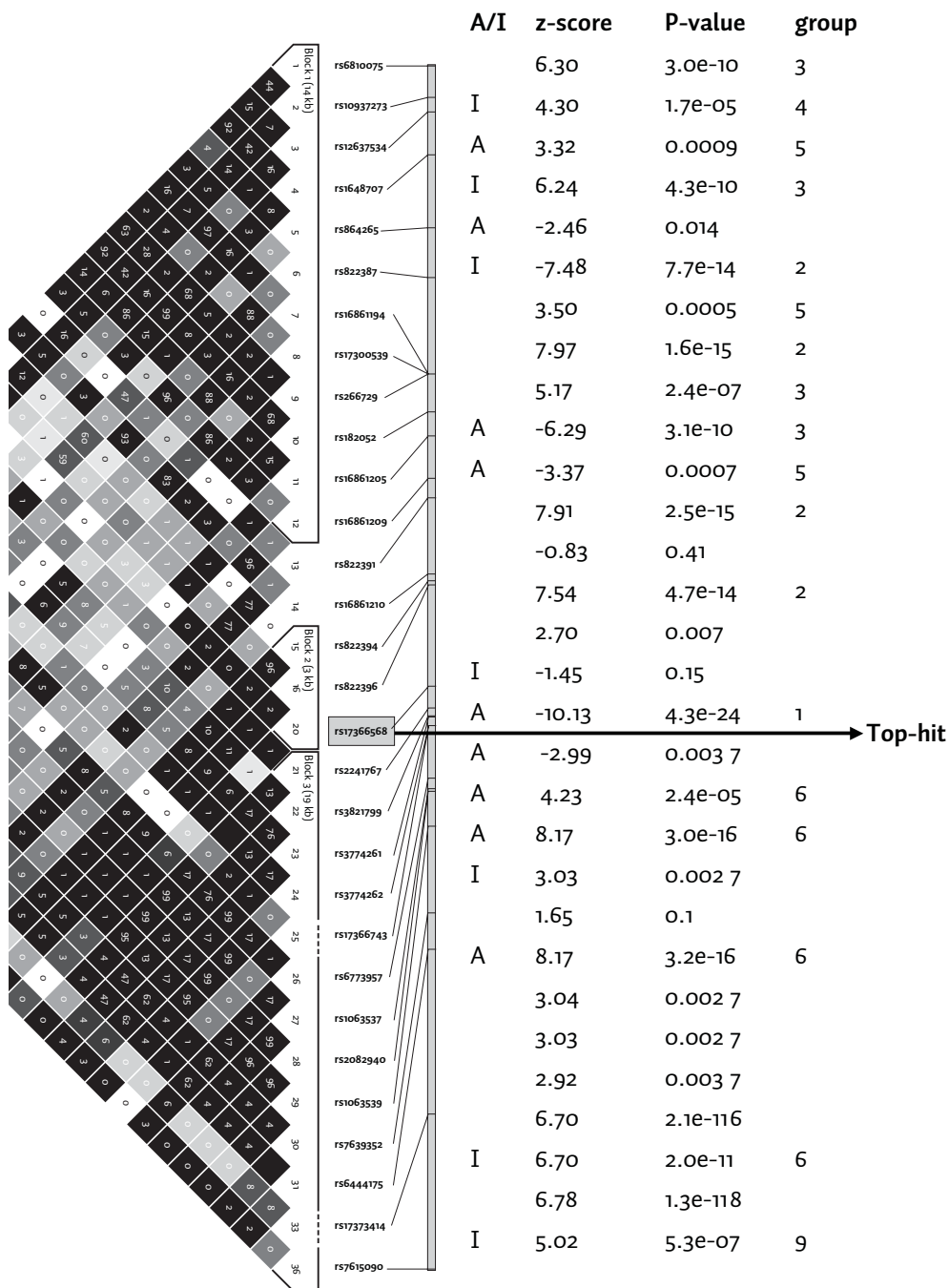


Fig.3. Linkage disequilibrium (LD) plot of SNPs in the ADIPOQ region spanning 50kb (positions 188030-188080kb). The grey shading of the diamonds represent the pair-wise D' and the numbers in the diamonds represent the pair-wise r between the two SNPs defined by the top left and the top right sides of the diamond. The figure clearly shows that the top-hit rs17366568 is located within its 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 $r > 0.60$ 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).

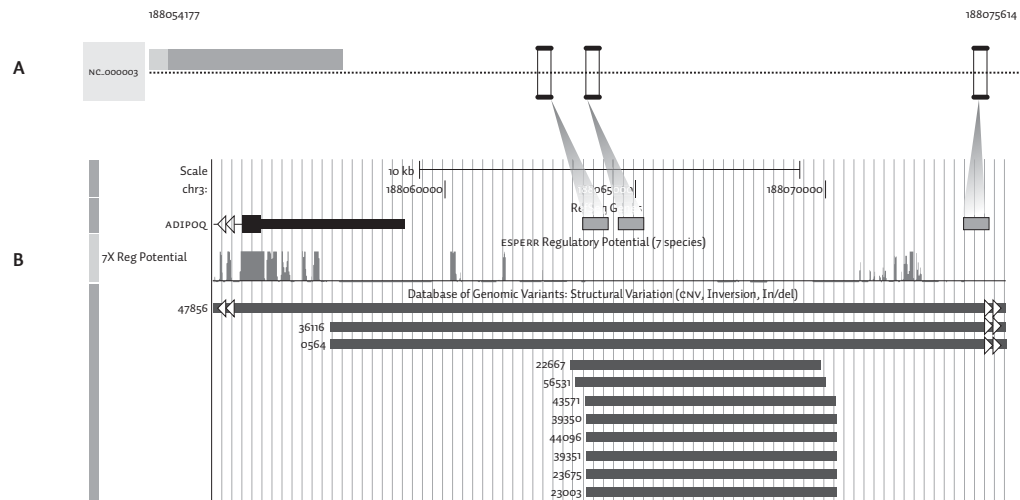


Fig. 4. Predicted regulatory regions in the *ADIPOQ* downstream region and their affection by copy number variations. Panel A: Genomatix Software Suite: Position of the regulatory promoter regions predicted by PromoterInspector (red boxes) and their position relative the *ADIPOQ* gene region (green box). 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 significance²⁸. 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 sites²⁹. Other studies showed that histone acetylation might influence the transcriptional regulation of the *ADIPOQ* gene³⁰ and that pioglitazone increases plasma adiponectin by posttranscriptional regulation³¹. 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 components³⁻⁵, but in-line with previous reports on a lack of association of the *ADIPOQ* SNPs with metabolic syndrome parameters¹⁰. 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 hormones⁵ or sex-specific epigenetic programming that could be transmitted to subsequent generations in a sex-specific manner leading to transgenerational effects as recently suggested³².

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 reports¹⁰. 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 adiponectin³⁴, 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 gossomes 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.

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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 height²) 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 and 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. $se_beta_men^2 + se_beta_women^2 + 2 \times corr(beta_men, beta_women) \times se_beta_men \times se_beta_women$). 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/>).

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Supplementary Table S1: Characteristics of Study Samples

| Study | # of subjects | Age (yrs) | | | BMI (kg/m ²) | | | Adiponectin (µg/ml) | | | Adiponectin |
|---|---------------|-----------|-----------|-----------|--------------------------|----------|----------|---------------------|-----------|----------|--------------------|
| | (% 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_i) for each of the three stage 1 studies KORA (n=1817), ERF (n=1195), or MICROS (n=1643).

| SNP | Chr | Pos (bp) | Allele | | Minor allele frequency | | | Rsqr | | |
|-----------------|-----|-----------|--------|-------|------------------------|------|--------|-------|-------|--------|
| | | | 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 | 15154095 | 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. Z-scores 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).

| SNP | Chr | Position (bp) | Nearest gene | Allele A1 A2 | Combined | | | | Women | | | | Men | | | |
|------------|-----|---------------|--------------|--------------|----------|-----------|---------|-----------|---------|-----------|---------|-----------|--------|---------|--------|---------|
| | | | | | Stage 1 | Stage 1+2 | Stage 1 | Stage 1+2 | Stage 1 | Stage 1+2 | Stage 1 | Stage 1+2 | | | | |
| | | | | | Zscore | P | Zscore | P | Zscore | P | Zscore | P | Zscore | P | | |
| 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 |
| rs17272041 | 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 |

| SNP | Chr | Position (bp) | Nearest gene | Allele A1 A2 | Combined | | | | Women | | | | Men | | | |
|--------------|-----|---------------|--------------|--------------|----------|---------|-----------|---------|---------|---------|-----------|---------|---------|--------|-----------|---|
| | | | | | Stage 1 | | Stage 1+2 | | Stage 1 | | Stage 1+2 | | Stage 1 | | Stage 1+2 | |
| | | | | | 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 | 0.909 | 5.038 | 4.7E-07 | 1.509 | 0.131 | |
| rs193073708 | 3 | 59443394 | FHIT | a g | -3.344 | 0.001 | -3.577 | 3.5E-04 | -1.073 | 0.283 | -1.796 | -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 | 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 | -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 | -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 | 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 | 4.121 | 3.8E-05 | 1.306 | 0.192 | |
| rs2328878 | 6 | 25405664 | LRRCL6A | a g | 2.269 | 0.023 | 0.784 | 0.433 | -0.110 | 0.913 | -1.026 | 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 | -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 | 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 | 0.314 | 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 | -0.066 | 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 | -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 | -0.166 | 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 | 0.280 | 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 | 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 | -0.542 | 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 | 0.870 | 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 | -0.413 | 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 | 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).

| Gene | Chr | Position | rsnumber | Alleles | f (%) | Reference | Results from literature | | p-values for adiponectin | | |
|------------------------|-----|-----------|------------|---------|-------|---------------------------|-------------------------|------------------------------------|--------------------------|-----------|------|
| | | | | | | | 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) | 1,656 | 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 |

| Gene | Chr | Position | rsnumber | Alleles | f (%) | Reference | Results from literature | | p-values for adiponectin | |
|--|-----|------------|------------|---------|-------|----------------------------|---|----------|--------------------------|------|
| | | | | | | | n / p / effect | combined | women | men |
| Triglycerides^e | | | | | | | | | | |
| TOMM40-APOE | 19 | 50106291 | r5439401 | G (A) | 32 | (Aulchenko et al., 2009) | 17,913 / 1.8x10 ⁻⁰⁹ / NA | 0.15 | 0.04 | 0.97 |
| NCAN, CILP2, PBX4 | 19 | 19523220 | r517216525 | 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 | r57819412 | 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 | r51558861 | T (C) | 18 | (Kooner et al., 2008) | ≈2,000 / 1.6x10 ⁻²³ / 0.08 SD | 0.64 | 0.81 | 0.64 |
| TRIB1 | 8 | 126560154 | r52954029 | 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 | r51260326 | 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 | r57120118 | 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 | r52624265 | 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 | r5714052 | 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 | r51167998 | 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 | r59939609 | 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 | r517782313 | 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 | r56548238 | 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 | r511084753 | 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 | r510938397 | 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 | r57498665 | 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 | r510838738 | 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 | r52815752 | 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 | r5987237 | 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 | r57826222 | 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 | r52605100 | 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 | r510146997 | 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

| Gene | Chr | Position | rsnumber | Alleles | f (%) | Reference | Results from literature | | p-values for adiponectin | |
|--|-----|-----------|------------|---------|-------|------------------------------|---|----------|--------------------------|------|
| | | | | | | | 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: 1.370.64 | | 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 |
| CDKN2A(z)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, CAMK1D | 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 | 16947394 | rs5560887 | A (G) | 30 | (Bouatia-Najji 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 |
| CYP7A1 | 10 | 104836168 | rs1191548 | 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 | rs5653178 | T (C) | 53 | (Newton-Cheh et al., 2009) | 79,661 / 3.0x10 ⁻¹⁸ / -0.46 mmHg DBP | 0.67 | 0.20 | 0.55 |

| Gene | Chr | Position | rsnumber | Alleles | f (%) | Reference | Results from literature | | p-values for adiponectin | |
|--------|-----|----------|------------|---------|-------|----------------------------|---|----------|--------------------------|------|
| | | | | | | | 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 GOKR 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 rs82239G and rs2241767).

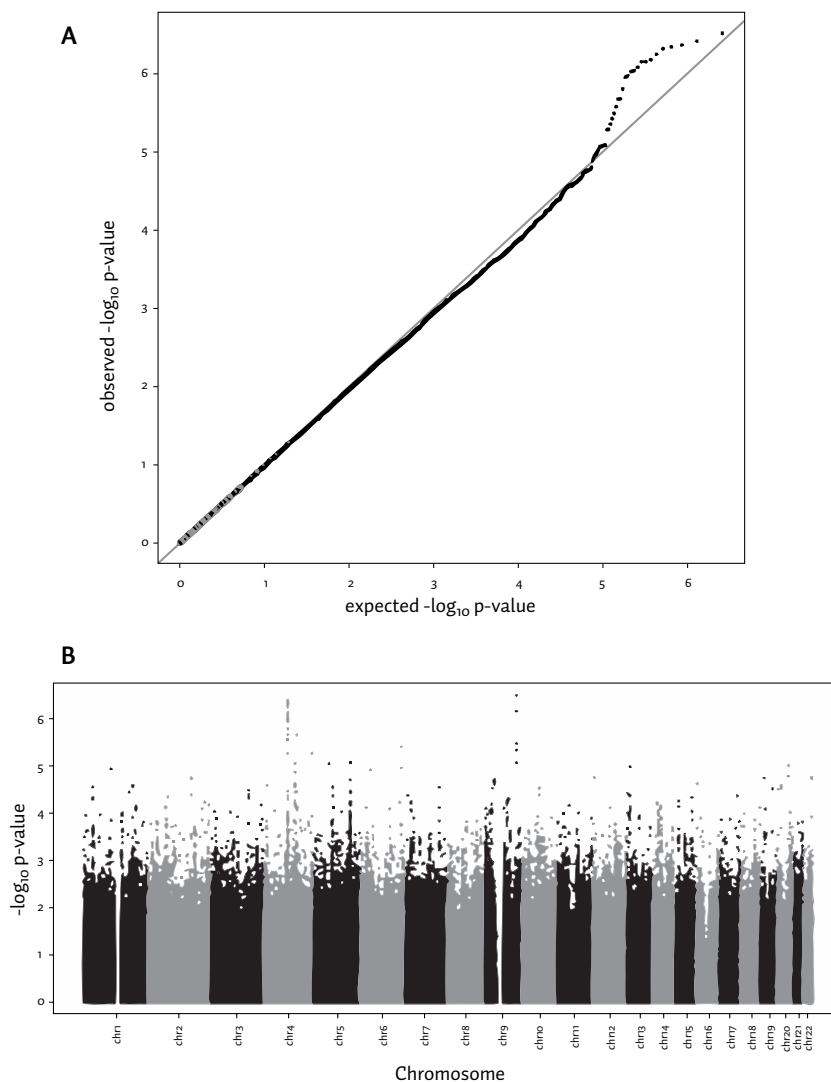
| SNP | Distance to rs17366568 | FASTSNP Prediction | FASTSNP Risk Score [min. 1 - max. 5] | Region | Genomatix Adipose-TFBS | PupaSuite | F-SNP | Visual SNP | HAPMAP |
|-------------------|------------------------|---|--------------------------------------|-----------------|------------------------|---------------------|-------------------------------|---------------------|------------|
| 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 |
| rs6262816 | 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.

| | Size [bp] | Position | | Distance from ADIPOQ |
|----------|-----------|-------------|-------------|----------------------|
| | | Start | End | |
| 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 $-\log_{10}$ P-value).

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