Single nucleotide polymorphisms of *ADIPOQ* gene associated with cardiovascular disease risk factors in European adolescents: the HELENA study

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Running head: *ADIPOQ* polymorphisms and CVD risk factors

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Abstract

Objectives: Cardiovascular diseases (CVDs) are responsible of 31% of all deaths worldwide. Genetic predisposition to CVDs in adolescents remains largely unknown. Aims of present research are to examine the association of *ADIPOQ* gene polymorphisms with cardiovascular disease risk factors in European adolescents.

Methods: A total of 14 polymorphisms in the *ADIPOQ* gene were genotyped in 1.057 European adolescents (12-18 years old) from the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. We measured serum lipids and a CVD risk score, along with weight, height, triceps and subscapular skinfold thickness, leptin, insulin and other markers of glucose regulation.

Results: The rs822393, rs822395 and rs7649121 polymorphisms of *ADIPOQ* gene were significantly associated with several CVD risk factors (i.e., high-density lipoprotein cholesterol [HDL-C], Apolipoprotein (Apo) A1, systolic blood pressure and CVD risk Score) in European adolescents. We also found an association of the TGAAGT *ADIPOQ* haplotype (rs822393, rs16861210, rs822395, rs822396, rs12495941 and rs7649121) with HDL-C and ApoA1 levels.

Conclusions: Several individual polymorphisms (rs822393, rs822395 and rs7649121) and a haplotype of *ADIPOQ* gene were significantly associated with cardiovascular disease risk factors in European adolescents.

Condensed Abstract

In this work we investigate the association between several genetic variants of *ADIPOQ* (adiponectin) and Cardiovascular disease (CVD) risk factors in European adolescents under a broad range of heritage models. We found that several individual polymorphisms (rs822393, rs822395, rs7649121) and a haplotype of *ADIPOQ* gene

were significantly associated with CVD risk factors in European adolescents. These results support the importance of *ADIPOQ* on the genetic architecture of cardiovascular physiopathology. Our findings are congruent with the role of ADIPOQ in the regulation of glucose and lipid metabolism, becoming a promising target against cardiovascular disease.

Keywords: *ADIPOQ*, cardiovascular disease, genetic susceptibility, adolescents, SNP

Introduction

Almost 18 million people die from cardiovascular disease (CVD) worldwide every year [1], which means 31% of global deaths. The exact cause of CVD is still unclear, but numerous risk factors are described, such as smoking, high blood pressure, high blood cholesterol levels, diabetes, inactivity, overweight/obesity, and family history of CVD, among others [2].

As most diseases, the development of CVDs is the result of a complex interplay of genetic and environmental factors. Some studies have shown a strong genetic cause of CVD as coronary artery disease [3] or sickle cell anemia [4]. Genetic susceptibility to CVD is commonly set up by multiple genes and polymorphisms acting together. A recent review [5] collected a list of genetic markers that influence HDL-C levels, LDL levels, triglycerides levels and other cardiovascular disease risk factors, as well as some heritable conditions such as coronary artery disease, atrial fibrillation or myocardial infarction. Other studies have described the influence of several genetic polymorphisms on stroke [6,7], myocardial infarction [8,9] and the whole set of cardiovascular complications [10].

Interesting single nucleotide polymorphisms (SNP) candidates would be those that have a wide range of physiological effects like glucose and lipid homeostasis. One such candidate is adiponectin (*ADIPOQ* gene), an adipocytokine secreted almost exclusively by adipocytes [11]. This hormone has a wide range of physiological benefits in the organism. Berg *et al*. [12] found that adiponectin improves insulin resistance, suggesting that this molecule inhibits glucose production and/or improves glucose oxidation independently of insulin levels. Others have described its role in lipid metabolism, increasing beta-oxidation and inhibiting lipid accumulation in the liver [13]. Indeed, low levels of adiponectin may contribute to the increased risk for

cardiovascular complications in obesity, insulin resistance and diabetes [14,15]. A recent meta-analysis [16] studied the association between *ADIPOQ* SNPs and coronary artery disease. The authors reported that the *rs1501299* polymorphism is associated with the susceptibility to coronary artery disease in Caucasians, East Asians and South Asians, while the *rs2241766* polymorphism only shows this association in East Asians. In another meta-analysis, Kanu *et al*. [17] examined the associations of three *ADIPOQ* SNPs (*rs266729*, *rs2241766*, and *rs1501299*) with several CVDs (atherothrombotic cerebral infarction, acute Coronary Syndrome, atherosclerosis, coronary artery disease, coronary heart disease (CHD), and myocardial infarction). They found a significantly increased CVD risk associated with the rs266729 and rs2241766 polymorphisms, but not with the rs1501299 polymorphism. Another study described that rs266729, rs822395, rs1501299 and rs2241766 polymorphisms were all significantly associated with the susceptibility to coronary artery disease [3] in certain populations [18]. Therefore, still some inconsistencies exist in research findings on the association between CVD and SNPs of *ADIPOQ*. Mostly, studies have focused solely on clinical end-point complications instead of on the early CVD risk factors, which makes it difficult to disentangle mechanistic pathways.

Data obtained within the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study provide an excellent opportunity to study the association between *ADIPOQ* gene polymorphisms with cardiovascular risk factors in European adolescents. The HELENA study was designed to provide reliable data on nutrition and health-related variables in a relatively large sample of European adolescents from 9 different countries and includes information on 14 single nucleotide polymorphisms (SNPs) of the *ADIPOQ* gene as well as CVD risk factors. Therefore, the aim of this

140 study was to examine the association between 14 *ADIPOQ* polymorphisms with CVD
141 risk factors in European adolescents.
142
143 **Material and methods**
144 *Participants*
145 The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross-
146 sectional study attempted to report the lifestyle and nutritional status of European
147 adolescents. A total of 3865 participants (12-18 year old) of 10 European cities in 9
148 countries (Greece, Germany, Belgium, France, Hungary, Italy, Sweden, Austria and
149 Spain) were selected to be part of this study. They were randomly selected from public
150 and private schools in each city between October 2006 and December 2007. We
151 collected blood samples of one-third of these participants (N=1155) with the
152 consequent genetic analysis and clinical biochemistry assays. Finally, 1057 (552 girls)
153 adolescents with *ADIPOQ* gene polymorphisms and CVD risk factors data were
154 included in this study. Adolescents and corresponding parents/guardians were fully
155 informed about objectives and methods of the study such as inclusion criteria [19,20],
156 and signed an informed written consent. Ethical guidelines of the Declaration of
157 Helsinki 1964 (revision of Edinburgh 2000), Good Clinical Practice, and legislation
158 about clinical research in humans in each of the participating countries were respected
159 in the study. Human research committees of each involved center approved the protocol
160 [21].
161
162 *Assessment of cardiovascular disease risk factors*
163 A total of 30 ml of venous blood was extracted between 8 and 10 am in fasting
164 conditions (ten hours after the last meal). Samples were centrifuged (3.500rpm/15min)

within 30 min and, thereafter, stored and transported (4-7ºC) to the central laboratory (Bonn, Germany). Serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density cholesterol (LDL-C), Apolipoprotein (Apo) A1, ApoB, triglycerides and glucose were measured in fresh samples on the Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany). Stability of samples had been tested before [22]. For the rest, samples were deposited at -80ºC until analysis, all in centralized laboratories.

Plasma insulin was measured by a solid-phase two-site chemiluminescent immunometric assay with an Immulite 2000 analyzer (DPC Biermann GmbH, Bad Nauheim, Germany). We computed the homeostatic model assessment (HOMA) as a marker of insulin resistance ([glycaemia X insulin]/22.5). In addition, the quantitative insulin sensitivity check index (QUICKI) was calculated as 1/[log(insulin) + log(glycaemia)]. Concentration of serum Leptin was measured using the RayBio® Human Leptin ELISA (Enzyme-Linked Immunosorbent Assay) kit. Blood pressure was measured with an automatic oscillometric device (OMRON M6). Adolescents quietly sat for 5 min before the measurements, conducted on the right arm in an extended position. Two measures were taken 5 min apart, and the mean of both values (in mmHg) was used in analyses. Anthropometric measures were collected according to standardised techniques [23]: weight was measured by an electronic scale (SECA 861), height with a telescopic instrument (SECA 225), and triceps and subscapular skinfolds with a skinfold caliper (Holtain). Body mass index (BMI) was calculated by dividing weight in kilograms by height in square meters. We computed a CVD risk score by adding the mean of the standardized value [(value-mean)/standard deviation] of the following variables: total cholesterol/HDL-C, triglycerides, HOMA, systolic blood

189 pressure, and triceps and subscapular skinfolds, as done previously in other pediatric
190 cohorts [24,25].
191
192 *Genotyping*
193 Blood for DNA extraction was collected in EDTA K3 tubes, stored at the Analytical
194 Laboratory at the University of Bonn and then sent to the Genomic Analysis Laboratory
195 at the Institut Pasteur de Lille (Lille, France). DNA was extracted from white blood
196 cells with the Puregene kit (Qiagen, Courtaboeuf, France) and stored at 20 °C. Samples
197 were genotyped using an Illumina System (Illumina, Inc, San Diego, California) with
198 the GoldenGate software (Inc, San Francisco, California). A high genotyping success
199 was achieved (≥99.8%) and each polymorphism respected the Hardy-Weinberg
200 equilibrium (HWE) (P≥0.01 in all cases; Table 1). Several polymorphisms of the
201 *ADIPOQ* gene were in linkage disequilibrium (Figure 1).
202
203 *Statistical analysis*
204 Deviations from HWE were determined by means of an exact test, considering a p value
205 of 0.01 as a threshold. Associations between genetic markers and CVD risk factors were
206 assessed through linear models. Five genetic models (dominant, recessive, additive,
207 codominant and overdominant) were used for all analyses, except in those where
208 rs16861210 and rs17366743 polymorphisms were involved. These polymorphisms
209 were analysed using only a dominant model due to the low number of minor
210 homozygotes (Minor allele frequency [MAF] < 0.1; Table 1). Adjustment variables
211 were BMI, age, sex and center. For each polymorphism, p values were computed using
212 the likelihood ratio test (LRT) between a model with the polymorphism and a null
213 model without it. These analyses were performed with the "SNPassoc" R package [26].

We considered the associations between all SNPs and each phenotype under a given heritage model as family test, i.e., the number of tests were equal to the number of SNPs. Therefore, a corrected p value for multiple comparisons following the Bonferroni method would be 0.0036 (0.05/14). We selected the significant associations to perform further haplotype analysis. Given the exploratory nature of these analyses and the reduced number of independent tests (markers are in linkage disequilibrium, see below), the Bonferroni correction could be too stringent [27]. Instead of this method, we performed an exploratory selection of associations using an approach that controls the expected proportion of false positives (False discovery rate [FDR]) [28,29]. Therefore, associations with FDR < 0.1 were used in haplotype analyses.

We also examined the statistical power of the analyses to detect true associations (Table S1). We calculated the statistical power to detect single phenotype-polymorphism associations under additive, dominant and recessive models, using the "genpwr" R package [30]. Power calculations were based on sample size, standard deviation of the phenotype and minor allele frequency of the polymorphism. In addition, we considered the significance threshold used to match FDR<0.1 and the explicative power of the polymorphism (R^2). We found adequate levels of power for those associations with high signification (and hence high R^2), suggesting that the capacity to detect weaker association could be limited in our study.

Linkage disequilibrium between polymorphisms and haplotype block structures were evaluated with Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview) and haplo.stats [31]. First, haplotype blocks were generated by the algorithm of four-gamete rules [32]. For each block, we tested if the observed frequencies of haplotypes were deviated from the expected under linkage equilibrium. Finally, we assessed the association between haplotypes and phenotypes by means of a permutation procedure.

Only additive and dominant models were considered given the low frequency of some haplotypes. For those significant associations, we performed regressions between haplotypes and phenotypes with the purpose of testing significant differences between the different haplotypes levels. Again, the FDR was calculated using the p values to check differences between the reference haplotype (the most frequent) and other haplotypes.

Results

The characteristics of the study sample are shown in Table 2.

Association between ADIPOQ polymorphisms and CVD Risk Markers

Three of the fourteen analysed SNPs (rs822393, rs822395 and rs7649121) showed significant associations with CVD risk factors after the Bonferroni correction (Figures 2, 3 and 4). The significant associations after a less stringent correction (FDR<0.05) for all markers are shown in supplementary appendix (Figures S2-S4), along with the p values for each association (Figures S5-S22).

We observed an association between the minor T allele of the rs822393 polymorphism and lower HDL-C levels under the dominant, codominant, recessive and additive models (p=0.0024, p=0.00036, p=0.00085 and p=0.00018, respectively) and with lower ApoA1 levels under the dominant, codominant, recessive and additive models (p=4e−04, p=0.00013, p=0.00134 and p=4e−05, respectively) (Figures 2 and 3).

Regarding the rs822395 polymorphism, we observed an association of the major A allele polymorphism with lower ApoA1 levels under the dominant, codominant, recessive and additive models (p=0.00234, p=0.00123, p=0.00314 and

p=0.00027, respectively), higher SBP under the dominant and additive models (p=0.00087 and p=0.00231, respectively), and higher CVD risk score under the recessive and additive models (p=0.00234 and p=0.00238, respectively) (Figures 3 and 4).

Finally, we observed an association of the minor T allele of the rs7649121 polymorphism with lower HDL-C levels under the dominant, codominant, overdominant and additive heritage models (p=0.00011, p=1e−04, p=0.0034 and p=2e−05 respectively), and with lower ApoA1 levels under the dominant, codominant, overdominant and additive models (p=0.00019; p=0.00026; p=0.00347 and p=5e−05, respectively) (Figures 2 and 3).

Association between ADIPOQ polymorphism haplotypes and CVD risk factors

ADIPOQ block 1 contains rs822393, rs16861210, rs822395, rs822396, rs12495941 and rs7649121 polymorphisms (Figure 1). Haplotype TGAAGT of *ADIPOQ* was significantly associated with lower HDL-C levels than CGAATA (global p=0.00335; difference between groups 0.04; 95CI = 0.07 - 0.01; P = 0.0042; FDR=0.02940; under the dominant model / / global p=0.0088; difference between groups 0.04; 95CI = 0.06 - 0.02; P = 0.0011; FDR=0.0077; under the additive model; differences between groups obtained from models with the log-transformed response variable). The TGAAGT haplotype was also associated with lower ApoA1 levels than the CGAATA haplotype (global p=0.0095; difference between groups 0.03; 95CI = 0.05 - 0.01; P = 0.0039; FDR=0.0273; under the dominant model / / global p=0.00375; difference between groups 0.03; 95CI = 0.05 - 0.01; P = 0.0026; FDR=0.0182; under the additive model).

Discussion

289 The primary findings of this study are the observed significant associations between the
290 rs822393, rs822395 and rs7649121 polymorphisms of *ADIPOQ* gene and several CVD
291 risk factors (i.e., HDL-C, ApoA1, SBP, CVD risk score) in European adolescents. We
292 also found a significant association of the TGAAGT *ADIPOQ* haplotype (rs822393,
293 rs16861210, rs822395, rs822396, rs12495941 and rs7649121) with HDL-C and ApoA1
294 levels. Taken together, these findings highlight the important genetic influence on
295 cardiovascular profile in European adolescents.

296 To our knowledge, this is the first study investigating the association between
297 *ADIPOQ* gene polymorphisms and CVD risk factors in European adolescents. In a
298 sample of 1457 Mexican children aged 9.24 ± 2.07 years-old, He et al. [33] did not find
299 any significant associations between four *ADIPOQ* polymorphisms (i.e., rs182052,
300 rs2241766, rs266729, rs822393) and neither cardio-metabolic traits, nor circulating
301 adiponectin concentrations. In agreement with these findings, in the current study the
302 rs182052 and rs2241766 polymorphisms were not associated with CVD risk factors.
303 However, in contrast with their results, we found strong associations between the
304 rs822393 polymorphism and HDL-C and ApoA1 serum levels under several genetic
305 models (dominant, codominant, recessive and additive models). Both studies have
306 similar sample sizes and were performed in non-adult populations, so these
307 discordances may be due to the fact that these polymorphisms may be population
308 dependent, with possibly different allele frequencies and penetrance in these
309 populations. Their study sample was based on urban population of central Mexico,
310 while children of nine different European countries set up our study.

311 A meta-analysis on the association between adiponectin gene polymorphisms
312 and coronary artery disease showed that the A allele of the rs822395 (-4034A>C)
313 polymorphism was significantly associated with a higher risk to suffer coronary artery

disease under the additive model (OR = 1.20, 95% CI = 1.02–1.43) in Caucasian adults [34]. These findings concur with our results. Our data show that the A allele of the rs822395 polymorphism is associated with lower ApoA1 levels, higher SBP and a higher CVD risk Score.

Another study based on a Chinese Han population [35] showed that homozygous carriers of the T allele of the rs1501299 polymorphism and the G allele of the rs2241766 polymorphism were associated with an increased CHD risk, and that the homozygous T allele carriers of the rs7649121 polymorphism had a decreased CHD risk. Moreover, the authors found a significant interaction between smoking and GG or GT genotypes of the rs1501299 polymorphism with higher risk of CHD. In our study, we did not find any significant association between the rs1501299 and rs2241766 polymorphisms and CVD risk factors; yet, we observed a robust association of the T allele of the rs7649121 polymorphism with lower HDL-C and ApoA1 levels. These findings are of importance due to the strong evidence of the inverse association between HDL-C levels and CHD [36]. Similarly, numerous studies have shown that ApoA1 provides identical or even improves the ability to identify patients at risk for future CHD and prognostic information as HDL-C [37]. However, causality of this association is still unclear. It is possible that this association is influenced by other confounding factors that are causally associated with CHD risk, as well as with HDL-C, and could be primarily responsible for these epidemiologic observations [38]. Nevertheless, genetic heritability might be population-dependent, with possibly different allele frequencies and penetrance in these populations, which could explain these discordances. Also, age differences between both studies are remarkable (mean age of 61.8 ± 12.1 years).

To our knowledge, fewer studies with unclear results regarding haplotypes of *ADIPOQ* and CVD risk have been performed. Pischon *et al*. [39] did not find an association between a five polymorphisms haplotype (rs266729, rs822395, rs822396, rs2241766 and rs1501299) and CHD. In contrast, we found that the TGAAGT haplotype of *ADIPOQ (*rs822393, rs16861210, rs822395, rs822396, rs12495941 and rs7649121) was significantly associated with lower HDL-C and ApoA1 serum levels. Sample size, age, genetic variants of the haplotype and the follow up period (6-8 years) could explain differences with our results.

Adiponectin regulates glucose and lipid metabolism, influencing the development of multiple metabolic disorders including obesity/overweight and diabetes mellitus. Therefore, it is evident that these two common metabolic disorders were associated with an increased risk of CVD [40]. A possible mechanism that may partially explain the observed associations in our study is that polymorphisms or haplotypes of *ADIPOQ* could alter adiponectin functions and therefore predispose to an increased CVD risk. Adiponectin increases serum HDL-C [41] through several mechanisms: i) via the increase of the hepatic production of ApoA1, which is the main apolipoprotein of HDL-C [42], and ii) via the activation of lipoprotein lipase and ATP-binding cassette transporter A1 and inhibition of hepatic lipase, which can also reduce triglycerides [43]. Overall, adiponectin seems to play a role in the development of CVD. Our result could be partially explained by the fact that these SNPs are intronic-variants with the potential capacity to modify the alternative-splicing pattern. It is well-known the importance of introns in final gene expression. In addition, intron-retention (not removed introns) has recently garnered attention as a major component of the global alternative splicing-mediated regulation and mRNA expression regulation, which are part of many mammalian programs of gene expression [44]. Indeed, Gumanova et al. [45] reported

363 the association between rs182052 and rs266729 *ADIPOQ* polymorphisms with
364 adiponectin levels in men. Overall, these lines of evidence suggest that *ADIPOQ*
365 polymorphisms may alter the expression levels and consequently adiponectin content.
366 This would explain the observed differences in CVD risk factors between *ADIPOQ*
367 genotypes. Of note is however that further research is needed to assess the link between
368 our studied genetic variants and adiponectin levels.

369 Some limitations of this work should also be acknowledged when interpreting
370 our findings. First limitation is the cross-sectional nature of our study, so causality
371 associations could not be determined. Second, the associations between *ADIPOQ*
372 polymorphisms and CVD risk factors could be modified by gene-gene and gene-
373 environmental interactions. Third, we have no information on patterns of relatedness
374 among the participants, and we do not know the ethnic/racial make-up of the sample.
375 Fourth, unfortunately we did not measure adiponectin blood concentration and the
376 rs266729 polymorphism in our study. In addition, blood pressure was measured twice,
377 and the mean of both measurements was considered for the analysis. It would have been
378 ideal to calculate the mean using three measurements instead. Our results should be
379 considered carefully, and studies with larger sample size and using SNPs that have
380 almost no variation in our sample could help to further confirm this possible genetic
381 predisposition.

382 In conclusion, we observed an association between single rs822393, rs822395,
383 rs7649121 polymorphisms and a six polymorphisms haplotype of the *ADIPOQ* gene
384 with CVD risk factors in European adolescents. With future prospects, these results
385 could be helpful to program clinical and health strategies, using SNPs of this and other
386 genes to support the individual classical risk factors determination and set up high-risk
387 groups in young population.

388

389 **Author Contribution**

390 IL, AIR, LC, LB, NM, MGG, YM, CPL, LAM, AM, MJC, JMP, DST and JRR

391 designed the study; DST performed all analyses; JM, DST and JRR wrote the initial

392 draft and all co-authors significantly contributed to the final version.

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532 **Tables**

SNP ID	**Major allele**	**Minor allele**	**MAF**	**HWE**
rs182052	G	A	0.32	0.14
rs822391	T	C	0.19	0.09
rs822393	C	T	0.21	0.51
rs16861210	G	A	0.09	0.86
rs822395	A	C	0.35	0.16
rs822396	A	G	0.18	0.12
rs12495941	G	T	0.38	0.74
rs7649121	A	T	0.15	0.27
rs2241766	T	G	0.13	0.78
rs1501299	C	A	0.29	0.01
rs3821799	C	T	0.48	0.24
rs3774261	G	A	0.42	0.05
rs17366743	T	C	0.02	0.10
rs1063537	C	T	0.12	0.39

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534 **Table 1**: Minor allele frequency (MAF) and results of exact test to assess deviations
535 from Hardy-Weinberg equilibrium (HWE) of *ADIPOQ* single nucleotide
536 polymorphisms (SNPs).

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Phenotype	All (n=1057)	Male (n=505)	Female (n=552)
Age (years)	14.71 ± 1.22	14.74 ± 1.25	14.68 ± 1.2
Weight (kg)	58.7 ± 12.7	61.86 ± 14.29	55.85 ± 10.17
Height (cm)	165.4 ± 9.3	169.5 ± 9.91	161.76 ± 6.98
BMI (kg/m^2)	21.34 ± 3.67	21.39 ± 3.99	21.3 ± 3.37
Cholesterol (mg/dL)	160.74 ± 27.69	154.03 ± 26.13	166.88 ± 27.68
HDL-C (mg/dL)	55.26 ± 10.67	53.17 ± 10.12	57.17 ± 10.81
LDL-C (mg/dL)	94.49 ± 25.09	90.78 ± 24.32	97.89 ± 25.33
Triglycerides (mg/dL)	69 ± 35.09	64.13 ± 31.65	73.46 ± 37.45
LDL-C/HDL-C	1.78 ± 0.63	1.78 ± 0.65	1.78 ± 0.6
Cholesterol/HDL-C	2.99 ± 0.66	2.98 ± 0.69	2.99 ± 0.63
Triglycerides/HDL-C	1.33 ± 0.88	1.29 ± 0.83	1.37 ± 0.92
ApoA1 (mg/dL)	1.5 ± 0.22	1.46 ± 0.21	1.55 ± 0.23
ApoB (mg/dL)	0.65 ± 0.16	0.63 ± 0.15	0.68 ± 0.16
ApoB/ApoA1	0.44 ± 0.13	0.44 ± 0.13	0.45 ± 0.13
apoB/LDL-C	0.27 ± 0.03	0.27 ± 0.03	0.27 ± 0.03
Leptin (ng/ml)	19.6± 22.2	9.55 ± 14.2	28.34± 24.1
Insulin (micro lU/mL)	10.31 ± 7.79	10.16 ± 8.82	10.46 ± 6.7
HOMA	2.35 ± 1.96	2.36 ± 2.24	2.34 ± 1.65
QUICKI	0.35 ± 0.03	0.35 ± 0.03	0.35 ± 0.03
SBP (mm Hg)	120 ± 13	124 ± 13	116 ± 11
DBP (mm Hg)	68.03 ± 8.84	67.52 ± 8.91	68.49 ± 8.76
CVD Risk score	-0.01 ± 0.61	-0.03 ± 0.66	0.02 ± 0.56

Table 2: Characteristics of the studied sample. Abbreviations: BMI: Body mass index; CVD: cardiovascular disease; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; Apo: Apolipoprotein; HOMA: Homeostatic model assessment; QUICKI: Quantitative insulin sensitivity check index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

Figure legends

Figure 1. Blocks 1 and 2 of *ADIPOQ* polymorphisms, which contains rs822393, rs16861210, rs822395, rs822396, rs12495941 and rs7649121 for block 1; rs2241766, rs1501299, rs3821799, rs3774261, rs17366743 and rs1063537 for block 2, according to genotyping data of this study. Boxes number referred to linkage disequilibrium (D') between polymorphisms, boxes with no number mean 100% linkage (D' = 1). Colour legend: i) Bright red = high D'; White = low D'; iii) Purple = High D' but low LOD score (see Haploview documentation for further details; http://www.broad.mit.edu/mpg/haploview).

Figure 2. Significant associations after the Bonferroni correction for *ADIPOQ* polymorphisms and high-density lipoprotein cholesterol (HDL-C). Heritage model, P value and false positive discovery rate are shown for each association. Values are adjusted with body mass index, center, sex, and age.

590 **Figure 3**. Significant associations after the Bonferroni correction for *ADIPOQ*
591 polymorphisms and apolipoprotein (Apo)A1 levels. Heritage model, P value and false
592 positive discovery rate are shown for each association. Values are adjusted with body
593 mass index, center, sex, and age.

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609 **Figure 4**. Significant associations after the Bonferroni correction for *ADIPOQ*
610 polymorphisms, systolic blood pressure (SPB) and cardiovascular disease (CVD) risk
611 score. Heritage model, P value and false positive discovery rate are shown for each
612 association. Values are adjusted with body mass index, center, sex, and age.

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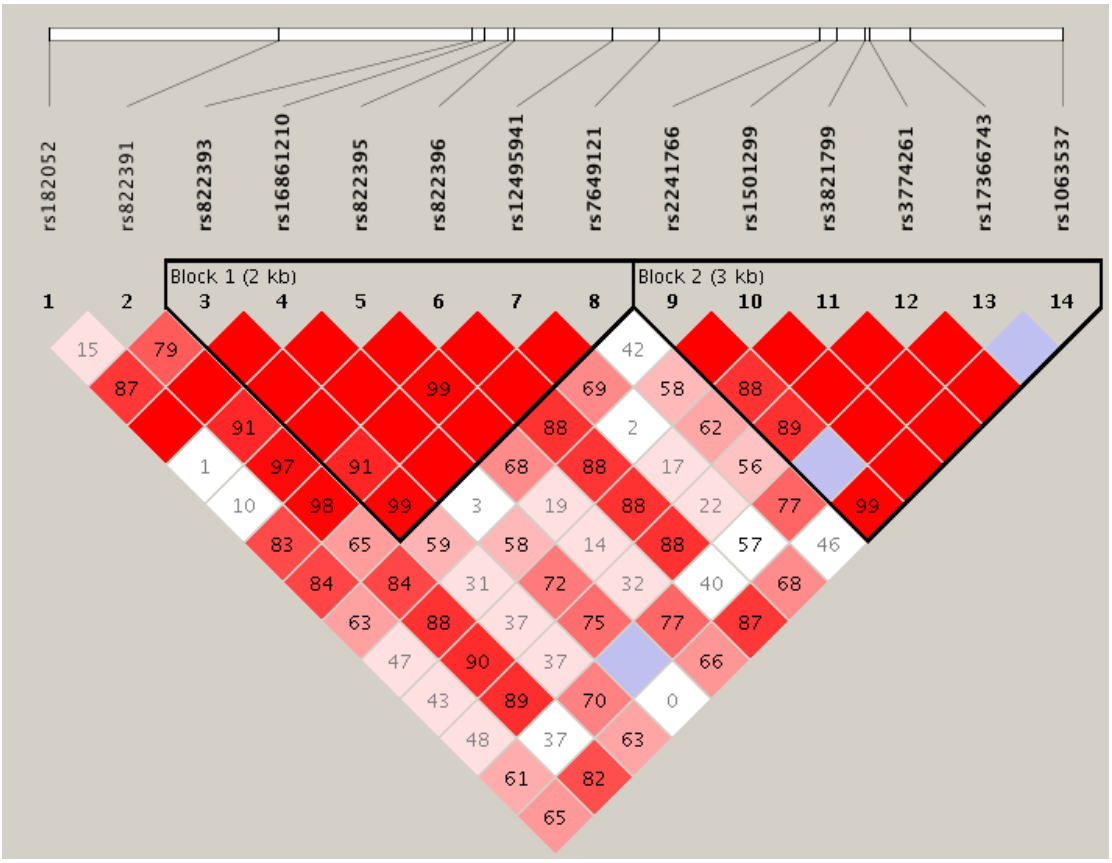
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rs822393
rs16861210
rs822395
rs822396
rs12495941
rs7649121
rs2241766
rs1501299
rs3821799
rs3774261
rs17366743
rs1063537
Block 1 (2 kb)
Block 2 (3 kb)
1 2 3 4 5 6 7 8 9 10 11 12 13 14
15 79 87 91 1 97 91 10 98 99 83 65 59 84 84 31 63 88 37 47 90 43 89 48 37 61 82 65
99 68 88 3 19 88 14 88 72 32 75 77 87 37 70 63
42 69 58 88 88 2 62 89 17 56 22 77 99 57 46 40 68 66 0

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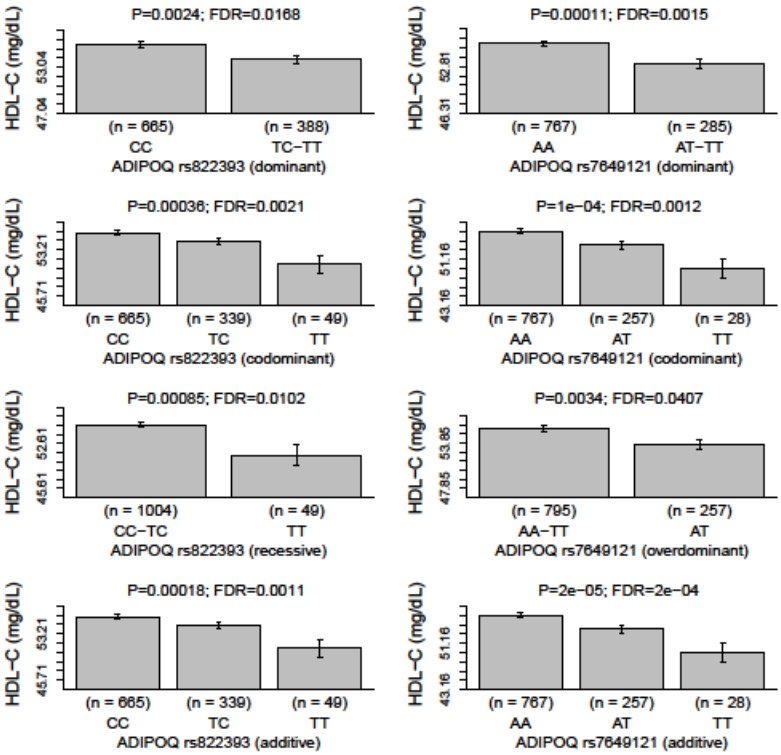
P=0.0024; FDR=0.0168
HDL−C (mg/dL)
47.04 53.04
(n = 665) (n = 388)
CC TC−TT
ADIPOQ rs822393 (dominant)
P=0.00011; FDR=0.0015
HDL−C (mg/dL)
46.31 52.81
(n = 767) (n = 285)
AA AT−TT
ADIPOQ rs7649121 (dominant)
P=0.00036; FDR=0.0021
HDL−C (mg/dL)
45.71 53.21
(n = 665) (n = 339) (n = 49)
CC TC TT
ADIPOQ rs822393 (codominant)
P=1e−04; FDR=0.0012
HDL−C (mg/dL)
43.16 51.16
(n = 767) (n = 257) (n = 28)
AA AT TT
ADIPOQ rs7649121 (codominant)
P=0.00085; FDR=0.0102
HDL−C (mg/dL)
45.61 52.61
(n = 1004) (n = 49)
CC−TC TT
ADIPOQ rs822393 (recessive)
P=0.0034; FDR=0.0407
HDL−C (mg/dL)
47.85 53.85
(n = 795) (n = 257)
AA−TT AT
ADIPOQ rs7649121 (overdominant)
P=0.00018; FDR=0.0011
HDL−C (mg/dL)
45.71 53.21
(n = 665) (n = 339) (n = 49)
CC TC TT
ADIPOQ rs822393 (additive)
P=2e−05; FDR=2e−04
HDL−C (mg/dL)
43.16 51.16
(n = 767) (n = 257) (n = 28)
AA AT TT
ADIPOQ rs7649121 (additive)

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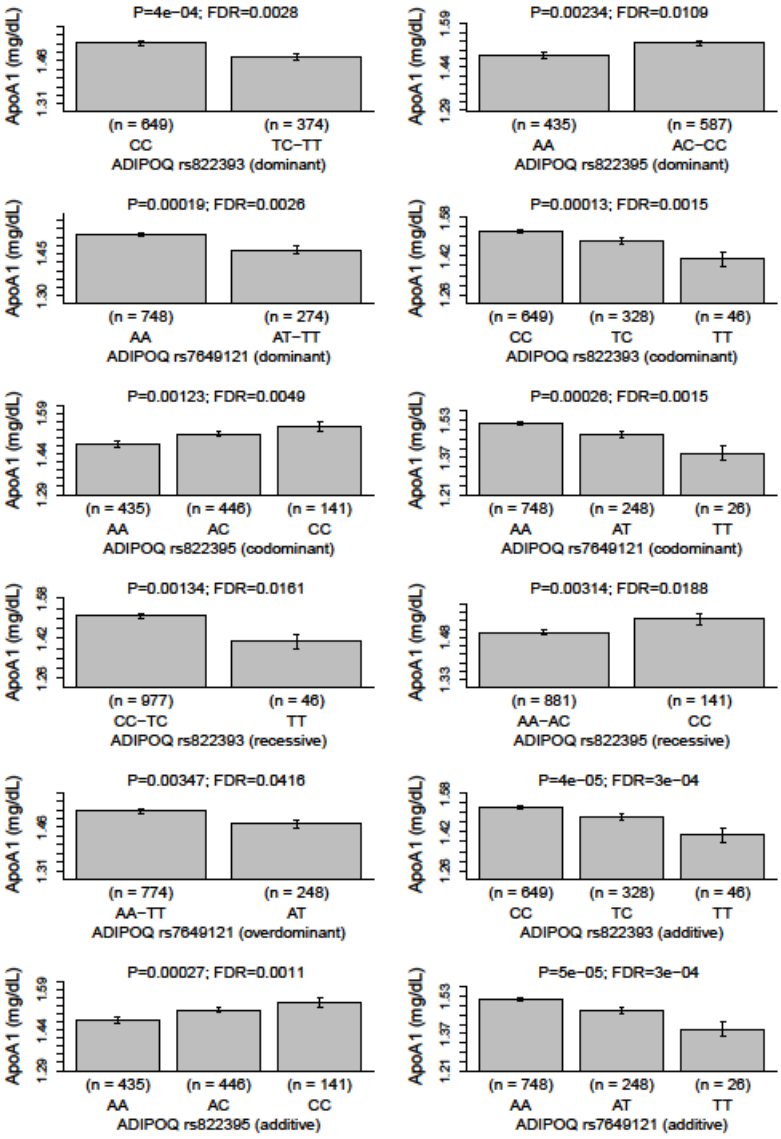
P=4e−04; FDR=0.0028
ApoA1 (mg/dL)
1.31 1.46
(n = 649) (n = 374)
CC TC−TT
ADIPOQ rs822393 (dominant)
P=0.00234; FDR=0.0109
ApoA1 (mg/dL)
1.29 1.44 1.59
(n = 435) (n = 587)
AA AC−CC
ADIPOQ rs822395 (dominant)
P=0.00019; FDR=0.0026
ApoA1 (mg/dL)
1.30 1.45
(n = 748) (n = 274)
AA AT−TT
ADIPOQ rs7649121 (dominant)
P=0.00013; FDR=0.0015
ApoA1 (mg/dL)
1.26 1.42 1.58
(n = 649) (n = 328) (n = 46)
CC TC TT
ADIPOQ rs822393 (codominant)
P=0.00123; FDR=0.0049
ApoA1 (mg/dL)
1.29 1.44 1.59
(n = 435) (n = 446) (n = 141)
AA AC CC
ADIPOQ rs822395 (codominant)
P=0.00026; FDR=0.0015
ApoA1 (mg/dL)
1.21 1.37 1.53
(n = 748) (n = 248) (n = 26)
AA AT TT
ADIPOQ rs7649121 (codominant)
P=0.00134; FDR=0.0161
ApoA1 (mg/dL)
1.26 1.42 1.58
(n = 977) (n = 46)
CC−TC TT
ADIPOQ rs822393 (recessive)
P=0.00314; FDR=0.0188
ApoA1 (mg/dL)
1.33 1.48
(n = 881) (n = 141)
AA−AC CC
ADIPOQ rs822395 (recessive)
P=0.00347; FDR=0.0416
ApoA1 (mg/dL)
1.31 1.46
(n = 774) (n = 248)
AA−TT AT
ADIPOQ rs7649121 (overdominant)
P=4e−05; FDR=3e−04
ApoA1 (mg/dL)
1.26 1.42 1.58
(n = 649) (n = 328) (n = 46)
CC TC TT
ADIPOQ rs822393 (additive)
P=0.00027; FDR=0.0011
ApoA1 (mg/dL)
1.29 1.44 1.59
(n = 435) (n = 446) (n = 141)
AA AC CC
ADIPOQ rs822395 (additive)
P=5e−05; FDR=3e−04
ApoA1 (mg/dL)
1.21 1.37 1.53
(n = 748) (n = 248) (n = 26)
AA AT TT
ADIPOQ rs7649121 (additive)

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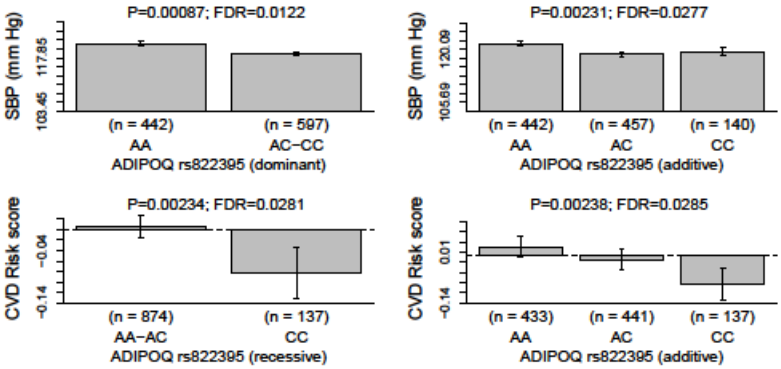
P=0.00087; FDR=0.0122
SBP (mm Hg)
103.45
117.85
(n = 442)
AA
(n = 597)
AC−CC
ADIPOQ rs822395 (dominant)
P=0.00231; FDR=0.0277
SBP (mm Hg)
105.69
120.09
(n = 442)
AA
(n = 457)
AC
(n = 140)
CC
ADIPOQ rs822395 (additive)
P=0.00234; FDR=0.0281
CVD Risk score
−0.14
−0.04
(n = 874)
AA−AC
(n = 137)
CC
ADIPOQ rs822395 (recessive)
P=0.00238; FDR=0.0285
CVD Risk score
−0.14
0.01
(n = 433)
AA
(n = 441)
AC
(n = 137)
CC
ADIPOQ rs822395 (additive)

627