HHS Public Access

Author manuscript

Int J Cancer. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Int J Cancer. 2015 July 1; 137(1): 154–164. doi:10.1002/ijc.29360.

Genetic variants of adiponectin and risk of colorectal cancer

Mingyang Song[1,2], **Jian Gong**[3], **Edward L. Giovannucci**[1,2,4], **Sonja I. Berndt**[5], **Hermann Brenner**[6,7], **Jenny Chang-Claude**[8], **Keith R. Curtis**[3], **Tabitha A. Harrison**[3], **Michael Hoffmeister**[6], **Li Hsu**[3,9], **Shuo Jiao**[3], **Loic Le Marchand**[10], **John D. Potter**[3,11,12], **Robert E. Schoen**[13], **Daniela Seminara**[14], **Martha L. Slattery**[15], **Emily White**[3,11], **Kana Wu**[1], **Shuji Ogino**[2,4,16,17], **Charles S. Fuchs**[4,17], **David J. Hunter**[1,2,4], **Shelley S. Tworoger**[2,4], **Frank B. Hu**[1,2,4], **Eric Rimm**[1,2,4], **Majken Jensen**[1], **Ulrike Peters**[3,11], and **Andrew T. Chan**[4,18]

[1]Department of Nutrition, Harvard School of Public Health, Boston, MA [2]Department of Epidemiology, Harvard School of Public Health, Boston, MA [3]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA [4]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA [5]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD [6]Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany [7]German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany [8]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany [9]Department of Biostatistics, School of Public Health, University of Washington, Seattle, WA [10]University of Hawaii Cancer Center, Honolulu, HI [11]Department of Epidemiology, School of Public Health, University of Washington, Seattle, WA [12]Centre for Public Health Research, Massey University, Wellington, New Zealand [13]Department of Medicine and Epidemiology, University of Pittsburgh Medical Center, Pittsburgh, PA [14]Division of Cancer Control and Population Sciences, National Cancer Institute, National Institutes of Health, Bethesda, MD [15]Department of Internal Medicine, University of Utah Health Sciences Center, Salt Lake City, UT [16]Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA [17]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA [18]Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA

Abstract

Circulating adiponectin has been associated with lower risk of colorectal cancer (CRC). Genome-wide association studies have identified several single-nucleotide polymorphisms (SNPs) associated with adiponectin levels. However, it is unclear whether these SNPs are associated with

Corresponding author: Andrew T. Chan, Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Gastroenterology Associates, 55 Fruit Street, Boston, MA 02114-2696, Phone: 617-726-3212, Fax: 617-724-6832, achan@partners.org.

Use of HUGO-designated official symbols: We use Human Genome Organisation (HUGO) Gene Nomenclature Committee (HGNC)-approved official symbols for genes and gene products (mRNA, peptides and proteins) including *ADIPOQ*, *ARL15*, *CDH13*, *FER*, and *ADIPOR1*, all of which are described at www.genenames.org.

Conflict of interest: None.

CRC risk. In addition, previous data on SNPs in the adiponectin pathway and their associations with CRC are inconsistent. Therefore, we examined 19 SNPs in genes related to adiponectin or its receptors and their associations with CRC using logistic regression among 7,020 cases and 7,631 controls drawn from 10 studies included in the Genetics and Epidemiology of Colorectal Cancer Consortium. Using data from a subset of two large cohort studies, we also assessed the contribution of individual SNPs and an adiponectin genetic score to plasma adiponectin after accounting for lifestyle factors among 2,217 women and 619 men. We did not find any statistically significant association between the 19 adiponectin-associated SNPs and CRC risk (multivariable-adjusted odds ratios ranged from 0.89 to 1.05, all $P > 0.05$). Each SNP explained less than 2.50% of the variance of plasma adiponectin, and the genetic score collectively accounted for 2.95% and 1.42% of the variability of adiponectin in women and men, respectively, after adjustment for age, body mass index, physical activity, smoking, alcohol consumption, regular use of aspirin or non-steroidal anti-inflammatory drug and postmenopausal hormone use. In conclusion, our findings do not support an association between known adiponectin-related common SNPs and CRC incidence. However, known common SNPs account for only a limited proportion of the interindividual variance in circulating adiponectin. Further work is warranted to investigate the relationship between adiponectin and CRC while accounting for other components in the pathway.

Keywords

adiponectin; single-nucleotide polymorphism; Mendelian randomization; colorectal cancer; lifestyle factors

Introduction

Increased adiposity is an established risk factor for colorectal cancer (CRC), with a stronger association observed in men than in women[1]. However, the mechanisms underlying this relationship remain unclear. A growing body of evidence suggests that adiponectin, an adipocyte-derived peptide hormone, may mediate the link between obesity and CRC[2, 3]. Adiponectin circulates in humans as a trimer, a hexamer, and a high molecular-weight (HMW) form. Circulating adiponectin levels are inversely associated with obesity, insulin resistance, and type 2 diabetes (T2D)[4, 5], which are all associated with increased risk of CRC. Suggested mechanisms through which adiponectin may influence CRC development include suppression of inflammation, improvement of insulin sensitivity, inhibition of cell growth and induction of apoptosis[2, 6].

Some[7–9] but not all[10, 11] prospective studies have shown an inverse association between circulating adiponectin concentrations and CRC risk. Using data from two large prospective cohorts, the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS), we previously reported that high adiponectin level was associated with a lower risk of CRC among men, but not among women[12]. However, the inherent limitations of observational studies, particularly their sensitivity to confounding, make it difficult to establish causality. A different approach exploits the genetic variation that may influence circulating adiponectin levels to provide causal evidence, as the independent assortment of

alleles during gamete formation, analogous to the randomized assignment in intervention trials, ensures the association of genetic variants with disease outcome is unconfounded by other common factors[13]. To our knowledge, this approach, known as Mendelian randomization, has not yet been employed to study the association between adiponectin and CRC.

Previous genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) in association with circulating adiponectin levels[14–20]. These SNPs have been mapped to the *ADIPOQ* (adiponectin, C1Q and collagen domain containing; HGNC ID; HGNC:13633) gene[14–20], the *ARL15* (ADP-ribosylation factor-like 15; HGNC ID; HGNC:25945) gene[18], the *CDH13* (cadherin 13; HGNC ID; HGNC:1753) gene which encodes a receptor for HMW forms of adiponectin[16, 19, 20], and the FER (fer[fps/fes related] tyrosine kinase; HGNC ID; HGNC:3655) gene involved in regulation of inflammation[18]. In addition, SNPs at the *ADIPOQ* loci have been associated with T2D[14, 19, 21], insulin sensitivity[22] and other metabolic traits[14, 17, 19], although the findings are mixed[15, 18, 23]. Candidate gene studies have also yielded inconsistent evidence on the association of variants at the *ADIPOQ and ADIPOR1* (adiponectin receptor 1; HGNC ID; HGNC:24040) genes with risk of CRC. Some studies reported a statistically significant association for rs266729[24], rs1063538[25], rs1342387[26], rs12733285[26] and rs2241766[27], whereas others failed to replicate these findings[28, 29]. Given the discrepant findings and limited sample size of prior studies, further investigation within a large cohort is needed.

To expand our knowledge on the role of adiponectin in CRC development, we examined genetic variants that influence either circulating adiponectin levels or adiponectin-related variants previously associated with CRC in relation to risk of CRC in 10 studies included in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO). In addition, with plasma and genetic information on adiponectin and detailed data on a range of lifestyle factors in a subset of samples from the NHS and HPFS, we had the unique opportunity to evaluate the relationships among circulating adiponectin, genetic variation, and lifestyle factors.

Methods and Materials

Study Population

We examined SNP-CRC associations among cases and controls from 10 studies within the GECCO[30]. To reduce the influence of population structure, we only included participants of European origin. Details of the individual studies are described in the Supplementary Materials. In brief, within each study CRC cases were identified and confirmed by medical records, pathologic reports, or death certificates. All participants provided informed consent and studies were approved by the relevant Institutional Review Boards. For the current study, a total of 7,020 CRC cases and 7,631 controls that had both genetic and lifestyle data were included.

For the SNP-plasma adiponectin association analysis, we examined genotype data and plasma adiponectin measurements among control participants from previous nested case-control studies of T2D[31], breast cancer[32] and CRC[33] within the NHS, and of myocardial

infarction[34] and CRC[33] within the HPFS. Detailed descriptions of the two cohorts can be found in the Supplementary Materials. Briefly, the NHS enrolled 121,701 registered female nurses who were aged 30–55 years in 1976 in the US, and the HPFS enrolled 51,529 US male professionals who were aged 40–75 years in 1986. In both cohorts, we updated medical, lifestyle, and other health-related information of participants through biennial follow-up questionnaires. The follow-up proportions have been approximately 90% in both cohorts.

We requested active participants to provide a whole blood sample in the NHS in 1989–1990 and in the HPFS in 1993–1995. A total of 32,826 women in the NHS and 18,225 men in the HPFS provided blood specimens on ice packs by overnight courier. The procedures of blood collection, handling and storage have been described previously[7]. Among participants who provided a blood sample, we used risk-set sampling matched on age, time of blood donation and other factors (e.g., smoking and fasting status) to randomly select up to 2 controls for each new case with a confirmed diagnosis of T2D[31], breast cancer[32], myocardial infarction[34] or CRC[33]. After excluding four outliers by the extreme Studentized deviate Many-Outlier procedure[35], we included a total of 2,217 women and 619 men who had both plasma adiponectin and genotype data for the present analysis.

SNP Selection

Based on the findings from previous GWAS in populations of European origin, we selected 16 SNPs within the *ADIPOQ* gene that are associated with circulating adiponectin concentrations[14–18] (also refer to GWAS catalog: http://www.genome.gov/gwastudies/). We limited our selection of genetic variants to those within the *ADIPOQ* gene that encodes the adiponectin protein to minimize the possibility of violation of the Mendelian randomization assumption that the genetic instrument should only affect the outcome through the exposure of interest. We also included 5 SNPs in adiponectin-related genes that were associated with CRC risk in the literature[24–26]. Pair-wise linkage disequilibrium (LD) was then assessed with information from the 1000 Genomics Pilot 1 CEU panel (http://www.broadinstitute.org/mpg/snap/ldsearch.php). Of the 21 SNPs, we excluded two SNPs (rs3774261, rs1648707) in LD with others as assessed by $r^2 \geq 0.80$ ($r^2 = 1.00$ between rs3774261 and rs6773957, $r^2 = 0.90$ between rs1648707 and rs6810075). Thus, a total of 19 SNPs were eligible for the final analysis, as shown in Supplementary Table 2.

Genotyping and Imputation

We used genotype data from 10 studies in the GECCO, including the Hawaii Colorectal Cancer Studies 2 and 3 (Colo2&3); Darmkrebs: Chancen der Verhütung durch Screening (DACHS); Diet, Activity, and Lifestyle Study (DALS); HPFS; Multiethnic Cohort (MEC); NHS; Physician's Health Study (PHS); Prostate, Lung, Colorectal Cancer, and Ovarian Cancer Screening Trial (PLCO); VITamins And Lifestyle (VITAL); and the Women's Health Initiative (WHI). Phase-one genotyping was conducted on a total of 1,709 colon cancer cases and 4,214 controls from PLCO, WHI, and DALS (PLCO Set 1, WHI Set 1, and DALS Set 1) using Illumina HumanHap 550K, 610K, or combined Illumina 300K and 240K. A total of 4,592 CRC cases and 4,478 controls from Colo2&3, DACHS, DALS Set 2, MEC, PMH, PLCO Set 2, VITAL, and WHI Set 2 were genotyped using Illumina

HumanCytoSNP. A total of 2,004 CRC cases and 2,244 controls from HPFS (2 sets), NHS (2 sets), and PHS (2 sets) were genotyped using Illumina HumanOmniExpress.

DNA was extracted from samples of white blood cells or, in a subset of the NHS, HPFS, DACHS, MEC and PLCO samples, and all VITAL samples from buccal cells using conventional methods. To monitor the quality of genotyping, each study included 1 to 6% blinded duplicates. Details on the QA/QC can be found in Supplemental Table 1. In brief, samples were excluded based on call rate, heterozygosity, unexpected duplicates, sex discrepancy, and unexpectedly high identity-by-descent or unexpected concordance (> 65%) with another individual. For missing SNP data, all included studies were imputed to HapMap II release 24. All SNPs met quality-control measures for Hardy-Weinberg Equilibrium in controls (HWE, $P \geq 10^{-4}$), and MAF ≥ 1% or imputation $R^2 > 0.3$.

Laboratory Assay of Adiponectin

Plasma adiponectin was measured among a subset of participants in the NHS and HPFS using the enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH)[31, 33] or competitive radio-imunoassay (Linco Research Inc, St Charles, Mo)[32, 34], as previously described. Quality-control samples were randomly interspersed and personnel who were blinded to quality-control status conducted all assays. The coefficients of variation from quality control samples ranged from 3.4–13.0%. To account for potential variation across batches, we corrected plasma adiponectin for measurement batch according to the average batch correction method with adjustment for age and body mass index (BMI)[36].

Covariate Assessment

All GECCO studies that were used for the present analysis collected data on smoking status, body weight and height (used to calculate BMI, weight/ height^2 [kg/m^2]), physical activity, alcohol consumption, regular use of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs), and hormone therapy in postmenopausal women. We adopted a flexible approach to retrospective covariate harmonization as previously described[37]. For the NHS and HPFS, to represent long-term lifestyle patterns for finer control of confounding, we used cumulative average covariate data collected from multiple biennial questionnaires before blood draw, as described in previous analyses[33].

Statistical Analysis

We described the basic characteristics of participants using mean for continuous variables and percentage for discrete variables, respectively. Given the sex difference observed in our previous analysis[33], we performed the statistical analyses in women and men separately. We examined the association between each SNP and CRC risk under an additive genetic model using two models of logistic regression: one was adjusted for age and the first 3 principal components from EIGENSTRAT (available at: http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm) to account for population substructure; the other was additionally adjusted for BMI, smoking status, alcohol consumption, regular use of aspirin or NSAIDs, and postmenopausal hormone therapy (in women only). To assess the aggregate association with CRC, for each individual we summed the number of alleles of the 16 SNPs that have been associated with increased level of adiponectin in previous GWAS (see SNP list in

Supplementary Table 2) and then examined its association with CRC using logistic regression. We first conducted the analyses within each study separately, and then used a meta-analytic approach to pool the results and assess potential heterogeneity across studies. As none of the *P* values for heterogeneity reached statistical significance, we present the results from the fixed-effects model.

We examined the association between SNPs and log-transformed adiponectin concentrations by linear regression under an additive genetic model. We present R^2 as a measure of the proportion of the variation in plasma adiponectin explained by SNPs, and the percent of change in adiponectin levels per one-allele increment as a measure of the magnitude of the association as shown in Table 3. To assess the aggregate effect of genetic variants on plasma adiponectin, we selected SNPs using $R^2 > 0.80\%$ and $P < 0.05$ as the criteria to create the weighted adiponectin genetic score. To obtain a more accurate population effect size, we used as the weight β-coefficients derived from the previously reported meta-analysis of GWAS in European populations (ref. 14). Thus, the adiponectin genetic score in women was calculated by the equation: genetic score = 1.78 * no. of A alleles for rs17300539 + 1.49 * no. of G alleles for rs17366568 + 0.70 * no. for A alleles of rs6773957 + 0.65* no. for T alleles of rs1063538. Because only 1 SNP demonstrated a statistically significant association with plasma adiponectin in men, the number of G alleles for rs17366568 alone was used to generate the score.

We then assessed the multivariable association of genetic score and lifestyle factors with log-transformed adiponectin levels using linear regression. The following lifestyle factors were included in the model in addition to age: BMI, physical activity, pack-years of smoking, alcohol consumption, and regular aspirin/NSAID use. Postmenopausal status and hormone use were additionally included in the model for women. To test for a possible nonlinear relationship, we added a quadratic term of each continuous covariate to the model. The test for nonlinearity was not statistically significant, and thus we report results from models without quadratic terms. We conducted stratified analyses to examine whether BMI and postmenopausal hormone use modified the association between genetic score and plasma adiponectin. To test for multiplicative interaction, we used likelihood ratio test to compare the model that included cross-product terms for stratification factors and genetic score to the model without these terms.

We used R (the R Foundation for Statistical Computing) and SAS version 9.3 (SAS Institute, Inc, Cary, NC) for the analyses. All statistical tests were two-sided and $P < 0.05$ was considered statistically significant.

Results

Table 1 summarizes the characteristics of participants studied in the SNP-CRC association analysis. A total of 7,020 cases and 7,631 controls from 10 studies were included with mean age ranging from 59 to 69 years old, BMI 25.0 to 27.9 kg/m^2, and alcohol consumption 5.3 to 17.9 g/d. About half of the participants ever smoked and 28–48% regularly took aspirin/NSAIDs. Analyses of each individual SNP in models first minimally and then fully adjusted did not show any statistically significant association with CRC risk in either women or men

(Tables 2–3). The multivariable-adjusted odds ratios (ORs) ranged from 0.89 to 1.05, with 95% confidence intervals (CIs) all including 1.0. We also examined the aggregate association with CRC of the 16 SNPs that have been related to circulating adiponectin in previous GWAS. The OR per 10-allele increment was 1.08 (95% CI: 0.95-1.22, $P = 0.24$) among women and 1.01 (95% CI: 0.90-1.13, $P = 0.87$) among men.

We then examined the association between SNPs and plasma adiponectin concentrations in a subset of NHS and HPFS participants. Women had statistically significantly higher adiponectin levels than men (median [interquartile range], ug/mL: 15.1 [11.3 to 18.6] in women; 11.2 [7.84 to 16.2] in men; $P < 0.001$ for gender difference). As shown in Table 4, genetic variants generally demonstrated a stronger association with adiponectin levels in women than men. Eleven of the 19 SNPs showed a statistically significant association with adiponectin measurements in women, with each explaining 0.32%-2.26% of the overall variance in plasma adiponectin. SNPs rs17366568 and rs17300539 were among the strongest associations ($R^2 = 2.26\%$ and 1.60%, respectively) with one-allele increments associated with 17.3%-19.8% alteration in adiponectin levels. In contrast, among men only one SNP, rs17366568, was associated with adiponectin concentrations ($P = 0.02$). This variant explained 0.94% of the overall variation of adiponectin levels, with men who carried one additional A allele of rs17366568 exhibiting 14.5% lower concentrations of adiponectin (95% CI: 2.96%-24.7%).

The genetic scores consisting of four SNPs in women and one SNP in men explained 2.82% and 0.94% of variation in adiponectin concentrations, respectively (Table 5). The inclusion of additional SNPs did not increase the explained variation. For example, the proportion of explained variation in women was 1.95% for an alternative genetic score that included all 11 SNPs that showed statistically significant associations with adiponectin. In women, age and alcohol consumption were statistically significantly associated with increased levels of adiponectin, whereas BMI was inversely associated with adiponectin. Postmenopausal women had higher adiponectin concentrations and menopausal hormone therapy was associated with still higher circulating levels. In men, in addition to age, BMI was the only factor that showed an association with adiponectin, with each 5-unit increase in BMI associated with 21.3% lower adiponectin levels (95% CI: 14.2%-27.8%). After adjusting for these lifestyle factors, the genetic score accounted for 2.95% and 1.42% of the variation of plasma adiponectin in women and men, respectively. The corresponding R^2 for the combination of genetic and lifestyle determinants was 15.5% in women and 6.65% in men. We then performed an analysis stratified by BMI and menopausal status. Neither BMI nor menopausal status appeared to modify the association between genetic score and plasma adiponectin (for BMI: $P_{interaction} = 0.25$ in women and 0.48 in men; for menopausal status: $P_{interaction} = 0.14$ in women).

Discussion

Previous studies have yielded inconsistent findings on the association between circulating adiponectin concentrations and risk of CRC. Low adiponectin has been associated with an increased risk of CRC in some studies[7–9], but not others[10, 11]. Given the close correlation between adiponectin and other metabolic factors, such as adiposity, inflammation and

hyperinsulinemia, it is difficult to disentangle specific influences and establish a causal relationship for adiponectin alone and CRC. Thus, studies examining the association of genetic determinants of adiponectin levels and CRC risk could provide important insight because polymorphisms should be minimally confounded by other factors. In the present analysis, we studied the common SNPs of the *ADIPOQ* gene that have been related to adiponectin concentrations in previous GWAS studies. Our results suggest that these genetic variants of circulating adiponectin are unrelated to risk of CRC.

There are several plausible interpretations of our findings. First, the relatively small proportion of variability in adiponectin concentration that is explained by known genetic variants makes it possible that a real but small association between adiponectin and CRC cannot be detected by utilizing these polymorphisms. In line with previous studies[14, 15], we found that compared to lifestyle factors, genetic variants accounted for less than 5% of the variance of adiponectin levels. Despite the moderate-to-high estimates of heritability (39–88%) for plasma adiponectin levels in twins and family-based studies, two meta-analyses of GWAS found that a multi-SNP score based on genome-wide significant SNPs explained only 5.0–6.7% of the variance of adiponectin concentrations[14, 15], indicating that genetic factors other than the known common variants may contribute to the variation of circulating adiponectin between individuals. In a previous study, we found that an approximate 40% difference in the risk of CRC was observed by contrasting men in the highest quartile of adiponectin with those in the lowest quartile[33], suggesting that the limited variation of adiponectin captured by the currently identified genetic variants may be inadequate to detect an inverse association with CRC. Recently, structural variation, rare variants, epigenetic inheritance and interactions between genetic variants have been suggested to explain the "missing heritability" phenomenon[38]. Therefore, further studies using novel sequencing technologies are needed to better determine the genetic contribution to the variation of adiponectin.

Second, other components unaccounted for in the present analysis may be required for adiponectin to exert an anti-cancer effect. Colonic tissues express two adiponectin receptors, ADIPOR1 and ADIPOR2, which mediate the biologic effects of adiponectin by activating AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-α (PPAR-α) pathways, respectively[2, 39]. Studies have found that disruption of ADIPOR1 and ADIPOR2 resulted in the abrogation of adiponectin signaling and abolished the suppressive effect of adiponectin on the growth of colon cancer cells[39]. Adiponectin treatment reduced mRNA expression of *ADIPOR2* in colorectal tumor tissues in mice[40]. In humans, ADIPOR1 and ADIPOR2 expression levels in colorectal tumors have been found to vary among individuals and been associated with tumor stage and differentiation of cancer cells[41]. In addition, a hypocaloric diet and exercise in older insulin-resistant adults increased skeletal muscle ADIPOR1 and ADIPOR2 expression without affecting serum total adiponectin levels[42]. Thus, it is possible that the variation in adiponectin receptor expression and circulating adiponectin interact to influence CRC development. Our inability to account for such interaction may contribute to the null results in the current study. Recently, we examined the association between plasma adiponectin and molecularly defined-subtypes of CRC. Interestingly, we observed that lower prediagnostic level of plasma adiponectin was

associated with an increased risk of *KRAS*-mutant CRC, but not with *KRAS*-wild-type CRC (unpublished data). Given the limited data linking adiponectin with specific molecular subtypes of CRC, further investigation is warranted.

In addition to adiponectin receptors, another adipokine, leptin, may also influence the association between adiponectin and colorectal carcinogenesis[2]. Leptin regulates energy balance through the central nervous system and modulates glucose and insulin homeostasis through activation in peripheral tissues[43]. Both adiponectin and leptin affect cellular behavior, but in an opposing manner. Adiponectin administration *in vivo* has been shown to decrease growth and proliferation, increase apoptosis, and decrease invasion in murine cancer models, whereas leptin has been found to increase proliferation, migration, and invasion of cancer cells[2]. There is some cross-talk between leptin and adiponectin pathways. Leptin-induced activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway can be antagonized by adiponectin-activated AMPK through protein phosphatase 2A (PP2A)[2]. Although the association between circulating leptin and CRC risk is unclear[33, 44, 45], there is some evidence suggesting that adiponectin and leptin interact to influence the risk of colorectal adenoma, and the inverse association between adiponectin and adenoma was stronger among individuals having high leptin levels[46].

Alternatively, the null results observed in the current study may reflect the fact that circulating adiponectin *per se* does not inhibit colorectal carcinogenesis. Although studies showed that the number of colon polyps were increased in adiponectin-deficient mice and growth of colon tumor was suppressed by adiponectin administration[40, 47], there was no difference in colon tumor incidence, number or size between the adiponectin transgenic mice that have constitutively elevated circulating adiponectin and wild-type mice[48]. In addition, improvement of insulin sensitivity by adiponectin has been hypothesized as a predominant mechanism against CRC. However, in our previous study, adjustment for C-peptide, a marker of insulin resistance, had no impact on the association of adiponectin with CRC[33]. In addition, in contrast with others[14, 17, 19, 22], several studies did not detect any association between the genetic variation of *ADIPOQ* and the risk of insulin resistance[18, 23, 49], metabolic syndrome[49] or T2D[18, 23]. Furthermore, although HMW adiponectin is the most biologically active form of adiponectin in relation to insulin sensitivity[50] and has been suggested to be more closely related to CRC than total adiponectin, a recent prospective study found that non-HMW adiponectin rather than HMW adiponectin was associated with CRC risk[8]. Therefore, further work is needed to investigate the role, if any, of adiponectin in colorectal carcinogenesis.

In the current study, we also examined several genetic variants of *ADIPOQ* and *ADIPOR1* that might not influence circulating adiponectin concentrations but have been associated with CRC in prior candidate gene studies. Kaklamani *et al.* reported an inverse association between rs266729 and CRC in a combined analysis of two case-control studies[24]. However, a subsequent study did not replicate this finding using data from two GWAS in the UK population[29]. Other SNPs in the *ADIPOQ* and *ADIPOR1* genes, including rs1063538, rs1342387, rs12733285 and rs2241766, have been reported to affect CRC risk in several relatively small studies among Chinese populations[25–27]. In the present study, we did not detect any statistically significant association for these SNPs using data from a large

consortium of populations of European descent. Therefore, differences in the ethnicity of the study population and the associated sample size may have contributed to the inconsistent findings.

Our study has several strengths, including a large sample size, available data on both genetic and plasma measurements of adiponectin, and detailed information on several lifestyle factors. One limitation of the current study is the inclusion of populations only of European descent, which limits the generalizability of our findings. However, the adiponectin-related SNPs that we examined were also identified from GWAS of European populations and thus the underlying genetic associations should hold in our study population. Moreover, limiting our analysis to European descent populations minimizes the potential for confounding by population structure. Another limitation is that we had plasma adiponectin data only on a subset of the two cohorts of the consortium, which precludes a simultaneous analysis of genetic and circulating adiponectin in relation to CRC among the same set of participants. Finally, the included genetic variants may have pleiotropic effects beyond their influence on circulating adiponectin, thus violating the assumption for the Mendelian randomization analysis[51]. However, this possibility was minimized by our restriction of SNPs to be those within the *ADIPOQ* gene. Furthermore, even if the influence by pleiotropy exists, in order to explain our null results, these SNPs must affect circulating adiponectin levels and CRC-related pathways in an opposite direction, which seems unlikely.

In conclusion, our findings do not support an association between known common SNPs related to circulating adiponectin and risk of CRC. This may be due to the fact that these SNPs account for only a small proportion of the variability of plasma adiponectin levels. Further studies are needed to integrate the expression status of adiponectin receptors and other biomarkers related to adiponectin pathway to elucidate any possible role of adiponectin in CRC development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This work was supported by the National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services [U01 CA137088 and R01 CA059045 to **GECCO**], the National Institutes of Health [R01 CA60987 to **COLO2&3**], the German Research Council [Deutsche Forschungsgemeinschaft, BR 1704/6-1, BR 1704/6-3, BR 1704/6-4 and CH 117/1-1 to **DACHS**], the German Federal Ministry of Education and Research [01KH0404 and 01ER0814 to **DACHS**], the National Institutes of Health [R01 CA48998 to **DALS**], the National Institutes of Health [P01 CA 055075, UM1 CA167552, R01 137178, and P50 CA 127003 to **HPFS**], the National Institutes of Health [R01 CA137178, R01 CA49449, P01 CA 087969 and P50 CA 127003 to **NHS**], the National Institutes of Health [R01 CA042182 to **PHS**], the National Institutes of Health [R37 CA54281, P01 CA033619, and R01 CA63464 to **MEC**], the National Institutes of Health [K05 CA154337 to **VITAL**], the National Institutes of Health [K24 DK098311 to **A.T.C**.]. M. S. is a trainee of the Harvard Transdisciplinary Research Center on Energetics and Cancer (TREC) which is supported by 1U54CA155626. A.T.C. is a Damon Runyon Clinical Investigator.

PLCO is supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics and supported by contracts from the Division of Cancer Prevention, National Cancer Institute, NIH, DHHS. Additionally, a subset of control samples were genotyped as part of the Cancer Genetic Markers of Susceptibility (CGEMS) Prostate Cancer GWAS (Yeager, M et al. Nat Genet 2007 May;39(5):645-9), Colon CGEMS pancreatic cancer scan (PanScan) (Amundadottir, L et al. Nat Genet. 2009 Sep;41(9):986-90 and Petersen, GM et al Nat

Genet. 2010 Mar;42(3):224-8), and the Lung Cancer and Smoking study. The prostate and PanScan study datasets were accessed with appropriate approval through the dbGaP online resource (http://cgems.cancer.gov/data/) accession numbers phs000207.v1.p1 and phs000206.v3.p2, respectively, and the lung datasets were accessed from the dbGaP website (http://www.ncbi.nlm.nih.gov/gap) through accession number phs000093.v2.p2. Funding for the Lung Cancer and Smoking study was provided by National Institutes of Health (NIH), Genes, Environment and Health Initiative (GEI) Z01 CP 010200, NIH U01 HG004446, and NIH GEI U01 HG 004438. For the lung study, the GENEVA Coordinating Center provided assistance with genotype cleaning and general study coordination, and the Johns Hopkins University Center for Inherited Disease Research conducted genotyping.

WHI is supported by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C.

DACHS: We thank all participants and cooperating clinicians, and Ute Handte-Daub, Renate Hettler-Jensen, Utz Benscheid, Muhabbet Celik and Ursula Eilber for excellent technical assistance.

GECCO: The authors would like to thank all those at the GECCO Coordinating Center for helping bring together the data and people that made this project possible.

HPFS, NHS and PHS: We would like to acknowledge Patrice Soule and Hardeep Ranu of the Dana Farber Harvard Cancer Center High-Throughput Polymorphism Core who assisted in the genotyping for NHS, HPFS, and PHS under the supervision of Dr. Immaculata Devivo and Dr. David Hunter, Qin (Carolyn) Guo and Lixue Zhu who assisted in programming for NHS and HPFS, and Haiyan Zhang who assisted in programming for the PHS. We would like to thank the participants and staff of the NHS and the HPFS, for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data.

PLCO: The authors thank Drs. Christine Berg and Philip Prorok, Division of Cancer Prevention, National Cancer Institute, the Screening Center investigators and staff of the PLCO, Mr. Tom Riley and staff, Information Management Services, Inc., Ms. Barbara O'Brien and staff, Westat, Inc., and Drs. Bill Kopp, Wen Shao, and staff, SAIC-Frederick. Most importantly, we acknowledge the study participants for their contributions to making this study possible. The statements contained herein are solely those of the authors and do not represent or imply concurrence or endorsement by NCI.

WHI: The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at: https://www.whi.org/researchers/SitePages/WHI%20Investigators.aspx

Abbreviations used

ADIPOQ	adiponectin
ADIPOR1	adiponectin receptor 1
ADIPOR2	adiponectin receptor 2
AMPK	AMP-activated protein kinase
ARL15	ADP-ribosylation factor-like 15
BMI	body mass index
CDH13	cadherin 13
CI	confidence interval
Colo2&3	Hawaii Colorectal Cancer Studies 2 and 3
CRC	colorectal cancer
DACHS	Darmkrebs: Chancen der Verhütung durch Screening
DALS	Diet, Activity, and Lifestyle Study

FER	fer(fps/fes related) tyrosine kinase
GECCO	Genetics and Epidemiology of Colorectal Cancer Consortium
GWAS	genome-wide association study
HMW	high molecular-weight
HPFS	Health Professionals Follow-up Study
LD	linkage disequilibrium
MAF	minor allele frequency
MEC	Multiethnic Cohort
NHS	Nurses' Health Study
NSAID	non-steroidal anti-inflammatory drug
OR	odds ratio
PHS	Physician's Health Study
PI3K	phosphoinositide 3-kinase
PLCO	Prostate, Lung, Colorectal Cancer, and Ovarian Cancer Screening Trial
PP2A	phosphatase 2A
PPAR	peroxisome proliferator-activated receptor
SNP	single-nucleotide polymorphism
T2D	type 2 diabetes
VITAL	VITamins And Lifestyle
WHI	Women's Health Initiative

References

1. World Cancer Research Fund / American Institute for Cancer Research, Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. AICR. 2007
2. Vansaun MN. Molecular pathways: adiponectin and leptin signaling in cancer. Clin Cancer Res. 2013; 19:1926–1932. [PubMed: 23355630]
3. Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. Nature reviews Cancer. 2011; 11:886–895.
4. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2001; 86:1930–1935. [PubMed: 11344187]
5. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999; 257:79–83. [PubMed: 10092513]
6. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006; 6:772–783. [PubMed: 16998510]

7. Wei EK, Giovannucci E, Fuchs CS, Willett WC, Mantzoros CS. Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. J Natl Cancer Inst. 2005; 97:1688–1694. [PubMed: 16288122]
8. Aleksandrova K, Boeing H, Jenab M, Bueno-de-Mesquita HB, Jansen E, van Duijnhoven FJ, Fedirko V, Rinaldi S, Romieu I, Riboli E, Romaguera D, Westphal S, et al. Total and high-molecular weight adiponectin and risk of colorectal cancer: the European Prospective Investigation into Cancer and Nutrition Study. Carcinogenesis. 2012; 33:1211–1218. [PubMed: 22431719]
9. Ho GY, Wang T, Gunter MJ, Strickler HD, Cushman M, Kaplan RC, Wassertheil-Smoller S, Xue X, Rajpathak SN, Chlebowski RT, Vitolins MZ, Scherer PE, et al. Adipokines Linking Obesity with Colorectal Cancer Risk in Postmenopausal Women. Cancer Res. 2012; 72:3029–3037. [PubMed: 22511581]
10. Stocks T, Lukanova A, Johansson M, Rinaldi S, Palmqvist R, Hallmans G, Kaaks R, Stattin P. Components of the metabolic syndrome and colorectal cancer risk; a prospective study. Int J Obes (Lond). 2008; 32:304–314. [PubMed: 17878894]
11. Lukanova A, Soderberg S, Kaaks R, Jellum E, Stattin P. Serum adiponectin is not associated with risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2006; 15:401–402. [PubMed: 16492937]
12. Song M, Zhang X, Wu K, Ogino S, Fuchs CS, Giovannucci EL, Chan AT. Plasma adiponectin and soluble leptin receptor and risk of colorectal cancer: a prospective study. Cancer Prev Res (Phila). 2013; 6:875–885. [PubMed: 23872505]
13. Clayton D, McKeigue PM. Epidemiological methods for studying genes and environmental factors in complex diseases. Lancet. 2001; 358:1356–1360. [PubMed: 11684236]
14. Dastani Z, Hivert MF, Timpson N, Perry JR, Yuan X, Scott RA, Henneman P, Heid IM, Kizer JR, Lyytikainen LP, Fuchsberger C, Tanaka T, et al. Novel loci for adiponectin levels and their influence on type 2 diabetes and metabolic traits: a multi-ethnic meta-analysis of 45,891 individuals. PLoS Genet. 2012; 8:e1002607. [PubMed: 22479202]
15. Heid IM, Henneman P, Hicks A, Coassin S, Winkler T, Aulchenko YS, Fuchsberger C, Song K, Hivert MF, Waterworth DM, Timpson NJ, Richards JB, et al. Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome-wide association analyses including 4659 European individuals. Atherosclerosis. 2010; 208:412–420. [PubMed: 20018283]
16. Ling H, Waterworth DM, Stirnadel HA, Pollin TI, Barter PJ, Kesaniemi YA, Mahley RW, McPherson R, Waeber G, Bersot TP, Cohen JC, Grundy SM, et al. Genome-wide linkage and association analyses to identify genes influencing adiponectin levels: the GEMS Study. Obesity (Silver Spring). 2009; 17:737–744. [PubMed: 19165155]
17. Qi L, Menzaghi C, Salvemini L, De Bonis C, Trischitta V, Hu FB. Novel locus FER is associated with serum HMW adiponectin levels. Diabetes. 2011; 60:2197–2201. [PubMed: 21700879]
18. Richards JB, Waterworth D, O'Rahilly S, Hivert MF, Loos RJ, Perry JR, Tanaka T, Timpson NJ, Semple RK, Soranzo N, Song K, Rocha N, et al. A genome-wide association study reveals variants in ARL15 that influence adiponectin levels. PLoS Genet. 2009; 5:e1000768. [PubMed: 20011104]
19. Chung CM, Lin TH, Chen JW, Leu HB, Yang HC, Ho HY, Ting CT, Sheu SH, Tsai WC, Chen JH, Lin SJ, Chen YT, et al. A genome-wide association study reveals a quantitative trait locus of adiponectin on CDH13 that predicts cardiometabolic outcomes. Diabetes. 2011; 60:2417–2423. [PubMed: 21771975]
20. Wu Y, Li Y, Lange EM, Croteau-Chonka DC, Kuzawa CW, McDade TW, Qin L, Curocichin G, Borja JB, Lange LA, Adair LS, Mohlke KL. Genome-wide association study for adiponectin levels in Filipino women identifies CDH13 and a novel uncommon haplotype at KNG1-ADIPOQ. Hum Mol Genet. 2010; 19:4955–4964. [PubMed: 20876611]
21. Hu FB, Doria A, Li T, Meigs JB, Liu S, Memisoglu A, Hunter D, Manson JE. Genetic variation at the adiponectin locus and risk of type 2 diabetes in women. Diabetes. 2004; 53:209–213. [PubMed: 14693717]
22. Gao H, Fall T, van Dam RM, Flyvbjerg A, Zethelius B, Ingelsson E, Hagg S. Evidence of a causal relationship between adiponectin levels and insulin sensitivity: a Mendelian randomization study. Diabetes. 2013; 62:1338–1344. [PubMed: 23274890]

23. Yaghootkar H, Lamina C, Scott RA, Dastani Z, Hivert MF, Warren LL, Stancakova A, Buxbaum SG, Lyytikainen LP, Henneman P, Wu Y, Cheung CY, et al. Mendelian randomization studies do not support a causal role for reduced circulating adiponectin levels in insulin resistance and type 2 diabetes. Diabetes. 2013; 62:3589–3598. [PubMed: 23835345]
24. Kaklamani VG, Wisinski KB, Sadim M, Gulden C, Do A, Offit K, Baron JA, Ahsan H, Mantzoros C, Pasche B. Variants of the adiponectin (ADIPOQ) and adiponectin receptor 1 (ADIPOR1) genes and colorectal cancer risk. Jama. 2008; 300:1523–1531. [PubMed: 18827209]
25. Liu L, Zhong R, Wei S, Yin JY, Xiang H, Zou L, Chen W, Chen JG, Zheng XW, Huang LJ, Zhu BB, Chen Q, et al. Interactions between genetic variants in the adiponectin, adiponectin receptor 1 and environmental factors on the risk of colorectal cancer. PLoS One. 2011; 6:e27301. [PubMed: 22087284]
26. He B, Pan Y, Zhang Y, Bao Q, Chen L, Nie Z, Gu L, Xu Y, Wang S. Effects of genetic variations in the adiponectin pathway genes on the risk of colorectal cancer in the Chinese population. BMC Med Genet. 2011; 12:94. [PubMed: 21749709]
27. Hu X, Yuan P, Yan J, Feng F, Li X, Liu W, Yang Y. Gene Polymorphisms of +45T>G, -866G>A, and Ala54Thr on the Risk of Colorectal Cancer: A Matched Case-Control Study. PLoS One. 2013; 8:e67275. [PubMed: 23826253]
28. Gornick MC, Rennert G, Moreno V, Gruber SB. Adiponectin gene and risk of colorectal cancer. Br J Cancer. 2011; 105:562–564. [PubMed: 21829206]
29. Carvajal-Carmona LG, Spain S, Kerr D, Houlston R, Cazier JB, Tomlinson I. Common variation at the adiponectin locus is not associated with colorectal cancer risk in the UK. Hum Mol Genet. 2009; 18:1889–1892. [PubMed: 19264763]
30. Peters U, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, Berndt SI, Bezieau S, Brenner H, Butterbach K, Caan BJ, Campbell PT, et al. Identification of Genetic Susceptibility Loci for Colorectal Tumors in a Genome-Wide Meta-analysis. Gastroenterology. 2013; 144:799–807. e24. [PubMed: 23266556]
31. Heidemann C, Sun Q, van Dam RM, Meigs JB, Zhang C, Tworoger SS, Mantzoros CS, Hu FB. Total and high-molecular-weight adiponectin and resistin in relation to the risk for type 2 diabetes in women. Ann Intern Med. 2008; 149:307–316. [PubMed: 18765700]
32. Tworoger SS, Eliassen AH, Kelesidis T, Colditz GA, Willett WC, Mantzoros CS, Hankinson SE. Plasma adiponectin concentrations and risk of incident breast cancer. J Clin Endocrinol Metab. 2007; 92:1510–1516. [PubMed: 17213279]
33. Song M, Zhang X, Wu K, Ogino S, Fuchs CS, Giovannucci EL, Chan AT. Plasma Adiponectin and Soluble Leptin Receptor and Risk of Colorectal Cancer: A Prospective Study. Cancer Prev Res (Phila). 2013
34. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. Jama. 2004; 291:1730–1737. [PubMed: 15082700]
35. Rosner B. Percentage Points for a Generalized ESD Many-Outlier Procedure. Technometrics. 1983; 25:165–172.
36. Rosner B, Cook N, Portman R, Daniels S, Falkner B. Determination of blood pressure percentiles in normal-weight children: some methodological issues. Am J Epidemiol. 2008; 167:653–666. [PubMed: 18230679]
37. Hutter CM, Chang-Claude J, Slattery ML, Pflugeisen BM, Lin Y, Duggan D, Nan H, Lemire M, Rangrej J, Figueiredo JC, Jiao S, Harrison TA, et al. Characterization of gene-environment interactions for colorectal cancer susceptibility loci. Cancer Res. 2012; 72:2036–2044. [PubMed: 22367214]
38. Eichler EE, Flint J, Gibson G, Kong A, Leal SM, Moore JH, Nadeau JH. Missing heritability and strategies for finding the underlying causes of complex disease. Nat Rev Genet. 2010; 11:446–450. [PubMed: 20479774]
39. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med. 2007; 13:332–339. [PubMed: 17268472]

40. Moon HS, Liu X, Nagel JM, Chamberland JP, Diakopoulos KN, Brinkoetter MT, Hatziapostolou M, Wu Y, Robson SC, Iliopoulos D, Mantzoros CS. Salutary effects of adiponectin on colon cancer: in vivo and in vitro studies in mice. Gut. 2013; 62:561–570. [PubMed: 22735569]
41. Byeon JS, Jeong JY, Kim MJ, Lee SM, Nam WH, Myung SJ, Kim JG, Yang SK, Kim JH, Suh DJ. Adiponectin and adiponectin receptor in relation to colorectal cancer progression. Int J Cancer. 2010; 127:2758–2767. [PubMed: 21351255]
42. O'Leary VB, Jorett AE, Marchetti CM, Gonzalez F, Phillips SA, Ciaraldi TP, Kirwan JP. Enhanced adiponectin multimer ratio and skeletal muscle adiponectin receptor expression following exercise training and diet in older insulin-resistant adults. Am J Physiol Endocrinol Metab. 2007; 293:E421–E427. [PubMed: 17488807]
43. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 372:425–432. [PubMed: 7984236]
44. Stattin P, Lukanova A, Biessy C, Soderberg S, Palmqvist R, Kaaks R, Olsson T, Jellum E. Obesity and colon cancer: does leptin provide a link? Int J Cancer. 2004; 109:149–152. [PubMed: 14735482]
45. Aleksandrova K, Boeing H, Jenab M, Bueno-de-Mesquita HB, Jansen E, van Duijnhoven FJ, Rinaldi S, Fedirko V, Romieu I, Riboli E, Gunter MJ, Westphal S, et al. Leptin and Soluble Leptin Receptor in Risk of Colorectal Cancer in the European Prospective Investigation into Cancer and Nutrition Cohort. Cancer Res. 2012; 72:5328–5337. [PubMed: 22926557]
46. Yamaji T, Iwasaki M, Sasazuki S, Tsugane S. Interaction between adiponectin and leptin influences the risk of colorectal adenoma. Cancer Res. 2010; 70:5430–5437. [PubMed: 20516125]
47. Fujisawa T, Endo H, Tomimoto A, Sugiyama M, Takahashi H, Saito S, Inamori M, Nakajima N, Watanabe M, Kubota N, Yamauchi T, Kadowaki T, et al. Adiponectin suppresses colorectal carcinogenesis under the high-fat diet condition. Gut. 2008; 57:1531–1538. [PubMed: 18676419]
48. Ealey KN, Archer MC. Elevated circulating adiponectin and elevated insulin sensitivity in adiponectin transgenic mice are not associated with reduced susceptibility to colon carcinogenesis. International Journal of Cancer. 2009; 124:2226–2230.
49. Peters KE, Beilby J, Cadby G, Warrington NM, Bruce DG, Davis WA, Davis TM, Wiltshire S, Knuiman M, McQuillan BM, Palmer LJ, Thompson PL, et al. A comprehensive investigation of variants in genes encoding adiponectin (ADIPOQ) and its receptors (ADIPOR1/R2), and their association with serum adiponectin, type 2 diabetes, insulin resistance and the metabolic syndrome. BMC Med Genet. 2013; 14:15. [PubMed: 23351195]
50. Oh DK, Ciaraldi T, Henry RR. Adiponectin in health and disease. Diabetes Obes Metab. 2007; 9:282–289. [PubMed: 17391153]
51. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. Int J Epidemiol. 2004; 33:30–42. [PubMed: 15075143]

Brief description of novelty and impact

Using data from the Genetics and Epidemiology of Colorectal Cancer Consortium, we did not find any association between known common genetic variants of adiponectin and colorectal cancer. However, analysis of the contribution of genetic variants to plasma adiponectin level after accounting for lifestyle factors suggests that genetic determinants account for only a limited proportion of the variance in adiponectin.

Table 1

Basic characteristics of study participants from GECCO*

Study	Abbreviation	Number of cases	Number of controls	% Female	Mean age (y)	Mean BMI (kg/m^2)	Mean alcohol use (g/d)	% Ever smokers	% Regular users of aspirin/NSAIDs
Diet, Activity and Lifestyle Study	DALS	1,100	1,165	44.9	64	27.1	10.0	54.0	37.8
Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial	PLCO	419	667	41.6	64	27.5	13.0	55.1	47.4
Women's Health Initiative	WHI	1,429	1,502	100	66	27.9	5.3	50.4	35.6
Darmkrebs: Chancen der Verhütung durch Screening	DACHS	2,247	2,075	39.9	69	26.7	15.2	57.8	28.2
Hawaii Colorectal Cancer Studies 2 & 3 (Colo2&3)	Colo2&3	75	104	44.8	65	26.1	12.4	54.7	38.1
Health Professionals Follow-up Study	HPFS	336	324	0	65	25.8	13.3	57.3	46.7
Multiethnic Cohort Study	MEC	304	316	46.4	63	26.4	17.9	64.8	40.5
Nurses' Health Study	NHS	486	841	100	60	25.5	5.9	57.2	35.4
Physicians' Health Study	PHS	375	386	0	59	25.0	8.7	58.4	48.0
VITamins And Lifestyle	VITAL	249	251	47.6	67	27.5	9.9	59.4	47.9

* Abbreviations: BMI = body mass index; GECCO = the Genetics and Epidemiology of Colorectal Cancer Consortium; NSAID = non-steroidal anti-inflammatory drug.

Table 2

Association between SNPs at the *ADIPOQ* loci and colorectal cancer risk among 3,739 cases and 4,184 controls in women from GECCO*

SNP	EA/NEA	EAF	Age and PCA-adjusted OR (95% CI) per allele†	*P*†	Multivariable-adjusted OR (95% CI) per allele‡	*P*‡	$P_{heterogeneity}$§
rs1063539	C/G	0.11	0.96 (0.86 to 1.07)	0.50	0.96 (0.86 to 1.08)	0.51	0.45
rs16861194	G/A	0.08	1.04 (0.91 to 1.18)	0.57	1.04 (0.92 to 1.19)	0.53	0.89
rs7615090	G/T	0.13	1.01 (0.88 to 1.15)	0.92	1.00 (0.87 to 1.15)	0.95	0.16
rs822394	A/C	0.19	1.02 (0.94 to 1.11)	0.65	1.02 (0.94 to 1.10)	0.70	0.32
rs17300539	A/G	0.07	0.98 (0.84 to 1.15)	0.82	0.98 (0.83 to 1.15)	0.77	0.34
rs17366568	A/G	0.08	1.03 (0.87 to 1.23)	0.70	1.04 (0.87 to 1.24)	0.67	0.20
rs17366743	C/T	0.05	0.96 (0.78 to 1.18)	0.71	0.95 (0.77 to 1.18)	0.66	0.77
rs6810075	C/T	0.35	0.99 (0.93 to 1.06)	0.84	1.00 (0.93 to 1.07)	0.92	0.99
rs6773957	A/G	0.39	1.00 (0.94 to 1.07)	0.98	1.00 (0.93 to 1.06)	0.89	0.13
rs822354	T/C	0.32	0.99 (0.92 to 1.07)	0.84	0.99 (0.91 to 1.06)	0.70	0.08
rs6444175	A/G	0.28	1.01 (0.94 to 1.09)	0.76	1.01 (0.94 to 1.08)	0.85	0.34
rs266717	A/G	0.50	1.02 (0.95 to 1.09)	0.61	1.01 (0.95 to 1.08)	0.72	0.06
rs1426810	C/T	0.36	1.04 (0.96 to 1.12)	0.35	1.04 (0.96 to 1.12)	0.35	0.33
rs1342387	A/G	0.45	1.04 (0.97 to 1.10)	0.28	1.03 (0.97 to 1.10)	0.37	0.89
rs12733285	T/C	0.29	1.04 (0.96 to 1.12)	0.33	1.03 (0.95 to 1.12)	0.44	0.57
rs266729	G/C	0.26	0.96 (0.89 to 1.04)	0.35	0.96 (0.89 to 1.04)	0.37	0.95
rs1501299	A/C	0.28	1.02 (0.95 to 1.10)	0.56	1.02 (0.94 to 1.09)	0.67	0.18
rs2241766//	G/T	0.11	0.96 (0.87 to 1.06)	0.42	0.96 (0.86 to 1.06)	0.41	0.44
rs1063538	T/C	0.39	1.00 (0.94 to 1.07)	0.99	0.99 (0.93 to 1.06)	0.88	0.14

*Abbreviations: CI = confidence interval; EA = effect allele; EAF = effect allele frequency; GECCO = the Genetics and Epidemiology of Colorectal Cancer Consortium; NEA = non-effect allele; OR = odds ratio; PCA = principal component analysis; SNP = single nucleotide polymorphism. A few women had missing information on genotype data of some SNPs: 1 on rs16861194, 23 on rs7615090, 6 on rs822394, 2 on rs17300539, 1 on rs6773957, 1 on rs6444175, 1 on rs266717, 3 on rs1426810, 9 on rs1342387, 1 on rs12733285, 2 on rs1501299, 1 on rs2241766, and 21 on rs1063538.

†Additive genetic model was used with adjustment for age and the first 3 principal components from EIGENSTRAT to account for population substructure.

‡Additionally adjusted for body mass index (<25, 25–30, ≥30 kg/m^2), smoking status (past or current smoking vs. never smoking), alcohol consumption (study-specific tertiles), regular use of aspirin or non-steroidal anti-inflammatory drugs, and postmenopausal hormone use (never users, ever users, premenopausal status).

§$P_{heterogeneity}$ across studies in the multivariable-adjusted model.

// The proxy rs3774262 (in pair-wise linkage disequilibrium with rs2241766: r^2=0.93, D' = 1.00) was used.

Table 3

Association between SNPs at the *ADIPOQ* loci and colorectal cancer risk among 3,281 cases and 3,447 controls in men from GECCO[*]

SNP	EA/NEA	EAF	Age and PCA-adjusted OR (95% CI) per allele[†]	*P*[†]	Multivariable-adjusted OR (95% CI) per allele[‡]	*P*[‡]	$P_{heterogeneity}$[§]
rs1063539	C/G	0.12	1.03 (0.91 to 1.16)	0.64	1.03 (0.91 to 1.16)	0.68	0.56
rs16861194	G/A	0.08	0.89 (0.78 to 1.02)	0.10	0.89 (0.77 to 1.02)	0.10	0.31
rs7615090	G/T	0.13	1.02 (0.88 to 1.19)	0.74	1.03 (0.88 to 1.19)	0.73	0.12
rs822394	A/C	0.18	0.98 (0.90 to 1.08)	0.72	0.97 (0.89 to 1.06)	0.53	0.42
rs17300539	A/G	0.08	0.96 (0.82 to 1.13)	0.62	0.96 (0.81 to 1.13)	0.61	0.46
rs17366568	A/G	0.08	0.97 (0.81 to 1.16)	0.73	0.97 (0.80 to 1.16)	0.72	0.21
rs17366743	C/T	0.05	1.08 (0.85 to 1.37)	0.51	1.09 (0.86 to 1.39)	0.48	0.91
rs6810075	C/T	0.35	0.94 (0.88 to 1.01)	0.11	0.95 (0.88 to 1.02)	0.14	0.21
rs6773957	A/G	0.40	1.03 (0.96 to 1.11)	0.36	1.04 (0.96 to 1.11)	0.34	0.62
rs822354	T/C	0.32	0.93 (0.86 to 1.01)	0.07	0.93 (0.86 to 1.00)	0.06	0.64
rs6444175	A/G	0.29	1.04 (0.96 to 1.12)	0.34	1.04 (0.97 to 1.13)	0.28	0.31
rs266717	A/G	0.50	1.04 (0.97 to 1.12)	0.26	1.04 (0.97 to 1.12)	0.31	0.53
rs1426810	C/T	0.37	1.05 (0.97 to 1.14)	0.19	1.05 (0.97 to 1.14)	0.25	0.80
rs1342387	A/G	0.46	0.98 (0.91 to 1.05)	0.58	0.99 (0.92 to 1.06)	0.76	0.90
rs12733285	T/C	0.30	0.98 (0.90 to 1.06)	0.64	0.98 (0.90 to 1.07)	0.65	0.78
rs266729	G/C	0.26	0.97 (0.89 to 1.05)	0.43	0.97 (0.89 to 1.06)	0.52	0.90
rs1501299	A/C	0.29	1.03 (0.95 to 1.11)	0.50	1.03 (0.95 to 1.11)	0.45	0.43
rs2241766[//]	G/T	0.12	1.03 (0.92 to 1.15)	0.60	1.03 (0.91 to 1.15)	0.66	0.55
rs1063538	T/C	0.40	1.03 (0.96 to 1.11)	0.36	1.04 (0.96 to 1.11)	0.34	0.61

[*] Abbreviations: CI = confidence interval; EA = effect allele; EAF = effect allele frequency; GECCO = the Genetics and Epidemiology of Colorectal Cancer Consortium; NEA = non-effect allele; OR = odds ratio; PCA = principal component analysis; SNP = single nucleotide polymorphism. A few men had missing information on genotype data of some SNPs: 1 on rs1063539, 1 on rs16861194, 17 on rs7615090, 3 on rs822394, 1 on rs17300539, 1 on rs17366568, 1 on rs17366743, 1 on rs6810075, 2 on rs6773957, 1 on rs822354, 4 on rs6444175, 3 on rs266717, 6 on rs1426810, 2 on rs1342387, 4 on rs12733285, 1 on rs266729, 1 on rs1501299, 2 on rs2241766, and 6 on rs1063538.

[†] Additive genetic model was used with adjustment for age and the first 3 principal components from EIGENSTRAT to account for population substructure.

[‡] Additionally adjusted for body mass index (<25, 25–30, ≥30 kg/m^2), smoking status (past or current smoking vs. never smoking), alcohol consumption (study-specific tertiles), and regular use of aspirin or non-steroidal anti-inflammatory drugs.

[§] $P_{heterogeneity}$ across studies in the multivariable-adjusted model.

// The proxy rs3774262 (in pair-wise linkage disequilibrium with rs2241766: r^2=0.93, D' = 1.00) was used.

Table 4

Association between SNPs and plasma adiponectin levels among women (Nurses' Health Study) and men (Health Professionals Follow-up Study)*

SNP	EA/NEA	EAF	Women (n = 2,217)			Men (n = 619)		
			R^2 (%)†	Relative change (95% CI), %‡	*P*	R^2 (%)†	Relative change (95% CI), %‡	*P*
rs1063539	C/G	0.11	0.02	1.74 (−3.14 to 6.86)	0.49	0.08	4.60 (−7.40 to 18.2)	0.47
rs16861194	G/A	0.07	0.37	−8.22 (−13.5 to −2.68)	0.004	0.23	8.53 (−5.00 to 24.0)	0.23
rs7615090	G/T	0.07	0.11	−4.64 (−10.1 to 1.13)	0.11	0.20	10.4 (−7.10 to 31.2)	0.26
rs822394	A/C	0.16	0.00	0.32 (−3.74 to 4.56)	0.88	0.06	3.64 (−7.64 to 16.3)	0.54
rs17300539	A/G	0.07	1.60	19.8 (12.9 to 27.0)	<0.001	0.08	5.50 (−9.42 to 22.9)	0.49
rs17366568	A/G	0.09	2.26	−17.3 (−21.5 to −12.9)	<0.001	0.94	−14.5 (−24.7 to −2.96)	0.02
rs17366743	C/T	0.03	<0.01	0.10 (−8.32 to 9.30)	0.98	0.09	−8.73 (−27.8 to 15.3)	0.44
rs6810075	C/T	0.33	0.34	−4.43 (−7.48 to −1.28)	0.006	0.08	−2.83 (−10.5 to 5.48)	0.49
rs6773957	A/G	0.39	0.82	7.04 (3.76 to 10.4)	<0.001	0.55	7.88 (−0.45 to 16.9)	0.07
rs822354	T/C	0.31	0.32	4.72 (1.25 to 8.30)	0.007	0.01	−0.81 (−8.77 to 7.84)	0.85
rs6444175	A/G	0.28	0.61	6.65 (3.07 to 10.4)	<0.001	0.21	5.00 (−3.46 to 14.2)	0.26
rs266717	A/G	0.49	0.45	5.01 (1.89 to 8.24)	0.002	0.01	1.10 (−6.54 to 9.35)	0.94
rs1426810	C/T	0.38	0.40	4.82 (1.61 to 8.14)	0.003	0.01	0.94 (−6.76 to 9.27)	0.82
rs1342387	A/G	0.45	0.05	1.73 (−1.40 to 4.95)	0.28	0.02	1.35 (−6.61 to 9.98)	0.75
rs12733285	T/C	0.29	0.04	1.66 (−1.69 to 5.11)	0.34	0.42	−7.22 (−15.3 to 1.63)	0.10
rs266729	G/C	0.25	0.09	−2.52 (−5.93 to 1.02)	0.16	0.46	−7.36 (−15.2 to 1.23)	0.19
rs1501299	A/C	0.28	0.61	6.66 (3.07 to 10.4)	<0.001	0.38	6.88 (−1.76 to 16.3)	0.19
rs2241766§	G/T	0.11	0.09	3.67 (−1.36 to 8.95)	0.16	0.10	4.86 (−6.91 to 18.1)	0.45
rs1063538	T/C	0.39	0.83	7.10 (3.81 to 10.5)	<0.001	0.55	7.88 (−0.45 to 16.9)	0.11

*Abbreviations: CI = confidence interval; EA = effect allele; EAF = effect allele frequency; NEA = non-effect allele; SNP = single nucleotide polymorphism.

†Percentage of variation of adiponectin concentrations explained by each SNP in the linear regression model.

‡Percent of changes in adiponectin levels per one-allele increment in the univariate linear regression.

§The proxy rs3774262 (in pair-wise linkage disequilibrium with rs2241766: r^2=0.93, D' = 1.00) was used.

Table 5

Association of genetic score and lifestyle factors with plasma adiponectin levels among women (Nurses' Health Study) and men (Health Professionals Follow-up Study)*

Variable	Women (n = 2,217)			Men (n = 619)		
	R^2 (%)†	Relative change (95% CI), %‡	*P*	R^2 (%)†	Relative change (95% CI), %‡	*P*
Genetic score§	2.82	5.56 (4.21 to 6.93)	<0.001	0.94	20.9 (6.78 to 36.9)	0.003
Age, y	1.98	3.00 (1.12 to 4.90)	0.002	0.59	3.09 (−0.18 to 6.46)	0.06
Body mass index, kg/m^2	10.2	−15.2 (−17.0 to −13.4)	<0.001	4.56	−21.3 (−27.8 to −14.2)	<0.001
Physical activity, MET-hours/wk	0.52	0.34 (−0.20 to 0.88)	0.21	0.29	0.34 (−0.62 to 1.31)	0.49
Pack-years of smoking	0.01	−0.22 (−0.80 to 0.36)	0.46	0.02	0.36 (−0.99 to 1.72)	0.61
Alcohol consumption, g/d	0.71	1.44 (0.35 to 2.53)	0.009	0.31	1.21 (−0.59 to 3.04)	0.19
Regular aspirin/NSAID use	0.01	1.15 (−2.90 to 5.37)	0.58	0.01	−0.56 (−10.8 to 10.8)	0.92
Postmenopausal hormone use	2.56			-		
Never		12.4 (4.37 to 21.1)	0.002		-	-
Ever		14.7 (6.83 to 23.1)	<0.001		-	-

*Abbreviations: CI = confidence interval; MET = metabolic equivalent = (caloric need per kilogram body weight per hour activity)/(caloric need per kilogram body weight per hour at rest); NSAID = non-steroidal anti-inflammatory drug.

†Percent of variation of adiponectin concentrations explained by each variable in the univariate linear regression model.

‡Percent of change in adiponectin levels per 1-point increment in genetic score and 5-unit increment for continuous covariates, estimated in linear regression model including all variables in the first column. For aspirin/NSAID use, percentage of change was calculated for regular users relative to non-users. For postmenopausal use, percentage of change was relative to adiponectin levels among premenopausal women.

§In women, the genetic score = 1.78 * no. of A alleles of rs17300539 + 1.49 * no. of G alleles of rs17366568 + 0.70 * no. of A alleles of rs6773957 + 0.65* no. of T alleles of rs1063538. In men, the genetic score = no. of G alleles of rs17366568.