

The adiponectin gene, *ADIPOQ*, and genetic susceptibility to colon cancer

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Abstract. In order to evaluate the contribution of polymorphisms of the adiponectin gene, *ADIPOQ*, to the risk of colon cancer, we conducted a case-control study of 60 colon cancer patients and 60 age, gender and ethnicity-matched controls in the Saudi population. We tested the hypothesis by analyzing the genotypes for two single nucleotide polymorphisms (SNPs), rs1501299 (G276T) and rs2241766 (T45G), in the *ADIPOQ* gene. In addition, the study was also designed to assess whether the two SNPs contribute to circulating adiponectin levels. We observed an increased risk of colon cancer associated with the 276T allele. The odds ratio (OR) was 2.64 [95% confidence interval (CI), 0.49-14.6]. The G allele at the T45G polymorphism was associated with a lower risk of colon cancer (OR=0.41; 95% CI, 0.19-0.86). Our results suggest that the risk of developing colon cancer may be partially explained by genetic polymorphisms in the *ADIPOQ* gene.

Introduction

Colon cancer is a major cause of cancer-related mortality in developed countries, and its incidence in developing countries is increasing. In Saudi Arabia, colon cancer incidences rank first among males and third among females after breast and thyroid cancer (1). Approximately 80% of colon cancer patients are over 40 years of age, and the peak of onset for most of the tumors is between 50 and 70 years of age (2). Epidemiological studies have revealed that environmental as well as genetic factors place certain individuals at a higher risk of developing colon cancer. To date, the numbers of genes linked to colon cancer are minuscule. To identify new colon cancer genes, genetic variation analysis could provide a window into the genetic landscape of human colon cancer. The most frequent type of variation in the human genome and an

excellent genotypic marker for research are single nucleotide polymorphisms (SNPs). Once the genes are identified, physicians would be able to use these genetic markers to identify individuals who are at high risk of the disease, and urge them to change their lifestyle and undergo more frequent physical screening tests in order to prevent colon cancer.

In the last 15 years, a number of investigators around the world have focused on understanding the correlation between the genetic role and biological regulation of adipocytokines. As a unique member of the adipocytokine family, adiponectin, which is an adipose-specific protein, appears to have an anti-atherogenic, anti-inflammatory and anti-diabetic effect. The adiponectin gene, *ADIPOQ*, is located on chromosome 3q27 and is comprised of three exons with two introns. It contains 244 amino acids, a signal peptide, a collagen-like domain at its N-terminus and a globular domain at its C-terminus, which shares sequence similarities with collagens X and VIII, as well as the complement factor C1q (3-5). In human plasma, the circulating adiponectin level ranges between 5 to 30 $\mu\text{g/ml}$ (6) and is reduced in patients with insulin resistance (7,8), type II diabetes (9-12), obesity (13), cardiovascular disease (14,15), gastric cancer (16) and colorectal adenomas and carcinoma (17,18). Low plasma adiponectin levels in these disease states are accompanied by reduced adiponectin gene expression in adipose tissue (7,19) and have been associated with *ADIPOQ* gene SNPs.

A number of SNPs have been identified in the *ADIPOQ* gene. Although the function of most SNPs remains unclear, it has been shown that two common SNPs, rs1501299 (G276T) and rs2241766 (T45G), may affect disease susceptibility. They have been associated with serum adiponectin (20-26), obesity (21,25,27,28), insulin sensitivity (18,21,29), type II diabetes (17,20,30) and coronary artery disease (24,31). However, some of these associations have been unable to be confirmed in other studies and the results have been controversial (32-34).

Given the significance of obesity in colon cancer development and the fundamental role of adiponectin in obesity, it is reasonable to hypothesize that *ADIPOQ* gene variation may play a role in colon cancer susceptibility. In this study, we sought to evaluate the association between two SNPs, G276T and T45G, in the *ADIPOQ* gene with the risk of colon cancer in the Saudi population. The study was also designed to assess whether the two SNPs contribute to circulating adiponectin levels.

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Materials and methods

Subjects. This was a case-control study involving patients (n=60) with a diagnosis of colon cancer and controls (n=60), who were recruited from King Abdulaziz Hospital and Oncology Center in Jeddah, Saudi Arabia. All cases (31 males and 29 females) had positive colonoscopic results for malignancy, histologically confirmed as colon cancer. Healthy, unrelated subjects (30 males and 30 females) were selected from the family clinic of King Abdulaziz Hospital, and they were judged to be in good health according to their medical history and colonoscopy preventive examination. None were taking any medication. Study subjects provided information on their body mass index (BMI), diabetes and family/personal history of cancer, using structured questionnaires. All participants gave written informed consent.

Anthropometric measurement and biochemical analyses. Anthropometric data including height, weight, BMI and waist and hip circumferences, which were measured using a standard technique: height without shoes by a stadiometer, weight in light clothes by a beam balance, waist circumference over the unclothed abdomen at the umbilicus at the end of a normal expiration and hip circumference at a maximal diameter by a non-stretchable standard tape. The BMI was calculated by dividing the weight in kilograms by height in meters squared. Venous blood was obtained following an overnight fast, and all serum samples were analyzed together at the end of the study. Serum adiponectin levels were measured by using an adiponectin (multimeric) EIA kit that was purchased from Alpco Diagnostics (Salem, NH, USA), with a measurement range of 0.075–4.8 ng/ml, with an observed value of 80–120%. All standards and unknown samples were analyzed in duplicate.

DNA extraction and *ADIPOQ* genotyping. Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA)-whole blood samples using a commercial kit (QIAamp DNA Blood Mini Kit; Hilden, Germany). DNA samples were genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays. The genotype assay was used for the analysis of the *ADIPOQ* gene, G276T and T45G SNPs. Two sets of primers (5.0 nmol) from TIB (TIB Molbiol Inc., Germany) were used. For exon 2 amplification, the forward primer (5'-GAAGTAGACTCTGCTGAGATGG-3') and the reverse primer (5'-TATCAGTGTAGGAGGTCTGTGATG-3') were used. For intron 2 region amplification, the forward primer (5'-CTACACTGATATAAACTATATGGAG-3') and the reverse primer (5'-CCCCAAATCACTTCAGGTTG-3') were used. The reactions were carried out in a final volume of 25 μ l, containing 1 μ l genomic DNA (0.2 μ g), 12.5 μ l HotStarTaq Master Mix, 10.1 μ l RNase free water and 0.2 μ l of each primer (0.1 μ M). Following the first denaturation for 5 min at 96°C, PCR was carried out for 40 cycles with denaturation at 96°C for 35 sec. The annealing temperature was at 63°C for G276T SNP and 53°C for T45G SNP, this was carried out for 35 sec and extension at 72°C for 45 sec, with a final extension for 4 min. PCR products that contain T45G and G276T SNPs were digested at 37°C with *HinfI* and *SmaI*, respectively, and electrophoresed on a 2% agarose gel.

Statistical analysis. All statistical analyses were performed with the SPSS (v.16) for Windows software. Continuous variables were expressed as the means \pm standard deviation (SD). In addition, one-way ANOVA was used. Genotype distributions, allele frequencies, odds ratio (OR) and risk ratio between the study groups were computed by 2x2 contingency table analysis. The Hardy-Weinberg equilibrium (HWE) was tested for the genotypes. The differences in frequencies of individual polymorphisms were assessed by frequent analysis, Fisher's exact tests and Chi-square (χ^2) tests. Results were considered statistically significant with a P-value <0.05.

Results

Subjects characteristics. The clinical characteristics of the patient and the control groups, including the serum concentration of adiponectin, are shown in Table I. In comparing the colon cancer patients to the controls, the results showed a highly significant difference between the patient and control groups regarding measurements of weight (P=0.0001), waist (P=0.01), hip (P=0.0001), BMI (P=0.004) and the adiponectin concentration (P=0.001).

PCR-RFLP analysis. The PCR technique was used to amplify the regions that contain G276T in intron 2 and T45G in exon 2 for all the collected samples. The amplified fragment that contains G276T showed a size of 107 bp. The normal (GG) genotype showed one band of size 107 bp. The heterozygous (GT) genotype showed three fragments of sizes 107, 82 and 25 bp. No homozygous (TT) genotype was found in the selected group (Fig. 1). The other amplified fragment that contains T45G showed a size of 372 bp. The genotype for the normal (TT) individuals produced one band of size 372 bp. The heterozygous (TG) genotype produced three bands of sizes 372, 219 and 153 bp. The homozygous (GG) genotype produced two bands of sizes 219 and 153 bp (Fig. 2).

Genotype distributions and allele frequencies of the *ADIPOQ* gene G276T SNP. The genotype and allele frequencies of the G276T variant were examined (Table II). The genotype frequencies of the patient group were 91.7% (n=55) normal (GG) and 8.3% (n=5) heterozygous (GT). In the control group, results revealed 96.7% (n=58) normal (GG) and 3.3% (n=2) heterozygous (GT). The frequency of the G and T alleles for the patient group were 95.9 and 4.1%, respectively. In the control group, the frequency of the G and T alleles were 98.4 and 1.6%, respectively. Genotype distributions for colon cancer patients (goodness-of-fit $\chi^2=0.12$, df=1, P=0.73) and the controls (goodness-of-fit $\chi^2=0.014$, df=1, P=0.91) were in HWE. The T allele at the G276T polymorphism was associated with a higher risk of colon cancer [OR=2.64; 95% confidence interval (CI), 0.49–14.6; P=0.44].

Genotype distributions and allele frequencies of the *ADIPOQ* gene T45G SNP. The genotype and allele frequencies of the T45G variant are shown in Table III. The genotype frequencies of the patients were 63.3% (n=38) normal (TT), 30% (n=18) heterozygous (TG) and 6.7% (n=4) homozygous (GG). In the controls, results revealed 45.4% (n=27) normal (TT), 53% (n=32) heterozygous (TG) and 1.6% (n=1) homozygous (GG).

Table I. Physical and biochemical comparison between the patients and the control group.

Variables	Patients	Controls	P-value
	Means \pm SD	Means \pm SD	
Age (years)			
All	54.28 \pm 12.32	50.77 \pm 12.26	0.09
Females	53.48 \pm 13.64	51.96 \pm 11.94	
Males	55.03 \pm 11.12	48.96 \pm 12.57	
Height (cm)			
All	160.79 \pm 9.14	163.50 \pm 9.70	0.12
Females	155.79 \pm 8.05	158.07 \pm 9.07	
Males	165.47 \pm 7.55	168.93 \pm 6.83	
Weight (kg)			
All	66.23 \pm 13.93	77.42 \pm 18.17	0.0001 ^a
Females	64.61 \pm 14.27	71.58 \pm 15.86	
Males	66.75 \pm 13.66	83.25 \pm 18.08	
Waist (cm)			
All	66.98 \pm 24.75	80.17 \pm 31.31	0.01 ^b
Females	61.02 \pm 28.05	84.63 \pm 30.78	
Males	72.55 \pm 20.11	75.69 \pm 31.70	
Hip (cm)			
All	66.04 \pm 30.18	88.56 \pm 35.66	0.0001 ^a
Females	66.81 \pm 32.11	93.80 \pm 34.66	
Males	65.32 \pm 28.77	83.32 \pm 36.44	
WHR			
All	1.09 \pm 0.43	0.98 \pm 0.47	0.15
Females	0.95 \pm 0.32	1.01 \pm 0.65	
Males	1.23 \pm 0.47	0.95 \pm 0.19	
BMI			
All	25.73 \pm 5.64	28.96 \pm 6.35	0.004 ^a
Females	26.77 \pm 6.36	28.82 \pm 6.70	
Males	24.75 \pm 4.77	29.10 \pm 6.08	
Adiponectin (ng/ml)			
All	5.05 \pm 3.49	7.59 \pm 4.87	0.001 ^a
Females	4.47 \pm 3.45	8.89 \pm 5.64	
Males	5.59 \pm 3.50	6.38 \pm 3.69	

^aHighly significant, ^bsignificant. BMI, body mass index; WHR, waist-hip ratio. Data are expressed as the means \pm SD. P-values were obtained by the Student's t-test. SD, standard deviation.

In the patient subjects, the frequency of the T and G alleles were 78.3 and 21.7%, respectively. In the control subjects, the frequency of the T and G alleles were 71.5 and 28.5%, respectively. Genotype distributions for the colon cancer patients (goodness-of-fit $\chi^2=0.33$, df=1, P=0.56) and the control group (goodness-of-fit $\chi^2=3.82$, df=1, P=0.50) were in HWE. The G allele at the T45G polymorphism was associated with a lower risk of colon cancer (OR=0.41; 95% CI, 0.19-0.86; P=0.22).

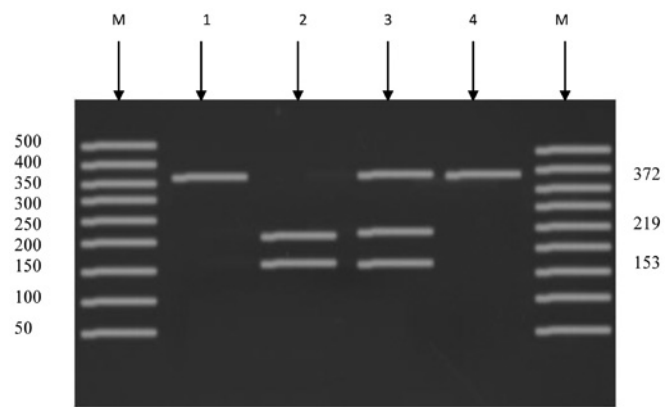


Figure 1. Representative RFLP analysis of the *Sma*I digest of the PCR product that contains position 276 of the *ADIPOQ* gene separated on a 2% agarose gel. Lane M: DNA marker. Lane 1: uncut PCR product of size 372 bp. Lane 2: Homozygous (GG) shows two bands of sizes 219 and 153 bp. Lane 3: Heterozygous (TG) shows three bands of sizes 372, 219 and 153 bp. Lane 4: Wild-type (TT) shows one band of size 372 bp. RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction.

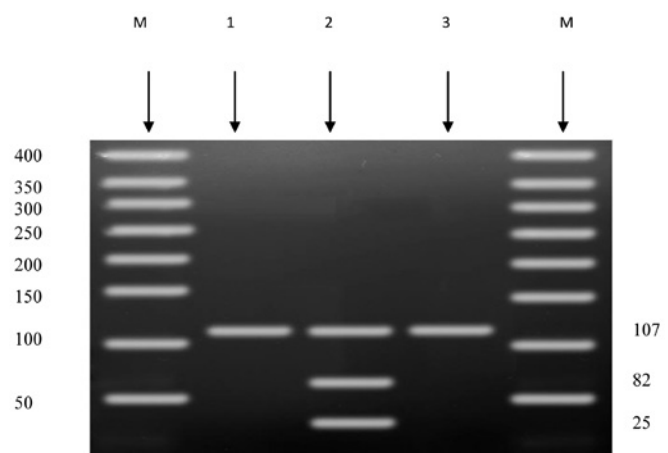


Figure 2. Representative RFLP analysis of the *Hinf*I digest of the PCR product that contains position 45 of the *ADIPOQ* gene separated on a 2% agarose gel. Lane M: DNA marker. Lane 1: uncut PCR product of size 107 bp. Lane 2: Heterozygous (GT) genotype that shows three bands of sizes 107, 82 and 25 bp. Lane 3: Wild-type (GG) genotype that shows one band of size 107 bp. RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction.

Association between the SNPs and circulating adiponectin concentration. The colon cancer group had significantly lower adiponectin concentrations than those in the control group (P=0.001). Further analysis revealed that the level of adiponectin of TG+GG genotype carriers was not significantly different from that of the TT genotype in colon cancer patients (P=0.30) and in the control group (P=0.37). The result of the association between the G276T SNP and circulating adiponectin concentration showed that the level of adiponectin of GT+TT genotype carriers was not significantly different from that of the GG genotype in colon cancer patients (P=0.32) and in the control group (P=0.84).

Table II. Allele distribution and genotype frequency of the *ADIPOQ* gene G276T SNP.

Group	n	Genotype frequency		Allele frequency	
		GG	GT+TT	G	T
Colon cancer	60	55 (91.7%)	5 (8.3%)	115 (95.9%)	5 (4.1%)
Control	60	58 (96.7%)	2 (3.3%)	118 (98.4%)	2 (1.6%)
		OR (95% CI), 2.64 (0.49-14.6)		OR (95% CI), 2.56 (0.49-13.94)	
		RR (95% CI), 2.50 (0.5-12.39)		RR (95% CI), 2.50 (0.49-12.64)	

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; RR, risk ratio.

Table III. Allele distribution and genotype frequency of the *ADIPOQ* gene T45G SNP.

Group	n	Genotype frequency		Allele frequency	
		TT	TG+GG	T	G
Colon cancer	60	40 (63.3%)	20 (36.7%)	94 (78.3%)	26 (21.7%)
Control	60	27 (45.4%)	33 (54.6%)	86 (71.5%)	34 (28.5%)
		OR (95% CI), 0.41 (0.19-0.86)		OR (95% CI), 0.76 (0.39-1.26)	
		RR (95% CI), 0.61 (0.39-0.93)		RR (95% CI), 0.69 (0.94-1.19)	

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; RR, risk ratio.

Discussion

The aim of this study was to evaluate the association between two SNPs, rs1501299 (G276T) and rs2241766 (T45G), in the *ADIPOQ* gene with the risk of colon cancer in Saudi patients. In addition, we focused on the effect of the two SNPs on serum adiponectin levels. We examined the hypothesis that the *ADIPOQ* gene polymorphisms may be one of the genetic factors that affect colon cancer susceptibility. Our results showed that carriers of the heterozygous (GT) genotype of G276T had more than a two-fold (OR=2.64; 95% CI, 0.49-14.6; P=0.44) higher risk of colon cancer than carriers of the normal (GG) genotype. By contrast, we found that the G allele in position 45 of the *ADIPOQ* gene had a lower risk (OR=0.41; 95% CI, 0.19-0.86; P=0.22) of colon cancer than carriers of the normal (TT) genotype. The results suggest that the G276T SNP of the *ADIPOQ* gene contributes to the genetic risk of colon cancer and that the presence of the G allele in position 45 of the *ADIPOQ* gene appears to act as a protective factor against colon cancer. To the best of our knowledge, this is the first study that has investigated the association between G276T and T45G polymorphisms of the *ADIPOQ* gene with colon cancer risk.

In the present study, although the patients with colon cancer had significantly lower height, weight, waist and hip circumferences than the controls, and differences of BMI were only of borderline significance, adiponectin levels were significantly (P=0.001) lower in the patient group than in the control group. Our results are in agreement with those from other studies that have shown an inverse association between adiponectin levels

and risk of cancer. Ishikawa *et al* observed lower adiponectin serum levels in patients with gastric cancer in comparison to the control group (16). Furthermore, they observed that adiponectin serum concentration inversely correlated with tumor size and cancer stage. Fukumoto *et al* observed in patients with adenoma a slightly lower adiponectin serum concentration than in healthy controls (17). Kumor *et al* observed in patients with colorectal adenomas and carcinoma lower serum adiponectin concentrations than in the control group (18). Those studies may give support to our data regarding the significantly lower serum adiponectin levels in colon cancer patients in comparison to the control group. We speculate that decreased serum adiponectin levels may be a factor involved in the development of colon cancer or a secondary effect of the metabolic derangements in colon cancer.

To explore the molecular mechanism underlying the role of adiponectin in colon cancer, we compared the levels of adiponectin between the heterozygous, homozygous and normal genotype carriers. Our results showed no significant difference in the levels of adiponectin between heterozygous or homozygous genotype carriers and the normal genotype carriers for the two SNPs (data not shown). Our results suggest that the two SNPs are non-functional and have no role in the expression of circulation levels of adiponectin. By contrast, previous have studies found that the T allele of SNP 276 is associated with higher adiponectin levels (19-27). However, some studies have found no association between the SNP 276 and circulating adiponectin levels (28-30). Furthermore, Filippi *et al* found significant associations between adiponectin levels and the SNP G276T and they suggested that the

adiponectin gene variant, or a mutation in linkage with it, determines lower adiponectin gene expression (31). Woo *et al*, found that the haplotypes formed by SNPs 45, 276 and 349 were associated with adiponectin levels in Caucasians but not in African-Americans (32). These results suggest that there may be multiple SNPs working in conjunction with each other and with the environment to affect adiponectin levels.

Despite the small number of colon cancer patients tested, the study's design was relatively strong owing to the fact that the controls were recruited from the same cohort as the colon cancer patients and were matched by age and gender.

In conclusion, our case-control study on the Saudi population showed that the risk of developing colon cancer may be partly explained by genetic polymorphisms in the *ADIPOQ* gene. Our data needs to be replicated in other populations and the mechanisms underlying the role of adiponectin in colon cancer disease require further investigation.

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