Colon cancer is a heterogeneous disease arising in association with abnormalities in different molecular pathways. Epidemiological studies have revealed that genetic susceptibility and environmental factors place certain individuals at a higher risk of developing colon cancer. To reduce colon cancer-related deaths, many studies aimed at identifying more genes linked to colon cancer and finding novel screening- and prognosis-related biomarkers. To date, there are no biomarkers that precisely predict the presence of the disease. Therefore, genetic variation analysis might provide a window into the genetic landscape of human colon cancer to identify new genes. The most frequent type of variation in the human genome and excellent genetic markers for research are single-nucleotide polymorphisms (SNPs). They serve as potential and unique genetic markers for association-based approaches to discover genetic components bearing complex traits. SNP profiles and patterns may find immense utility in identifying a comprehensive collection of genes that contribute to the development and susceptibility of complex diseases such as cancer. The presence of SNPs in several genes (MLH1, MSH2, PMS2, APC, MUTYH, SMAD7, STK11, XRCC3, DNM1, MTHFR, RAD51, XRCC2, EXO1, XRCC1, VDR, and RETN) was reported to be associated with colon cancer. Therefore, identifying these SNPs is important for scientists to ascertain the gene or genes that predispose individuals to the colon cancer risk.

Resistin gene (RETN) codes a peptide hormone called resistin, which is a 12.5–kDa, C-terminal, cysteine-rich signaling peptide. Former researches suggested that resistin in humans is secreted predominantly by adipose tissue, in particular, from adipocytes and macrophages. Subsequent studies suggested that resistin may not come directly from adipocytes, and may rather originate from inflammatory cells infiltrating fat tissue. The release of resistin appears to be stimulated by inflammatory processes and has been linked to insulin resistance and inflammation. Several single-nucleotide polymorphisms (SNPs) have been identified in the human resistin gene (RETN). This study aims to investigate the association of RETN rs1862513 (C-420G) and rs3745367 (G+299A) SNPs with the colon cancer risk in Saudi patients.
by inflammation, lipopolysaccharide, interleukin–6, hyperglycemia, growth, and gonadal hormones. While released within the fat tissue, resistin acts on adipocytes themselves leading to insulin resistance. Resistin exists in 3 forms: a trimer, a hexamer, and a monomer; the lower molecular weight form is the most active.23 Since the discovery of resistin in 2001, there has been controversy regarding the role of resistin in humans. There has been strong data for rodent (mice) on the pathophysiological effects of resistin in mediating a multitude of metabolic impairments, including insulin resistance and type II diabetes.24 Unfortunately, these findings have not been necessarily translatable to humans. A clear finding regarding resistin physiology in humans suggests that resistin adipose tissue expression and resistin serum levels increase in obese subjects.25-27 Consistent with this, there are strong positive correlations between plasma resistin levels and increases in both body mass index (BMI) and the quantity of abdominal visceral adipose tissue.25,28-31

Given the importance of obesity in colon cancer development and the fundamental role of resistin in obesity, it is reasonable to hypothesize that RETN gene SNPs may play a role in colon cancer susceptibility. SNPs rs1862513 (C-420G) and rs3745367 (G+299A) in the RETN gene were selected because of their previous association with resistin circulating levels.15,32 The rs1862513 (C-420G) SNP, in the promoter region of the RETN gene, has been associated with obesity, insulin sensitivity, and insulin resistance.33-35 Here, the study investigated the effect of rs1862513 (C-420G) and rs3745367 (G+299A) SNPs in RETN gene on the risk of colon cancer.

SUBJECTS AND METHODS

Participants
A case-control study was conducted among Saudi adult patients (n=60) with a diagnosis of colon cancer. The patients were recruited from King Abdulaziz Hospital and Oncology Center in Jeddah, Saudi Arabia. They had positive colonoscopic results for malignancy, histologically confirmed as colon cancer. Unrelated subjects (controls) (n=60) were recruited in the same period as the cases 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. Both colon cancer patients and controls were excluded if they had diabetes or high blood pressure, or if they were under any treatment course. All volunteers and patients signed an informed consent for participation. The ethical committees of the King Abdulaziz University had approved the study.

Anthropometric analyses
Anthropometric parameters represented by weight and height were measured for each patient included in the study. The BMI was calculated by applying the following formula: BMI=weight (kg)/height (m²). Data about the medical history of each patient was also collected.

Genotyping
The SNP rs1862513 (C-420G), the one that showed an association with colorectal cancer in the study by Pechlivanis and his team, was selected for the analysis.36 Furthermore, SNP rs3745367 (G+299A) was selected from dbSNP (http://www.ncbi.nlm.nih.gov). Total genomic DNA was extracted from peripheral blood (5 mL) samples using a DNA extraction kit (QiAamp DNA Blood Mini Kit; Hilden, Germany), applying the manufacturer’s protocol. The polymorphisms were identified using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Two sets of primers (5.0 nmol) from TIB (TIB Molbiol Inc., Germany) were used. For promoter region amplification, the forward primer 5’-TTTTGTACGTTGCAACGC-3’ and reverse primer 5’-GGGCTCAGCTAACCAAAT-3’ were used. For 3’-untranslated region amplification, the forward primer 5’-AGAGTCCACGCTCCTGTGTT-3’ and reverse primer 5’-CATCTCAGTTTTATTTTCCAG-3’ were used. The amplification was performed in a volume of 25 µL, containing 0.2 µg genomic DNA, 1X PCR buffer, 2.5 mM MgCl2, 0.2 mM of each dNTP, 0.1 µM of each primer, and 0.5 U Taq DNA poly-

| Variables | Patients n=60 Mean (SD) | Controls n=60 Mean (SD) | P value |
|-----------|------------------------|-------------------------|---------|
| Age (y)   | 50.5 (12.1)            | 54.3 (12.3)             | .08     |
| Height (cm)| 163.2 (9.1)           | 160.8 (9.0)             | .14     |
| Weight (kg)| 77.4 (10.1)           | 66.4 (13.3)             | .001b   |
| Waist (cm)| 80.1 (31.1)           | 67.1 (24.8)             | .01a    |
| Hip (cm)  | 88.6 (25.6)            | 66.0 (30.2)             | .0001b  |
| WHR       | 0.96 (0.3)             | 1.08 (0.3)              | .19     |
| BMI       | 28.6 (6.1)             | 25.3 (5.4)              | .005b   |

2-test was used in the comparison.
BMI: body mass index; SD: standard deviation; WHR: waist-to-hip ratio.
*Significant; +Highly significant.
merase (Promega Corporation, Madison, WI, USA). The PCR initial denaturation was done at 96°C for 5 minutes, 40 cycles at 96°C for 35 seconds and at 51°C for 35 seconds (SNP C-420G), 55°C for 30 seconds (SNP G+299A), 72°C for 35 seconds, and a final elongation step at 72°C for 4 minutes. A total of 5 µL of the PCR products was digested with restriction endonucleases (BpiI for SNP C-420G and AluI for SNP G+299A) for 2 hours at 37°C. The PCR products of digestion were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide.

Statistical analysis
The statistical analyses were performed using the SPSS version 16.0 (SPSS Inc., Chicago, USA). Continuous variables were expressed as the means (standard deviation [SD]). In addition, Mann-Whitney U/Wilcoxon Rank Sum test (Z-test) was used to compare between the 2 groups. The Hardy-Weinberg equilibrium (HWE) was tested for genotype distributions and allele frequencies. The differences in genotype distributions and allelic frequencies between colon cancer patients and controls were examined using the Fisher exact and the chi-square tests. The odds ratios (OR) and 95% confidence interval (95% CI) ranges were calculated by using logistic regression. Significance was considered at a P value <.05.

RESULTS

Subjects’ characteristics
The mean age was 50.5 years for colon cancer patients and 54.3 years for controls. No significant difference was found for body height, waist-to-hip ratio, and age between the 2 groups. However, the body weight (P=.001), waist (P=.01), hip (P=.0001), and BMI (P=.005) were significantly higher in the colon cancer patients than in the controls. The characteristics of the patient and the control groups are shown in Table 1.

PCR-RFLP analysis
The amplified fragment that contains C-420G SNP showed a size of 330 bp. The wild (CC) genotype showed 2 bands of sizes 202 and 128 bp. The heterozygous (CG) genotype showed 3 fragments of sizes 330, 202, and 128 bp. The homozygous (GG) genotype produced 1 fragment of size 330 bp (Figure 1). The other amplified fragment that contains G299A showed a size of 373 bp. The genotype for the wild (GG) individuals produced 2 bands of sizes 243 and 55 bp. The heterozygous (GA) genotype produced 4 bands of sizes 243, 158, 85, and 55 bp. The homozygous (AA) genotype

Figure 1. Photograph of a 2% agarose gel showing the result of BbsI digest. Lane M: DNA marker. Lane 1: uncut PCR product of size 330 bp. Lane 2: heterozygous (CG) genotype that shows 3 bands of size 330, 202, and 128 bp. Lane 3: homozygous (GG) genotype that shows an uncut band of size 330 bp. Lane 4: normal (CC) genotype that shows 2 bands of size 202 and 128 bp.

Figure 2. Photograph of a 2% agarose gel showing the result of AluI digest. Lane M: DNA marker. Lane 1: uncut PCR product of size 373 bp. Lane 2: normal (GG) genotype that shows 2 bands of size 243 and 55 bp. Lane 3: heterozygous (GA) genotype that shows 4 bands of size 243, 158, 85, and 55. Lane 4: homozygous (AA) genotype that shows 3 bands of size 158, 85, and 55 bp.
produced 3 bands of sizes 158 85, and 55 bp (Figure 2).

In the recruited control group, the genotype distribution of C-420G was out of HWE ($X^2 = 6.18, df=1, P=.01$). In the patients, the genotype distribution of C-420G was in HWE ($X^2 = 0.67, df=1, P=.41$). When the C-420G genotype frequencies were compared between the healthy control and patient groups, the CC genotype occurred with a lower frequency in colon cancer patients than in controls, thus suggesting its protective effect against colon cancer (see Table 2). However, CG heterozygosity and G alleles were more prevalent in patients and might, therefore, convey susceptibility for the disease.

In regard to G299A SNP, the genotype distributions for the controls ($X^2 = 6.55, df=1, P=.01$) and the patients ($X^2 = 29.83, df=1, P=.0001$) were out of HWE, which is possibly because of the small sample size. As shown in Table 3, the wild GG genotype occurred with a lower frequency in patients (5%) than in controls (25%). The prevalence of GA genotype was 85% in patients and 65% in healthy individuals (OR=6.5, $P=.002$). A further association analysis was conducted to identify the interactions of the 2 SNPs, C-420G and G299A, and their impact on the colon cancer risk. The genotype combinations CC/GA, CG/GA, and GG/GA for C-420G and G299A were associated with a higher risk of colon cancer (Table 4).

**DISCUSSION**

Adipokines, secreted by the adipose tissue, are strong candidates for the link between obesity and colon cancer risk. To investigate the influence of rs1862513 (C-420G) and rs3745367 (G+299A) SNPs in RETN gene on the colon cancer risk, the SNPs were genotyped in a cohort of cases and controls from Saudi Arabia. In the single SNP analysis, the data of the present study showed that both rs1862513 (C-420G) and rs3745367 (G+299A) SNPs had an association with the colon cancer risk (OR 2.48, 95% CI 1.07-5.74 and OR 6.33, 95% CI 1.77-24.18, respectively).

The promoter SNP rs1862513 (C-420G) has been investigated by many groups; some researchers examined its association with resistin levels and adiposity. However, only 3 publications with contrasting findings have addressed the question of an association between rs1862513 (C-420G) SNP and colorectal cancer (CRC) risk. The present study confirms the results of the 2 studies that reported an association between RETN SNP rs1862513 (C-420G) and colon cancer risk.

Although a few studies were performed on the association of rs1862513 (C-420G) SNP with CRC, no study has been reported on the association of rs3745367 (G+299A) SNP in RETN gene with CRC risk. The present study observed that carriers of the heterozygous (GA) genotype of SNP 299 (OR=6.5, 95% CI 1.77-24.18, $P=.002$) had a significantly higher colon cancer risk than carriers of the wild (GG) geno-
Cancer is a multigenic disease in which single SNP may only have a modest independent effect, and multiple SNPs may provide a more accurate representation of the risk. The present study explored the interaction of RETN G299A and C-420G SNPs in 60 controls and 60 cases of colon cancer. The odds ratio (OR) was used to evaluate the colon cancer risk in terms of the best combination of SNP-SNP interactions. Compared to their corresponding non-SNP combinations, the estimated OR of the patients with CG/GA (OR=18.76; CI: 2.22–158.36; \( P = .0007 \)) and GG/GA (OR=9.63; CI: 0.98–94.54; \( P = .04 \)) combinations were characterized by a higher colon cancer risk. This combination effect could be explained by the additive effect of the 2 genotypes.

Although a single SNP may have a moderate effect on the development of cancer, several SNPs together can exert a significant influence. The pondering question is “how many SNPs are needed?”. The answer to this question is not simple due to the fact that the effect of genotype on disease phenotype varies between diseases and populations, owing this to genetic and environmental heterogeneity. A strategy utilizing biomarkers to stratify patients into appropriate screening programs can potentially prevent colon cancer. Future work approaching epistatic relationships from the SNP level is a need for a preventive strategy.

Although the number of subjects was small in this study limiting the statistical power, the finding of this study should be considered. Despite this limitation, the design of this study was relatively strong because the controls were recruited from the same cohort as the colon cancer patients. Also the cases and controls were matched by age and sex.

In summary, the present data indicates a potential role for RETN G299A and C-420G polymorphisms in the genetic predisposition to colon cancer disease in the Saudi population. It also demonstrated a combined effect of SNPs RETN C-420G and G+299A and showed an increased chance of the colon cancer risk. The interaction was not previously reported. Future research should be conducted with a larger sample population and a combination approaches to further test SNP-SNP interactions in adipocytokine genes associated with the colon cancer risk.
REFERENCES

1. Kruk J, Czernecki U. Physical activity and its relation to colon cancer risk: updating the evidence. Asian Pac J Cancer Prev. 2013; 14(7): 3993-30.

2. Peters U, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, et al. Identification of genetic susceptibility loci for colorectal tumors in a genome-wide meta-analysis. Gastroenterol ology. 2013; 144(4): 799-807.

3. Tse G, Elick GD. Cruciferous vegetables and risk of colorectal neoplasms: a systematic review and meta-analysis. Nutr Cancer. 2014; 66(1): 128-39.

4. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, et al. The human mutator gene homology MSH2 and its association with hereditary nonpolyposis colon cancer. Cell. 1993; 75(5): 1027-38.

5. Uthoff SM, Eichenberger MR, McAuliffe TL, Hamilton-Gi, Lalandik S. Wingless-type frizzled receptor signaling and its putative role in human colon cancer. Mol Carcinog. 2001; 31(1): 56-62.

6. Gunter MJ, Leitzmann MF. Obesity and colorectal cancer: epidemiology, mechanisms and candidate genes. J Nutr Biochem. 2006; 17(3): 149-58.

7. Kupka R, Slivinskis T, Wisniweska-Jarosinska M, Chojnacki J, Wasylecka M, Dzik L, et al. Polymorphisms in RAD1, ARCC2 and ARCC3 genes of the homolog repair complex in colorectal cancer—a case control study. Mol Biol Rep. 2011; 38(4): 2849-54.

8. Lurie G, Zhang W, Lenz HJ. Molecular prognostic markers in locally advanced colon cancer. Clin Colorectal Cancer. 2007; 6(10): 683-90.

9. Fain JE. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm. 2006; 74: 433-77.

10. Bastard JP, Maaschi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw. 2006; 17 (1): 4-12.

11. Wisam H, Al-Daghrir NM, Cheeth R, McTernan PG, Barnett AH, Kumar S. Relationship of serum adiponectin and resistin to glucose intolerance and fat topography in South-Asians. Cardiovasc Diabetol. 2008; 7: 10.

12. Patel SD, Rajala MW, Rossetti L, Scherer PE, Shapiro L. Disulfide-dependent multimeric assembly of resistin family histones. Science. 2004; 304: 1514-58.

13. Gatamani M, Szewergold NR, Gneves DR, Ahi ma RS, Lazar MA. Macrophage-derived human resistin exacerbates adipose tissue inflammation and insulin resistance in mice. J Clin Invest. 2009; 119: 531-39.

14. Degawa-Yamauchi M, Bovenkerk JE, Julier BE, Watson W, Kerr K, Jones R, et al. Serum resistin (FIZZ3) protein is increased in obese humans. J Clin Endocrinol Metab. 2003; 88(11): 4542-55.

15. Vendrell J, Broch M, Vilarrasa N, Molina A, Gómez JM, Gutiérrez C, et al. Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. Obes Res. 2004; 12 (6): 662-71.

16. Schlaffner A, Böchler C, Müller-Ladner U, Herfarth H, Ehling A, Paul G, et al. Identification of variables influencing resistin serum levels in patients with type 1 and type 2 diabetes mellitus. Horm Metab Res. 2004; 36(10): 702-07.

17. Azuma K, Katsukawa F, Oguchi, S, Murata M, Yamazaki H, Shimada A, et al. Correlation between serum resistin level and adiposity in obese individuals. Obes Res. 2005; 13(9): 1197-1203.

18. Yannakoulia M, Yannakouris N, Blüher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. J Clin Endocrinol Metab. 2003; 88(4): 1730-36.

19. Vozarova de Courten B, Degawa-Yamauchi M, Considine RV, Tatarnarri PA. High serum resistin is associated with increased in adiposity but not a worsening of insulin resistance in Pima Indians. Diabetes. 2004; 53: 1279-84.

20. Piestrzeniewicz K, ?uczk? K, Komorowski J, Maciejewski M, Jankiewicz Wik?, Sock J. Resistin increases with obesity and atherosclerotic risk factors in patients with myocardial infarction. Metabolism. 2008; 57: 488-93.

21. Osawa H, Yamada K, Onuma H, Ochi M, Murakami A, Yamauchi J, Takasaki T, et al. Resistin SNP-420 determines its monocyte mRNA and serum levels inducing type 2 diabetes. Biochem Biophys Res Commun. 2005; 335: 596-92.

22. Mennegi C, Cocco A, Salvemini L, Thompson R, De Cosmo S, Doria A, et al. Heritability of serum resistin and its genetic correlation with insulin-resistance-related features in non diabetic Caucasians. J Clin Endocrinol Metab. 2006; 91(7): 2792-5.