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Genetic variation in the *ADIPOQ* gene and serum adiponectin increase the risk of bladder cancer

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Abstract
Bladder cancer (BC) is the 10th most common cancer worldwide. Genetic studies estimated 30% heritability in BC risk. Adiponectin is an adipocytokine that has important roles in the regulation of energy metabolism. Recent evidence suggests dysregulation of adiponectin levels in BC tissues. Serum level of adiponectin is influenced by single nucleotide polymorphisms (SNPs) in the *ADIPOQ* gene. However, limited evidence is available regarding the association between adiponectin serum levels or SNPs in *ADIPOQ* and BC risk. This study aimed to assess whether adiponectin serum levels or SNPs in *ADIPOQ* may modify BC risk. In this case-control study, 114 BC patients were recruited along with 114 controls. Study subjects were genotyped for variations in *ADIPOQ* SNPs, namely rs17300539, rs266729, rs2241766, and rs1501299. Adiponectin levels were measured from the serum of study subjects. Our analysis showed that the G allele and the GG genotype of rs1501299 were significantly more frequent in BC patients compared to those in the control group (p-value < 0.05). Moreover, two *ADIPOQ* haplotypes containing the above G allele were associated with increased BC risk (p-value < 0.05). Multivariate analysis showed that increased serum adiponectin, smoking or age were all significant predictors of BC (p-value < 0.05). The data supports use of serum adiponectin and the G allele of rs1501299 SNP in *ADIPOQ* as potential biomarkers and/or targets in BC. To further validate findings in this study, larger populations of various ethnicities and/or genetic backgrounds are required. More investigations on the functional role of adiponectin in BC will also provide better understanding of potential targeting adiponectin for BC treatment.

Keywords: Adiponectin; *ADIPOQ*; Bladder cancer; rs1501299; Single nucleotide

Highlights:
- Serum adiponectin is associated with bladder cancer risk.
- The G allele and the GG genotype of rs1501299 of *ADIPOQ* gene are associated with bladder cancer risk.
- Serum adiponectin and the G allele of rs1501299 SNP in *ADIPOQ* can be used as potential biomarkers of bladder cancer.

Abbreviations:
AdipoR: adiponectin receptors; BC: Bladder cancer; BMI: body mass index; CI: confidence interval; DM: diabetes mellitus; EDTA: Ethylene-Diamine-Tetra-Acetic acid; ELISA: Enzyme Linked Immuno-sorbent Assay; OR: odds ratio; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism; SNPs: single nucleotide polymorphisms.

Introduction

Bladder cancer (BC) ranks as the 10th most common cancer worldwide, with an estimated 549,000 new cases and 200,000 deaths per year, according to recent Global cancer statistics 2018 (Bray et al., 2018). Cigarette smoking remains a major risk factor for the development of BC and it is estimated that 50% of all BC patients smoked cigarettes (Cumberbatch et al., 2018; van Osch et al., 2016). Smoking is also associated with a more invasive BC phenotype at the time of diagnosis, faster tumor progression, and lower survival rates (Jiang et al., 2012). Exposure to other environmental factors also increases the risk of BC, including exposure to arsenic and chlorinated byproducts that may be present in drinking water (Hrudey et al., 2015).

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Despite the strong association between smoking and BC, not all BC patients are smokers or ex-smokers (Redondo-Gonzalez et al., 2015). Moreover, epidemiological data suggested that only a minor fraction of individuals exposed to any of the environmental factors mentioned above will eventually develop BC (Chu et al., 2013). Indeed, there is evidence that BC risk may also be affected by genetic polymorphisms (Franekova et al., 2008; Giedl et al., 2016; Sankhwar et al., 2016; Yin et al., 2018). In addition, epidemiological data showed a 2-fold increase in BC risk in first degree relatives (Aben et al., 2002), while Lichtenstein et al. (2000) reported a 31% genetic heritability for BC based on a large population-based twin study.

Adiponectin is a hormone secreted by adipose tissues and it appears to have a variety of functions (Achari and Jain, 2017; Di Zazzo et al., 2019; Lee and Shao, 2014). It mediates its effects through interactions with two receptors: AdipoR1 and AdipoR2. Adiponectin is primarily known for its role in the regulation of energy metabolism and insulin resistance (Achari and Jain, 2017; Lee and Shao, 2014). Indeed, it is generally accepted that adiponectin enhances insulin sensitivity and increases energy expenditure in target tissues. Not surprisingly, lower levels of adiponectin were found to be associated with multiple metabolic disorders, including obesity, prediabetes, and polycystic ovarian syndrome (Alfaqih et al., 2018a, b; Balsan et al., 2015; Lee and Shin, 2020; Shirazi et al., 2021).

Observations made as early as the 1920s by Otto Warburg indicated that cancer cells harbor perturbations in energy metabolism compared to normal non-malignant cells (Liberti and Locasale, 2016). Evidence continues to shift the paradigm of how the scientific community views cancer biology as the cumulative product of simple mutations in proto-oncogenes or tumor suppressor genes to a complete reprogramming of energy metabolism in cancer cells (Liberti and Locasale, 2016). Given the well-documented role of adiponectin in energy metabolism, it is not surprising that there is an accumulating body of literature demonstrating that perturbations in adiponectin levels could modify the risk of several tumors (Cui et al., 2018; Tan et al., 2015; Wei et al., 2005; Zeng et al., 2015).

Most evidence supports a tumor suppressor role of adiponectin in modifying cancer risk. For example, in prostate cancer, Tan et al. (2015) observed that adiponectin levels are lower in high grade prostate cancer tissues and proposed that this could be the result of gene silencing through promoter hypermethylation. Moreover, *in vitro* studies demonstrated that direct treatment of the breast cancer cell line, MCF7, with adiponectin suppressed its growth (Jardé et al., 2009). In line with the above evidence, several observational studies demonstrated that low levels of serum adiponectin were linked with an increased risk of breast, colon, esophageal, endometrial, and renal cell carcinoma (de Martino et al., 2016; Izadi et al., 2012; Miyoshi et al., 2003; Wei et al., 2005; Yıldırım et al., 2009; Zeng et al., 2015).

Despite this, the role of adiponectin in modifying cancer risk is not that clear and there is evidence supporting its role as a tumor promoter. For example, epidemiological evidence indicated that higher serum levels of adiponectin were associated with increased risk of liver and prostate cancers (Arano et al., 2011; Zhang et al., 2018). It can therefore be concluded that the role of adiponectin in cancer is not that well-understood, and there could be differences between the effects of endogenous or locally produced adiponectin with systemic adiponectin found in the serum. Another explanation is that the exact effect of adiponectin could be tissue specific or dependent on tumor stage.

In BC, conflicting evidence is present in the role of adiponectin. Recent findings from Kashiwagi et al. (2020) using immunohistochemistry staining of bladder tissues revealed higher expression of adiponectin, AdipoR1, and AdipoR2 in bladder tumors compared to benign urothelial tissues, which supports a tumor promoter role for endogenously produced adiponectin. However, the same study reported a lower risk of recurrence for tumors that have an adiponectin positive non-muscle-invasive type (Kashiwagi et al., 2020). In addition, *in vitro* experiments showed that treatment of two bladder cancer cell lines with a synthetic adiponectin inhibited their migration (Kashiwagi et al., 2020). Therefore, the exact role of adiponectin in modifying BC risk is not that well understood.

A study that compared the levels of adiponectin between fraternal and identical twins concluded that adiponectin was significantly associated with genetic variance and heritability, which is independent of the body mass index (BMI) (Cesari et al., 2007). The same study found that plasma adiponectin levels could be heavily affected by single nucleotide polymorphisms (SNPs) in the gene that codes for adiponectin protein known as *ADIPOQ* (Cesari et al., 2007).

Given the fact that variations in plasma adiponectin levels are strongly influenced by genetic variation in *ADIPOQ* (Dhillon et al., 2011), and the preliminary evidence of the role of adiponectin in determining the risk of recurrence of BC (Kashiwagi et al., 2020), we hypothesized that serum adiponectin and SNPs in the *ADIPOQ* gene (rs17300539, rs266729, rs2241766 and rs1501299) could be associated with BC. Accordingly, this study aimed to (1) evaluate the association of adiponectin levels with the risk of bladder cancer and (2) evaluate the association of certain SNPs in *ADIPOQ* gene with the risk of bladder cancer.

Materials and methods

Study design and subject description

This was a case-control study involving 114 Jordanian BC patients who attended the Urology clinics of King Abdullah University Hospital (KAUH). All patients included in the study had a confirmed diagnosis of BC and were actively being treated for their tumor at the time of recruitment.

Patient recruitment involved a short interview with a clinical research coordinator. In this interview, the coordinator briefly explained the objectives of the study and that participation would involve the collection of demographic and clinical data, anthropometric measurements, and a future blood withdrawal. Patients who agreed to participate in the study were asked to sign an informed consent, which also reiterated the information detailed above.

During the patients' next visit to the clinic the following data were collected: (a) demographic data including age, gender and smoking status, and (b) anthropometric data including weight [in kilograms (kg)] and height [in meters (m)] which was used to calculate the Body Mass Index (BMI) of the patient using the following formula; BMI (kg/m^2) = weight (kg)/ square of the height (m). Demographic and anthropometric data collected by the research coordinator were then entered into excel spreadsheets.

The control group consisted of 114 subjects selected from the same institution. These were patients who attended family medicine clinics of KAUH for routine visits and were free from bladder illness – according to their medical history. This included the absence of any urology related symptoms (diffi-

culty urinating, blood and other discharges in the urine, burning upon urination).

Blood sample collection

Two blood samples (5 ml each) were collected from each of the study subjects by a certified phlebotomist. Subjects were requested to fast for 15 hours prior to blood withdrawal, which was performed the following morning at around 9 am. For DNA extraction, one sample was placed in an Ethylene-Diamine-Tetra-Acetic acid (EDTA) tube (AFCO, Jordan), and then kept at 4 °C. The other sample was placed in a plain tube with gel clot activator (AFCO, Jordan) and used to obtain serum following centrifugation for 5 minutes at 4000 × g. The supernatant (i.e., serum) was then transferred into an Eppendorf tube which was stored in a –80 °C freezer.

Biochemical measurements

The serum stored at –80 °C was later used for the measurement of adiponectin levels. An Enzyme Linked Immuno-sorbent Assay (ELISA) was used for this purpose. The kit used to measure adiponectin levels was purchased from Mybiosource (San Diego, CA, USA). The instructions of the manufacturer were followed for the measurement.

DNA extraction and genotyping

Genomic DNA was extracted from whole blood samples collected in EDTA tubes. QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was used in the procedure. DNA purity was then checked using an ND-2000 Nanodrop (Thermo Scientific, Waltham, MA, USA). A Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based approach was used to determine the genotypes of four SNPs of *ADIPOQ* gene (rs17300539, rs266729, rs2241766 and rs1501299). The concentrations of the reagents used in the PCR reaction and the final reaction volume were as described in previous publications (Alfaqih et al., 2018b). The forward and reverse primer sequences for each SNP can be found in Table 1. The location of each SNP on the *ADIPOQ* gene, the size of the PCR amplicon, the restriction enzyme used in the assay, and the size of the products following restriction enzyme digestion are listed in Table 1. The undigested PCR products and the DNA fragments which resulted from restriction enzyme treatment were run on a 3% agarose gel stained with SYBR™ Safe DNA Gel Stain (ThermoFisher Scientific, Waltham, MA, USA) and then visualized under blue light.

Table 1. *ADIPOQ* SNPs information

SNP ID	Location and base change	Forward primer Reverse primer	PCR program (34 cycles)	PCR product size (bp)	Restriction enzyme, incubation temperature & time	RFLP product (bp)
rs17300539	Promoter* region (AG)	CAGGAAGCTGAGGCTTTACA ACCCACTTAGGTGTTCCTAGA	95 °C 30 s 58.5 °C 30 s 72 °C 1 min	342	Alu I 37 °C, 1 h	AA: 236, 106 GA: 342, 236, 106 GG: 342
rs266729	Promoter* region (CG)	ACTGTGGAGATGATATCTGG CATTTTGACAGCTACCTTGG	95 °C 30 s 72 °C 1 min	412	Hha1 37 °C, 1 h	GG: 170, 243 CG: 170, 243, 412 CC: 412
rs1501299	Intron 2* (GT)	TGACCAGGAAACCACGACTC CCATCTACACTCATCCTTGG	95 °C 30 s 58 °C 30 s 72 °C 1 min	341	BsmI 65 °C, 1 h	GG: 229, 112 GT: 341, 229, 112 TT: 341
rs2241766	Exon 2* (TG)	AGTAGACTCTGCTGAGATGG ACATTCTTACCTGGATCTCC	95 °C 30 s 59 °C 30 s 72 °C 1 min	333	BspH1 37 °C, 1 h	TT: 153, 180 TG: 333, 153, 180 GG: 333

* All SNP information was obtained from the NCBI dbSNP database; SNP: single nucleotide polymorphism; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Studies (SPSS) software (version 23, IBM, NY). Differences in age, BMI, and adiponectin levels between BC patients (cases) and control groups were assessed using a student *t*-test, while differences in gender distribution, smoking and diabetes mellitus (DM) status were assessed using Pearson's Chi-square. The association between allele or genotype categories with BC risk was assessed using Pearson's Chi-square. SHEsis software was used to run haplotype analysis (http://analysis.bio-x.cn/myAnalysis.php).

Multivariate logistic regression analysis was performed to delineate relationship between serum adiponectin, rs17300539, or rs1501299, and BC risk in the presence of age, gender, smoking, DM and BMI as potential confounders. All values of $p \leq 0.05$ with a confidence limit at 95% were considered significant.

Results

Baseline characteristics of the study subjects and serum levels of adiponectin

Baseline characteristics of the study subjects are shown in Table 2. BC patients were significantly older than control subjects (p-value = 0.011). Gender distribution showed that there was a slightly higher percentage of males among BC patients compared to control subjects and there was a higher percentage of BC patients who were smokers, but these differences were not statistically significant (p-value > 0.05). Moreover, there were no differences between BC patients and control groups in BMI or in DM status. Interestingly, our findings indicated that serum levels of adiponectin were significantly higher in BC patients relative to disease free subjects (p-value = 0.0015).

Table 2. Baseline characteristics of the study subjects and serum levels of adiponectin

Variable	Control (n = 114)	Bladder cancer (n = 114)	p-value[a]
Gender (n) (%)			
Males	101 (88.60%)	98 (85.96%)	0.551
Females	13 (11.40%)	16 (14.04%)	
Smoking (n) (%)			
Yes	62 (54.39%)	75 (65.79%)	0.079
No	52 (45.61%)	39 (34.21%)	
DM (n) (%)			
Yes	42 (36.84%)	42 (36.84%)	1
No	72 (63.16%)	72 (63.16%)	
Age (years)[b]	60.60 ± 12.12	64.71 ± 12.40	0.011*
BMI (kg/m^2)[b]	28.30 ± 5.70	28.10 ± 4.90	0.977
Adiponectin (μg/ml)	1.60 ± 0.90	2.03 ± 1.12	0.0015*

DM: diabetes mellitus; BMI: body mass index. The p-values were calculated by Pearson Chi-square test for gender, smoking and DM[a] (%), while the student's t-test was used for Age, BMI and adiponectin[b]. Data are presented as the mean ± standard deviation; * indicates significant difference.

Association between rs1501299 and rs2241766 in ADIPOQ *with bladder cancer*

Considering that serum adiponectin was higher in BC patients and that this difference could be influenced by genetic variation among individuals, we then tested the association between several SNPs in *ADIPOQ* gene and BC. We used a PCR-RFLP based approach to determine the genotype of study subjects for the following SNPs: rs17300539, rs266729, rs2241766, and rs1501299. The results of this analysis are shown in Table 3. Our findings indicated that rs2241766 or rs1501299 were significantly associated with BC (p-value < 0.05).

In our analysis of genotype distribution of rs2241766, it was determined that the percentage of individuals of the heterozygous TG genotype was lower in the control group (Table 3). Regarding rs1501299, our results showed that the percentage of individuals with the TT genotype was significantly higher in the control group, while the percentage of individuals of the GG genotype was significantly higher among BC patients (Table 3).

Interestingly, upon testing the association of alleles of rs1501299 with BC (Table 4), it was found that the frequency of the G allele was significantly higher among BC patients while the frequency of the T allele was significantly higher in the control group.

Haplotype association between ADIPOQ *SNPs and BC*

Given the above findings on the association of individual SNPs in *ADIPOQ* with BC, we then examined the relationship between the haplotypes of all four SNPs (rs17300539, rs266729, rs2241766, rs1501299) and BC.

Our findings (see Table 5) demonstrated that three haplotypes in the *ADIPOQ* gene were significantly associated with BC (p-value < 0.05). Two of the haplotypes significantly increased the risk of the disease. Namely, the GCGG haplotype (OR 3.04; CI 1.58–5.83; p-value = 0.0005) and the GGGG haplotype (OR 4.65; CI 1.34–16.10; p-value = 0.008), while the GCGT haplotype significantly decreased BC risk (OR 0.21; CI 0.11–0.41; p-value < 0.0001).

Table 3. Genotype frequencies of rs17300539, rs266729, rs2241766, and rs1501299 SNPs in control and bladder cancer subjects

SNP ID	Genotype	Control n (%)	Bladder cancer n (%)	p-value[a]
rs17300539	GG	103 (90.40%)	105 (92.10%)	0.640
	GA	11 (9.60%)	9 (7.90%)	
	AA	0 (0.00%)	0 (0.00%)	
rs266729	CC	62 (54.40%)	66 (57.90%)	0.670
	CG	45 (39.50%)	39 (34.20%)	
	GG	7 (6.10%)	9 (7.90%)	
rs2241766	TT	52 (45.60 %)	41 (36.00%)	0.044*
	TG	52 (45.60%)	69 (60.50%)	
	GG	10 (8.80%)	4 (3.50%)	
rs1501299	GG	6 (5.30%)	26 (22.80%)	0.0004*
	GT	87 (76.30%)	76 (66.70%)	
	TT	21 (18.40%)	12 (10.50) %)	

[a] The p-values were calculated by the Pearson Chi-square test; * indicates significant difference.

Table 4. Allele frequencies of rs17300539, rs266729, rs2241766, and rs1501299 SNPs in control and bladder cancer subjects

SNP ID	Allele	Control *n* (%)	Bladder cancer *n* (%)	*p*-value[a]
rs17300539	G A	217 (95.18%) 11 (4.82%)	219 (96.05%) 9 (3.95%)	0.647
rs266729	C G	169 (74.10%) 59 (25.90%)	171 (75.00%) 57 (25.00%)	0.830
rs2241766	T G	156 (68.40%) 72 (31.60%)	151 (66.20%) 77 (33.80%)	0.618
rs1501299	G T	99 (43.40%) 129 (56.60%)	128 (56.10%) 100 (43.90%)	0.007*

[a] The *p*-values were calculated by the Pearson Chi-square test; * indicates significant difference.

Table 5. Haplotype frequency of SNPs rs17300539, rs266729, rs2241766, and rs1501299 in control and bladder cancer subjects

Haplotype	rs17300539	rs266729	rs2241766	rs1501299	Control	Bladder cancer	OR (95% CI)	*p*-value[a]
1	G	C	G	G	0.06	0.16	3.04 (1.58–5.83)	0.0005*
2	G	C	G	T	0.21	0.05	0.21 (0.11–0.41)	<0.000*
3	G	C	T	G	0.17	0.22	1.36 (0.85–2.17)	0.199
4	G	C	T	T	0.25	0.28	1.17 (0.77–1.78)	0.467
5	G	G	G	G	0.01	0.06	4.65 (1.34–16.10)	0.008*
6	G	G	G	T	0.02	0.05	2.05 (0.69–6.09)	0.185
7	G	G	T	G	0.16	0.10	0.59 (0.34–1.04)	0.066
8	G	G	T	T	0.07	0.04	0.50 (0.21–1.19)	0.109

OR: odds ratio; CI: confidence interval; [a] *p*-values were calculated by the Pearson Chi-square test; * indicates significant difference.

Multivariate analysis of adiponectin and ADIPOQ *SNPs with bladder cancer*

The levels of serum adiponectin could be influenced by age and smoking status. BC patients of this population were significantly older than those in the control group. There was also a higher frequency of smokers (although not significant in univariate analysis). Therefore, we conducted a multivariate regression analysis to test if adiponectin or any of the *ADIPOQ* SNPs significantly affects BC risk following adjustment with other confounders.

In this analysis (shown in Table 6) it was observed that serum adiponectin significantly increased the risk of BC by approximately 1.5-fold (OR 1.65; CI 1.19–2.30; *p*-value = 0.003). In this model, smoking and age were also significant predictors of an increased risk of BC (*p*-value < 0.05).

Multivariate analysis also demonstrated that rs1501299 SNP significantly influenced BC risk. We showed that the GT heterozygous genotype of rs1501299 significantly reduced BC risk by around 90% relative to the homozygous GG genotype (OR 0.10; CI 0.03–0.34; *p*-value < 0.001). Furthermore, the homozygous TT genotype of rs1501299 reduced the risk by around 83% (OR 0.17; CI 0.06–0.46; *p*-value < 0.001).

Table 6. Multivariate regression analysis of study subjects

Variable	OR	95% CI	*p*-value[a]
Gender	1.57	0.63–3.94	0.336
Age (years)	1.03	1.01–1.06	0.014*
Diabetes mellitus	0.96	0.52–1.78	0.896
Smoking	2.08	(1.11–3.90)	0.023*
BMI (kg/m^2)	1.00	(0.94–1.06)	0.923
Adiponectin (μg/ml)	1.65	(1.19–2.30)	0.003*
rs2241766			
GG	1		
GT	2.70	(0.61–11.95)	0.190
TT	4.29	(0.98–18.67)	0.053
rs1501299			
GG	1	–	
GT	0.10	0.03–0.34))	<0.001*
TT	0.17	(0.06–0.46)	<0.001*

OR: odds ratio, CI: confidence interval; [a] *p*-values were calculated by binomial logistic regression analysis; * indicates significant difference.

Discussion

The findings of this study indicate that elevated serum levels of adiponectin and genetic variations in *ADIPOQ* (the gene that codes for the adiponectin protein) are associated with BC risk. To our best knowledge, this report is the first to demonstrate that BC risk could be modified by serum adiponectin, rather than endogenously produced adiponectin. Furthermore, it is the first study to report a link between SNPs in *ADIPOQ* and BC.

The above findings will help to increase our understanding of the pathophysiology of BC and its risk, and highlight several approaches – both pharmacological and non-pharmacological – that can be used to prevent BC and/or slow down its progression. Moreover, the results of this study add to a growing body of evidence that supports the notion that the risk of BC can be affected by environmental factors and genetic polymorphisms (Franekova et al., 2008; Giedl et al., 2016; Jiang et al., 2012; Sankhwar et al., 2016; Yin et al., 2018).

An increase in adiponectin levels was observed in BC patients recruited to participate in this investigation. This result is in agreement with the findings made by Kashiwagi et al. (2020) who observed higher expression levels of adiponectin protein in BC tissue microarrays, although the authors of the latter study did not measure the serum levels of adiponectin and only stained intratumoral adiponectin protein using an immunohistochemistry-based approach.

It is interesting to note that although our findings and those of Kashiwagi et al. (2020) demonstrated a tentative role of adiponectin in increasing the risk of BC, the same report observed that tumors with an adiponectin positive non-muscle-invasive type have a lower risk of recurrence. In addition, treatment of two BC cell lines with AdipoRon, a synthetic adiponectin, reduced migration of the two lines. The two results above indicate a protective role for adiponectin in BC that contradicts our data supporting a tumor promoter role for serum adiponectin (Kashiwagi et al., 2020). The literature is rich in data supporting dual roles (tumor suppressor and tumor promoter) for many genetic/biochemical factors (Datta et al., 2020; Yang and Moses, 2008). As detailed in the introduction, the association of adiponectin with cancer risk might depend on the source of adiponectin (endogenous vs. exogenous), the stage of the tumor, or the target tissue (i.e., tissue-specific).

Here we also have studied SNPs in the *ADIPOQ* gene and found that two SNPs, rs1501299 and rs2241766 were associated with BC risk in univariate analysis. In our multivariate regression model, only rs1501299 remained associated with BC. This is not the first investigation in Jordan to establish a link between rs1501299 and a disease of chronic nature. Indeed, genetic variations in the above SNP were shown to be associated with polycystic ovarian syndrome (Alfaqih et al., 2018b), prediabetes (Alfaqih et al., 2018a), and longevity (Khabour et al., 2010).

The uniformity of the above observations merit further testing of the role of rs1501299 across larger, multi-center disease cohorts. If a more comprehensive evaluation of rs1501299 is in agreement with the findings of this report, it is suggested that genetic variations in rs1501299 are ascertained as part of a risk prediction algorithm that contains other genetic and/or environmental risk factors.

The involvement of rs1501299 SNP in multiple disease processes could be explained by the presence of a common mechanism involved in the pathophysiology of all these disorders. It also indicates that prevention and/or treatment of these disorders could be partially achieved through adopting similar approaches or pharmacologically targeting the same pathway(s).

In this investigation, several lines of evidence support the notion that the G allele of rs1501299 could be a high-risk allele for BC. Firstly, allele frequency analysis demonstrated a significantly higher frequency of the G allele in BC patients compared to controls. Secondly, genotype frequency analysis revealed a significantly higher frequency of the GG genotype of rs1501299 among BC patients. In agreement with these observations, haplotype analysis revealed that two of the haplotypes that increase the risk of BC have the G allele of rs1501299. Given the above observations, it is recommended to further investigate the role of the G allele using *in vitro* experiments in cell line/animal models of BC.

Conclusions

This study is the first to establish the association between serum levels of adiponectin and genetic variants in *ADIPOQ* and BC risk, using both univariate and multivariate models. The data points to the potential clinical application of using serum adiponectin and the G allele of rs1501299 SNP in *ADIPOQ* as biomarkers and/or targets in BC.

However, the conclusions of this report might be limited by the small number of patients involved in the analysis. Another limitation is the absence of survival data of the patients and that which tests the correlation between intratumoral levels of adiponectin and BC risk and/or patient survival.

Authors' contributions

Conception: LE and MA; Funding acquisition: SB; Methodology: LE, MA, and KM; Interpretation or analysis of data: KM, HB, and YK; Writing original draft: LE, MA and KP; Revision for important intellectual content: OH, SB, HB, and KP; Supervision: LE, MA, OH and SB. All authors have critically reviewed and approved the final draft and are responsible for the content.

Conflict of interests

The authors have no conflict of interests to declare.

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Availability of data and materials

Study data is available from the corresponding author upon reasonable request.

Role of the funding source

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Ethical statement

This study was approved by the Deanship of Research and the Institutional Review Board of Jordan University of Science and Technology (IRB number 50/132/2020). Consent forms were obtained from all subjects who participated in this study. The study was conducted in accordance with the Declaration of Helsinki.

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