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An increased risk of breast cancer associated with Val109Asp polymorphism in *omentin* gene

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Abstract

Breast cancer is the most common malignancy in women. Recent evidence has shown that the combination of factors such as obesity, insulin resistance and alternation in adipokines is associated with the risk and prognosis of breast cancer. Omentin is a newly identified adipokine that is highly expressed in visceral adipose tissue. The plasma omentin level was inversely correlated with obesity and insulin resistance. This study was aimed to investigate the impact of Val109Asp *omentin* gene polymorphism with the risk of breast cancer. This case-control study was done on 149 breast cancer patients and 150 healthy women as control. Genotyping was determined by restriction fragment length polymorphism (RFLP) analysis of amplified DNA fragments. Our finding showed that there are significant association between 109Asp/Val genotypes and breast cancer risk (OR: 1.7, 95%CI: 1-2.8, p: 0.02). The Val allele increased the risk of breast cancer (OR: 1.5, 95%CI: 1-2.2, p: 0.04) as compared to the Asp allele. In conclusion, our data suggest for the first time a significant association between Val109Asp *omentin* gene polymorphism and breast cancer susceptibility in Iranian women. Further studies with large sample size and different ethnicities are required to validate our findings.

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Introduction

Breast cancer is the most common malignancy in women representing 1.38 million new cases in 2008 worldwide (Ferlay *et al.,* 2012). In Iran, breast cancer ranks first among female cancers comprising 24.4% of all malignancies in women (Taheri *et al.,* 2012). Overweight and obesity are associated with an increased risk of developing breast cancer and of recurrence or death in breast cancer patients (Duggan *et al.,* 2011). Several studies have shown an association between obesity and breast cancer risk (Sinicrope and Dannenberg, 2011; Lorincz and Sukumar, 2006; Kaviani *et al.,* 2013). Obesity is commonly associated with hyperinsulinemia, insulin resistance, and alterations in levels of adipokines (Duggan *et al.,* 2011). Adipose tissue is not only an inert energy storage organ, but is now known to express and secrete a variety of hormones, and cytokines, known as adipokines, which act at both the local and systemic levels (Wolk *et al.,* 2001; Kwon and Pessin, 2013). Omentin is a newly identified adipokine that is highly and selectively expressed in visceral adipose tissue relative to subcutaneous adipose tissue (Yang *et al.,* 2006; Kralisch *et al.,* 2005). There are two highly homologous isoforms of omentin, omentin-1 and omentin-2; however, omentin-1 is the major circulating form in human plasma (Kralisch *et al.,* 2005). Omentin-1 has been identified in other tissues at lower expression levels and also named omentin, intelectin, intestinal lactoferrin receptor and endothelial lectin (Yang *et al.,* 2006). In vitro studies have shown that omentin increases insulin signal transduction by activating the protein kinase Akt/protein kinase B and enhancing insulin-stimulated glucose transport in isolated human adipocytes. Thus omentin may play an important role in modulating insulin (De Souza Batista *et al.,* 2007). Increased breast cancer risk associated with hyperinsulinaemia has been attributed to synergistic interaction between elevated free estrogen concentrations and aberrant insulin signaling (Stoll and Secreto, 1992). Moreover, as omentin can activate both AKT, a key survival factor, activation of AKT could be a mechanism of omentin-mediated cell proliferation and cancer (Yamawaki *et al.,* 2011).

Schäffler *et al* in 2007 identified for the first time, single nucleotide missense polymorphism in exon 4 *omentin* gene and described that the nucleotide +326 is polymorphic (A/T). Thus, the codon GAC was replaced by GTC changing the amino acid Asp to Val at position 109 (Schäffler *et al.,* 2007). Whereas, the significance of this polymorphism in the human omentin gene in connection with breast cancer has not been studied, the objective of this study was to determine the relation of Val109Asp *omentin* gene polymorphism and breast cancer risk.

Materials and methods

Subjects

From May 2012 to August 2012, 149 female patients (mean age ±SD: 49.9±11) who were newly diagnosed with breast cancer at an Imam Hussein Hospital in Esfahan, Iran were enrolled into the study as patient group. Among women who visited the same hospital for annual health examinations, we selected 150 women (mean age ±SD: 50±12) with normal mammographic findings and no previous history of any kind of cancer as age (±5)-matched controls. Informed consent was obtained from each subject before the study. The study was approved by the local ethical committee of Esfahan University of Medical Sciences.

Genotyping

Blood samples were collected from the subjects. Genomic DNA was isolated by the salting-out method from the whole blood of the samples (Miller *et al.,* 1988). Polymerase Chain Reaction (PCR)-based Restriction Fragment Length Polymorphism (RFLP) was done to identify the *Omentin* Val109Asp gene polymorphism as described previously. PCR primer sequences used for +326A/T polymorphic site detection were 5´-gAg CCT TTA ggC CAT gTC TCT-3´ (forward) and 5´-CTC TCC TTC TTC TCC AgC CCA T-3´ (reverse) (Schäffler *et al*., 2007). Three-step PCR for 40 cycles was carried out with an initial denaturation at 95°C for 4 minutes, followed by cycling at 94°C for 1 minutes, 62°C annealing for 1

minutes, 72°C for 1 minute extension and a final extension at 72°C for 5 minutes. The amplified PCR products were subjected to RFLP using the *AccI* (Fermentas, USA) restriction enzyme for enzyme digestion, and visualized on 2% agarose gel stained with ethidium bromide (Fig. 1).

Statistical analysis

Data analysis was performed using SPSS software version 17. Differences in the demographic variables were evaluated using independent sample t-test. Hardy–Weinberg analysis was performed to compare the observed and expected genotype frequencies by χ^2 test. Unconditional logistic regression was used to calculate ORs and 95% CI for comparison of selected characteristics and different genotypes between cases and controls. The probability level of <0.05 was used as the criterion of significance and all statistical tests were two-sided.

Results

Demographic and biochemical characteristics of the case-control study are shown in Table 1. There is no significant difference between patients and control groups regarding age (p=0.78). Detailed genotype and allele distributions are summarized in Table 2. Among controls (χ^2=0.4, df=1, p>0.05) and patients (χ^2=1.12, df=1, p>0.05) the observed genotype frequencies of the *omentin* gene polymorphism did not deviate significantly from those expected from the Hardy–Weinberg equilibrium.

Table 1. Demographic and biochemical characteristics of the case and control.

Variables	Controls	Patients	t	P
Age (years)	50±12	49±11	0.2	0.78
Height(cm)	159.1±7.3	157.3±7.4	1.9	0.05
Weight(kg)	67.7±11.6	70±13.3	1.4	0.14
Body mass index(kg/m²)	26.7±4	28±4.7	2.3	0.01
Age at menarche (years)	13.7±0.5	13.6±1.6	0.1	0.88
FBS (mg/dL)	94.07±23.1	111.5±39.1	2.5	0.01
Total cholesterol (mg/dL)	178.9±49.9	198±36	2.08	0.04
LDL-C (mg/dL)	95.6±34.5	117.9±30	3.03	0.003
HDL-C(mg/dL)	42.5±13	47.4±8	2.04	0.04

Frequencies of Asp/Asp, Asp/Val and Val/Val genotypes among patients were 54.4%, 40.9% and 4.7%, respectively, that differed from those among controls (Asp/Asp: 67.3%, Asp/Val: 28.7% and Val/Val: 4%); there was no significant difference in genotype frequencies between two groups (χ^2=5.4, df=2, p: 0.07).

Table 2. Genotype and allele distribution of the *omentin* Val109Asp polymorphism in the patients and controls.

Omentin Val109Asp polymorphism	Patients(%)	Control(%)	OR(95% CI)	p	[a]OR(95% CI)	[a]p
genotypes						
Asp/Asp	81(54.4)	101(67.3)	0.58(0.36-0.92)	0.02	0.52(0.31-0.87)	0.01
Asp/Val	61(40.9)	43(28.7)	1.7(1-2.8)	0.02	2.07(1.2-3.5)	0.008
Val/Val	7(4.7)	6(4)	1.4(0.47-4.4)	0.51	1.3(0.34-3.7)	0.8
Val/Val +Asp/Val	68(45.6)	49(32.7)	1.7(1-2.7)	0.02	1.9(1.1-3.2)	0.02
Alleles						
Asp	223(75)	246(82)	0.66(0.45-0.98)	0.04		
Val	75(25)	55(18)	1.5(1-2.2)	0.04		

Significant differences were observed in Asp/Val genotype frequencies between the patients and control groups (OR: 1.7, 95%CI: 1-2.8, p: 0.02). In the dominant effect of the Val allele (comparison between Val/Val+Asp/Val vs. Asp/Asp), Val/Val+Asp/Val genotypes was associated with an increased risk of breast cancer (OR: 1.9, 95% CI: 1–2.7, p: 0.02), in the other word, there was significant association between Val allele and increased risk of breast cancer. Conversely, the Asp allele was a protective against breast cancer (OR: 0.66, 95%CI: 0.45-0.98, p: 0.04). The association remained almost unchanged after adjusting for BMI (Table 2).

Discussion

Although alternation of the omentin levels in related to different disease reported in several studies, the role of *omentin* gene polymorphisms has fewer considered. Our study is the first investigation to examine the association of Val109Asp *omentin* gene polymorphism and breast cancer risk. Results showed that Asp/Val genotypes are associated with increased risk of breast cancer; in addition, we evaluated the breast cancer risk using dominant models of the 109Val allele. Our present findings indicate that the 109Val allele, acts as a dominant allele in relation to increased risk of breast cancer (Table 2).

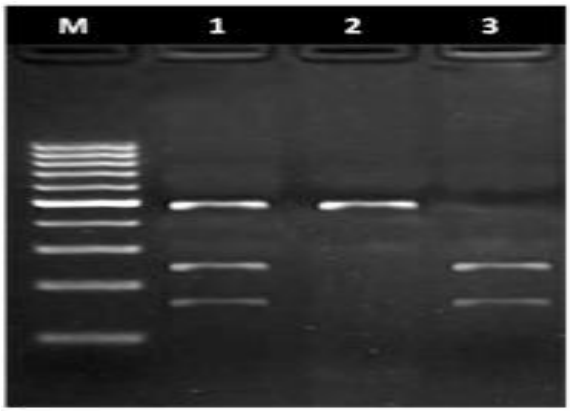

Fig. 1. Photograph of the Val109Asp *omentin* polymorphism by using PCR-RFLP method. The product sizes were 471 bp for Asp allele, 197 bp and 274 bp for Val allele. M:DNA Marker; Lane 1:Asp/Val genotype, Lane 2:Asp/Asp genotype and Lane 3:Val/Val genotype.

Omentin-1 is a novel fat depot-specific adipokine that increases insulin sensitivity and is expressed in visceral adipose tissue (Choi *et al.,* 2011). In vitro experiments have shown that treatment with recombinant omentin-1 enhanced insulin-stimulated glucose uptake in human subcutaneous and omental adipocytes, triggering Akt signaling in both the absence and the presence of insulin (Yang *et al.,* 2006). Moreover, Serum concentrations of omentin and mRNA expression of omentin in visceral adipose tissue are decreased in obese patients (Auguet *et al.,* 2011). Recent evidence has shown the constellation of obesity, insulin resistance and adipokines is associated with the risk and prognosis of breast cancer especially in postmenopausal women (Dalamaga, 2013). Thus, according to the role of omentin as an adipokine, our study examines the association of Val109Asp *omentin* gene polymorphism and breast cancer risk. In several studies, the relationship between Val109Asp polymorphism in *omentin* gene and diseases such as diabetes (Schäffler *et al.,* 2007), psoriasis (Turan *et al.,* 2013), and coronary artery disease (Yörük *et al.,* 2013) was investigated. Despite the importance of omentin in the etiology of these diseases, no significant association has been observed between Val109Asp *omentin* gene polymorphism and these diseases.

Our results showed a significant association between Val109Asp *omentin* gene polymorphism and breast cancer risk. Tsuji *et al.,* 2001 have reported that the Val/Asp109 site lays carboxyterminally outside of a fibrinogen domain spanning from amino acid 38 to amino acid 82. Amino acid position 109 is conserved between humans, mice and chimpanzee. However, when compared to the homologous proteins XCGL (*Xenopus laevis* cortical granule lectin), LSL (*L. japonica* lamprey serum lectin) and αGSL (*H. roretzi* ascidian glactose-specific lectin), the site at position 109 does not represent a completely conserved consensus amino acid position (Tsuji *et al.,* 2001), whereas the direct adjacent site at 108 contains alanine as a highly conserved amino acid. Although there do not exist experimental data on the functional consequence of the Val109Asp mutation on protein

function, the proximity of the mutated amino acid to the highly conserved amino acid at site 108 might be of functional relevance (Schäffler *et al.,* 2007).

In conclusion, this study showed that Val109Asp polymorphism in *omentin* gene may contribute to susceptibility to breast cancer. Larger studies with different ethnicities are required to confirm our finding.

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