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Correlation of A G to A Nucleotide Substitution in Upstream Region of Leptin with Breast Cancer Susceptibility

Vahid Arab-Yarmohammadi[1], Mehran Sharifi[2]

[1]Student Research Committee, School of Nursing and Midwifery, Shahroud University of Medical Sciences, Shahroud, Iran, [2]Department of Internal Medicine, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran

Abstract

Single nucleotide polymorphisms in leptin gene may increase the risk of breast cancer. The aim of this study was to investigate the association of leptin G-2548A gene polymorphism with breast cancer risk in an Iranian population. In a case-control study, 98 subjects including 45 women with breast cancer and 53 healthy women were recruited. The genotypes of G-2548A polymorphism were detected by polymerase chain reaction-restriction fragment length polymorphism. Our data revealed that there is a significant association between AG homozygote genotype and breast cancer risk (OR= 2.41, 95%CI= 1.05-5.52, *p*= 0.037). Also, we observed that G allele marginally increases the breast cancer risk (OR= 1.92, 95%CI= 1.00-3.70, *p*= 0.049). Based on our preliminary study, leptin G-2548A gene polymorphism may be a genetic risk factor for breast cancer and further studies with larger sample size are required to obtain more accurate results.

Keywords: *Breast cancer: Leptin gene; G-2548A variety.*

Introduction

Breast cancer is one of the common malignancy among women around the world[1, 2]. The breast cancer is a heterogenic disease and many environmental and genetic factors are involved in the development of it[3, 4]. Single nucleotide polymorphisms (SNPs) as genetic variations could be involved in susceptibility and development of diseases including breast cancer[5, 6, 7]. Genes such as BRCA-1, BRCA-2, and P53 are most well-known genes in breast cancer. Women with mutations in these genes are diagnosed with breast cancer during their lifetimes which most of them can be recognized at a young age before menopause[8, 9]. Leptin gene is another gene which may have an important role in breast cancer risk[10].

Leptin as an adipocytokine, encodes by the human LEP gene and it secrets by adipocytes[11]. Some studies revealed that this protein and its receptor have an increased expression in breast cancer, particularly in high-grade tumors. Also, it is associated with development of breast cancer[12]. Leptin is essential for the growth of tumor cells and its effects arises from discerning binding to the receptor. The receptor of this protein is expressed in various tissues, such as the mammary gland[13].

The LEP gene is located on chromosome 7 (7q32.1) with three exons. There are many polymorphisms in this gene that the rs7799039 is one of the common polymorphisms of this gene. This polymorphism is located at the upstream of LEP gene, and it results in G to A nucleotide at -2548 position (G-2548A). The aim of this study was to investigate the association of aforementioned polymorphism with breast cancer risk in an Iranian population.

Materials and method

Subjects

In a case-control study, we employed 98 subjects (including 45 women with breast cancer and 53 age-matched healthy women) from Shahid Beheshti Hospital (Kashan, Iran). The breast cancer was approved by pathology tests. The control subjects were healthy women without any familial history of malignancy. After obtaining signed informed consent form, we collected 2 ml peripheral blood samples from each subjects into sterile tubes containing EDTA anticoagulant agent.

DNA extraction and SNP genotyping

The genomic DNA was extracted from blood samples by a general salting out procedure[14]. The G-2548A SNP genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers sequences were obtained from previous study[15]. For primer checking, at first the whole genomic sequence of LEP gene was deduced from national center for biotechnology information (NCBI) databank. The primer sequences were analyzed around the G-2548A polymorphism position by Oligo7 software. The forward and reverse primers sequences were 5'-TTTCCTGTAATTTTCCCATGAG-3' and 5'-AAAGCAAAGACAGGCATAAAA-3', respectively. PCR was carried out in 25µl total volume containing 2.5µl 10X PCR buffer, 0.75µl of each primer (10pm/µl), 0.75µl $MgCl_2$ (50 mM), 0.5 µl dNTP (10 mM), 0.3U Taq DNA polymerase, and 60 ng template DNA. The PCR was performed in a Peqlab thermal cycler system with the following program: initial denaturation at 95°C for 5 min, followed by 35 repetitive cycles including denaturation at 95°C for 45 secs, annealing at 51°C for 45 secs, and extension at 72°C for 1min and a final extension at 72°C for 10 min. The 242bp amplified fragments were treated by *Hha*I restriction enzyme (Fermentas CO., Germany) according to the manufacturer protocol. The digested fragments were separated by 3% agarose gel electrophoresis which stained by ethidium bromide and visualized in UV light. It should be noted that the presence of G nucleotide at G-2548A position created *Hha*I restriction site.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was evaluated by Chi-square test in both case and control groups. Also, the similar test was used to assess the differences in the distribution of alleles and genotypes between case and control groups. The association of G-2548A polymorphism with breast cancer risk was also was evaluated by odds ratios (ORs) with 95% confidence intervals (CIs). A p value less than 0.05 was considered statistically significant. SPSS software version 19.0 (SPSS, Inc., Chicago IL, USA) was used for the all statistical analyses.

Results

The distribution of allele and genotype frequencies is presented in table 1. The genotype distribution showed that control group was at Hardy-Weinberg equilibrium (p> 0.05). Our data revealed that there is a significant association between AG genotype and breast cancer risk (OR= 2.41, 95%CI= 1.05-5.52, p= 0.037), whereas there was no significant association between GG genotype and breast cancer risk (OR= 3.67, 95%CI= 0.31-43.27, p= 0.302). Also, carriers of G allele (AG+GG) were at a high risk for breast cancer risk (OR= 2.48, 95%CI= 1.10-5.59, p= 0.029). Allele analysis showed that the frequency of A allele is 67.78% and 80.19% for case and control groups, respectively. Therefore, the frequency of G allele was 32.22% and 19.81% for cases and controls, respectively. Thereby, we observed a marginal significant association between G allele and breast cancer risk (OR= 1.92, 95%CI= 1.00-3.70, p= 0.049).

Table 1. Genotype and allele frequencies of G-2548A in cases and controls

Genotype	No. and Percentage		OR (95% CI)	*p*-value
	Control (n=53)	Case (n=45)		
AA	33 (62.26%)	18 (40.00%)	-	-
AG	19 (35.85%)	25 (55.56%)	2.41 (1.05-5.52)	**0.037**
GG	1 (01.89%)	2 (04.44%)	3.67 (0.31-43.27)	0.302
AG+GG	20 (37.74%)	27 (60.00%)	2.48 (1.10-5.59)	**0.029**
Allele				
A	85 (80.19%)	61 (67.78%)	-	-
G	21 (19.81%)	29 (32.22%)	1.92 (1.00-3.70)	**0.049**

OR: odds ratio, CI: confidence interval

Significant differences between the case and control groups are bolded

Discussion

In this study we evaluated the association of LEP G-2548A gene transition with risk of breast cancer in an Iranian population. Our data revealed that there are significant associations between AG genotype and G allele and risk of breast cancer. Also, we observed that carriers of G allele (AG+GG) were at a high risk for breast cancer. Some previous studies showed similar results. For example[15] reported that AG, GG and G varieties are associated with risk of breast cancer in population of south of Iran. In addition[16] reported that GG genotype and G allele are associated with breast cancer risk in north of Iran. While[17] reported that there is no significant association between LEP G-2548A polymorphism and risk of breast cancer in a population from center of Iran. The different results in aforementioned studies may result in environmental and ethnic factors[18].

Leptin is considered as an important protein for the human mammary gland growth, as far as latest studies have revealed that the protein might play a pivotal role in breast cancer[19]. Expression of leptin gene in cell lines of breast cancer, solid tumors, and normal breast tissue have been reported. Overexpression of leptin in the most breast cancers was reported whereas this overexpression has not been reported in cell of healthy Moreover, leptin can cause breast cancer cells to become malignant forms[20, 21]. Also, it is reported that leptin and leptin receptor overexpress tumorous tissues[19]. Leptin plays some roles such as growth of cells, cell differentiation, and angiogenesis. Also, it could increase some products of endothelial cells including nitric oxide and overexpression of VEGFR-2, FGF2, and VEGF[22]. In some cells such as malignant or normal epithelial cells, the leptin can act as a migration and mitogenic factor[23]. Leptin also could induce cancer cells growth in breast cancer epithelium via aromatase expression and estrogen production or activation[24]. Evidences show overexpression of leptin and leptin receptor in initial breast cancer with metastases[25].

Single nucleotide polymorphisms are a group of genetic variations that could happen in every region of genes. The influence of SNPs is dependent on the location of them on genes[26, 27]. SNPs in promoter region of genes could affect the gene expression[28]. LEP G-2548A transition as an upstream polymorphism may alter gene expression. In silico analysis is a useful tool for evaluation of effects of SNPs[29, 30, 31]. Therefore, it is suggested that further studies are focused on the molecular effects of LEP G-2548A by computational analysis.

Finally, there are some limitations in our study that we should be mentioned in this section. At first, we don't evaluate gene-gene and gene-environment interactions. Also, the sample size of our study was very small.

Conclusion

Leptin G-2548A gene transition may be a genetic risk factor for breast cancer, but further studies with larger sample size with regard to mentioned interactions are need to obtain more accurate results.

Ethical Clearance- All the participants' informed written consent and this study confirmed by the principles outlined in the Declaration of Helsinki and approved by the Shahid Beheshti Hospital's Ethics Committee.

Source of Funding- This study was performed by a personal expense.

Conflict of Interest- The authors report no conflicts of interest.

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