ASSOCIATION OF E-SELECTIN S128R POLYMORPHISM WITH HEREDITARY BREAST CARCINOMA SUSCEPTIBILITY IN TURKISH PATIENTS WITHOUT BRCA1/2 GERMINE MUTATIONS

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ABSTRACT

Inherited genetic factors play an important role in breast cancer susceptibility. The BRCA1 and BRCA2 mutations are the most well-known genetic factors associated with increased risk of breast cancer. E-selectin is a cell surface glycoprotein and its serum levels are known to increase in various cancers. The present retrospective study aimed to evaluate whether E-selectin S128R polymorphism (NG_012124.1: g.7161A>C, NM_000450.2: c.445A>C, NP_000441.2: p.Ser149Arg), which is known to have a role in cancer risk, is associated with breast cancer susceptibility in BRCA1/2 mutation non-carriers with breast cancer. The study included 90 patients with breast cancer and 270 healthy controls. All breast cancer patients were screened for BRCA1/2 mutations and confirmed to be BRCA1/2 mutation non-carriers before inclusion in the study. Genotyping for the E-selectin S128R polymorphism was performed using real-time polymerase chain reaction (PCR) analysis. The frequencies of the AA, AC and CC genotypes were 70.0, 25.5 and 4.5%, respectively, in the patient group and 79.25, 19.25 and 1.5%, respectively, in the controls. The frequencies of A and C alleles were 84.8 and 15.2% in the patient group, respectively, and 88.9 and 11.1%, respectively, in the controls. No significant differences were determined in the genotype and allele frequencies of the E-selectin S128R polymorphism between the patient and control groups (p = 0.095). The S128R (A/C) polymorphism was not found to be associated with an increased risk of breast cancer [odds ratio (OR) = 0.69; 95% confidence interval (95% CI): 0.43-1.10; p = 0.1248]. There was no association between the S128R polymorphism and breast cancer susceptibility in BRCA1/2 mutation non-carriers with breast cancer in the studied Turkish population. Further studies with larger sample sizes are needed to validate our findings.

Keywords: Breast carcinoma; Cancer susceptibility; E-selectin; rs5361

INTRODUCTION

Breast cancer is one of the most common invasive cancers in the female population worldwide and in Turkey [1]. Breast cancer is a multifactorial disease driven by both genetic and non genetic etiological factors. Non genetic environmental factors such as pregnancy, hormone replacement therapies, obesity and alcohol consumption are known to be closely linked to breast cancer risk [2]. The well-characterized hereditary form of breast cancer is caused by germline pathogenic variants in BRCA1/2 [3]. Early studies conducted on selected multiple-case families have estimated that approximately 80.0% of BRCA1/2 mutation carriers would develop breast cancer by age 70 [4,5]. However, in a later meta-analysis study that pooled the data of studies including patients unselected for family history, it was reported that the observed risk for breast cancer development in BRCA1 and BRCA2 mutation carriers by age 70 was 65.0 and 45.0%, respectively, which were lower than the estimated value [6,7]. Additionally, individuals of high-risk families who do not carry BRCA1/2 mutations are also at an increased risk for developing breast cancer [4,7]. All these data strongly suggest that other than the well-studied BRCA1/2 genes, additional genetic factors are also involved in a predisposition to breast cancer. However, not much is known about the genes contributing to
breast cancer susceptibility in non carriers of the \textit{BRCA1/2} mutations.

Selectins are adhesion molecules, which are expressed by endothelial cells, thrombocytes and leukocytes, and have three subsets, namely L-, P- and E-selectins [8]. Structurally, selectins contain an N-terminal, calcium-dependent lectin domain, an epidermal growth factor-like domain, regulatory elements, a transmembrane domain, and a short cytoplasmic tail [9]. Intracellular and extracellular interactions mediated by adhesion molecules are critical for the dissemination of metastatic tumor cells. Loss of cell-cell and/or cell-matrix adhesions allows malignant tumor cells to escape from their primary microenvironment and to acquire a more motile and invasive phenotype, and thereby enables them to migrate to the other sides of the body. Consistent with this, E-selectin is involved in migration and metastasis [8,10-18] that are two critical steps in carcinogenesis.

E-selectin is a cell surface glycoprotein expressed on endothelial cells after activation by cytokines. Several studies have demonstrated that serum E-selectin levels are elevated in patients with a variety of cancers, including ovarian, breast and gastric cancers [14]. Numerous single nucleotide polymorphisms (SNPs) of the \textit{E-selectin} gene have been identified, among which the most common variant is the g.7161A/C or A561C missense variant (rs5361) leading to a serine to arginine substitution in exon 4 at position 128 (S128R) (NG_012124.1:g.7161A>C, NM_000450.2: c.445A>C, NP_000441.2: p.Ser149Arg) [19]. The E-selectin S128R (A/C) polymorphism alters the binding specificity of the extracellular domain and thus facilitates ligand binding, which in turn improves the adhesion of lymphoid and myeloid cells to the endothelium [20-22]. In the \textit{E-selectin} gene variants, the S128R polymorphism is of particular interest as it is clinically associated with increased cancer risk [23].

The present study aimed to investigate whether the S128R polymorphism of the \textit{E-selectin} gene contributes to development of breast cancer in patients with breast cancer but without \textit{BRCA1/2} mutations in the Turkish population. To the best of our knowledge, this study is the first to investigate the correlation between the S128R polymorphism and breast cancer in the absence of \textit{BRCA1/2} mutations in the Turkish population.

\textbf{MATERIALS AND METHODS}

\textbf{Subjects.}\ The present study included 360 genetically unrelated females between 40-50 years of age who were referred to a regional reference laboratory between 2013 and 2016 for genetic counseling and testing. Of these females, 90 were diagnosed with breast carcinoma, clinically resembling the hereditary type according to the National Comprehensive Cancer Network (NCCN) guidelines of genetic/familial high-risk assessment for breast and ovarian cancers [24]. These patients were otherwise healthy. All patients were screened for \textit{BRCA1/2} mutations by next generation sequencing (NGS). Briefly, targeted amplification of all coding exons of \textit{BRCA1} and \textit{BRCA2} was performed using the \textit{BRCA MASTR Dx} kit from Multiplicom, Agilent Technologies (Santa Clara, CA, USA), as described by the manufacturer and the amplicon pool was sequenced on the Illumina MiSeq sequencing platform. Data analysis was performed with SEQ powered by Genomize (https://seq.genomize.com). Patients in whom normal results were obtained with no pathogenic variants were included. The control group consisted of 270 females who did not belong to an at-risk population with higher \textit{BRCA} mutation carrier frequencies, such as Ashkenazi Jewish descent, who had no previous cancer diagnosis and no family history of cancer, or cardiovascular diseases hypothesized to be related with increased \textit{SELE} polymorphism frequencies. The present study was approved by the Clinical Research Ethics Committee of Maltepe University, Istanbul, Turkey, and written informed consent was obtained from all participants. Histopathological data obtained from patient records revealed that all patients had invasive ductal carcinoma.

\textbf{Genotyping.}\ For genotyping analysis, DNA was extracted from ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral blood samples of all consenting subjects using the Qiagen DNA Blood Mini Kit and a QiaCube robotic device (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions. All patients and controls were examined for the S128R (A/C) SNP of the \textit{E-selectin} gene [19] by real-time (RT) polymerase chain reaction (PCR) analysis using TIB Molbiol LightSNP Genotyping Assay (TIB Molbiol GmbH, Berlin, Germany).

The reaction master mix used in the study was commercially obtained from TIB Molbiol GmbH. The RT-PCR reactions were performed using 50 ng genomic template DNA. The Qiagility robotic instrument (Qiagen GmbH) was used to prepare the reagent mix. The RT-PCR cycling conditions used for the S128R polymorphism were as follows: 10 min. of initial denaturation at 95 °C, 45 cycling reactions of 10 seconds at 95 °C, 10 seconds at 60 °C, 15 seconds at 72 °C, melting curves at 95 °C, 40 °C, 75 °C, and cooling to 40 °C. Repeatability of the reactions was checked for internal quality control by repeating the procedure using randomly chosen samples. Melting peaks were obtained at 59 °C for the A allele and at 64 °C for the C allele.
Statistical Analyses. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Statistics for Windows version 21.0 (IBM Corporation, Armonk, NY, USA). Data were expressed as frequencies and percentages. Genotype frequencies in the patient and control groups were compared using the ζ² test. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to examine the effect of the S128R polymorphism on breast cancer susceptibility in non carriers of the BRCA1/2 mutations. A p value of <0.05 was considered statistically significant.

RESULTS

In the present study, we genotyped 360 subjects (90 BRCA1/2 mutation non carrier patients diagnosed with breast carcinoma and 270 controls with no previous cancer history) for the S128R polymorphism of the E-selectin gene. The genotype and allele distributions of the patients and controls are presented in Table 1.

No significant differences were determined in the genotype and allele frequencies of the E-selectin S128R (A/C) polymorphism between the patient and control subjects (Table 1). Of the 90 BRCA1/2 mutation non carrier patients, 63 (70.0%) had the AA genotype and 23 (25.5%) had AC genotypes, and four (4.5%) had the CC genotype. Of 270 controls, 214 (79.25%) had the AA genotype, 52 (19.25%) had the AC genotype, and four (1.5%) had the CC genotype. The frequency of the pathogenic C allele was 31/203 (15.2%) in the patient group and 60/540 (11.1%) in the control group (Table 1); the patient and control groups did not differ in the distribution of the pathogenic C allele. The S128R (A/C) polymorphism was not found to be associated with an increased risk of breast cancer (OR = 0.69; 95% CI: 0.43-1.10; p = 0.1248).

DISCUSSION

E-selectin is involved in cancer metastasis by regulating the adhesion of circulating cancer cells to the endothelial cells of blood vessels [10]. Currently, several SNPs of the E-selectin gene have been identified. The S128R polymorphism (A>C variation), the most common SNP of the E-selectin gene, facilitates ligand binding, which in turn increases the adhesion of lymphoid and myeloid cells to the endothelium. The E-selectin S128R polymorphism is associated with an increased risk of several cancers including gastric [25,26], colorectal [27] and pancreatic [28] cancers. However, a very limited number of studies investigating the association of the E-selectin S128R polymorphism with breast cancer susceptibility have revealed contradictory results. Kontogianni et al. [29] reported that the E-selectin S128R genotypes (AA, AC, and CC) were not associated with any of the tumor parameters in a Caucasian population. On the other hand, Naidu et al. [30] reported that women carrying the E-selectin S128R polymorphism showed a significantly increased breast cancer risk in an Asian population. Different results from these studies may imply that the effects of the S128R polymorphism on breast cancer susceptibility may be population-dependent.

In the present study, we examined whether the S128R polymorphism is associated with susceptibility to breast carcinoma in BRCA1/2 mutation non carriers with breast cancer in the Turkish population. This study is the first to investigate the association between the E-selectin S128R polymorphism and breast cancer in the Turkish population as well as to investigate the association of the E-selectin S128R polymorphism with development of breast cancer in the absence of BRCA1/2 mutations. For this purpose, 90 BRCA1/2 mutation non carriers with breast cancer and 270 controls with no previous cancer history, were genotyped using RT-PCR. The results of the present study demonstrated that the E-selectin S128R polymorphism was not associated with an increased risk of breast cancer in BRCA1/2 mutation non carriers with breast cancer in the Turkish population. Further studies with larger sample sizes are required to reveal the association between the E-selectin S128R polymorphism and breast cancer risk in the absence and presence of BRCA1/2 mutations.

ACKNOWLEDGMENTS

The authors would like to thank Mustafa Karabızici (Izmir Biomedicine and Genome Center, Izmir, Turkey), for his helpful comments on E-selectin polymorphisms;
Günay Karataş and Serdar Kasakyan (Duzen Laboratories Group, Istanbul, Turkey), for their assistance in operating the Bio-Rad Laboratories (Hercules, CA, USA) instrument and the Qiagility robotic system (Qiagen GmbH), respectively. Finally, we would like to thank Dr. Erdal Cosgun and Dr. Kimberly Cannady (Medical University of South Carolina Charleston, Charleston, SC, USA) for reviewing the statistical analysis and critical review of the manuscript. The authors declare that they all participated in the design, execution, and analyses of the study, and that they approved the final version of the manuscript.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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