

# The single nucleotide polymorphism +936 C/T VEGF is associated with human epidermal growth factor receptor 2 expression in Moroccan breast cancer women

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**Abstract** The vascular endothelial growth factor (VEGF), a potent regulator of angiogenesis, is involved in the development and progression of breast cancer (BC). The functional +936C/T polymorphism of the VEGF-A gene has been implicated in BC susceptibility; however, published data are conflicting. In the current case–control study, we analyzed the association of the +936C/T polymorphism with BC risk and tumor markers expression, human epidermal growth factor receptor 2 (HER2/neu) and cancer antigen 15.3 (CA 15.3) in Moroccan women. We genotyped the DNA of 70 BC patients and 70 healthy women by TaqMan SNP assays. The  $\chi^2$  test and Fisher's exact test were used for statistical analyses. The overall results revealed that there is no association between the +936C/T polymorphism and BC risk [ $p = 0.8$ ; OR 0.87, 95 % CI (0.32–2.42)]. However, when we stratified the

group of patients according to the status of tumor markers, a statistical significant association of +936C/T SNP and HER2/neu expression was observed ( $p = 0.009$ ). In contrast, no association with the other tumor marker, CA 15.3, was found ( $p = 0.090$ ). Thus, the +936C/T polymorphism seems to have a correlation with HER/neu expression in BC disease.

**Keywords** Angiogenesis · VEGF · SNP · Breast cancer susceptibility · HER2 · CA 15.3

## Abbreviations

BC	Breast cancer
CI	Confidence interval
CA 15.3	Cancer antigen 15.3
DNA	Deoxyribonucleic acid
HER2	Human epidermal growth factor receptor 2

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mRNA	Messenger ribonucleic acid
OR	Odds ratio
SNP	Single-nucleotide polymorphism
UTR	Untranslated region
VEGF	Vascular endothelial growth factor

## Introduction

Angiogenesis, the process of new capillary blood vessel formation, is required for tumor growth and metastasis and constitutes an important control point in the progression of cancer [1]. Extensive laboratory data suggest that angiogenesis plays an essential role in breast cancer (BC) [2].

The major mediator of angiogenesis is the vascular endothelial growth factor-A (VEGF-A) (also known as VEGF) [3]. It is a potent pro-angiogenic factor that stimulates endothelial cell proliferation, migration and survival and also increases vascular permeability [4].

VEGF-A gene contains eight exons separated by seven introns and is located at chromosome 6p21.3 [5]. It is highly polymorphic [6]. The main commonly studied polymorphism of VEGF-A is +936C/T (rs3025039) and is located at the 3'-untranslated (3'UTR) region.

Alteration of C to T at position 936 had protective effect against BC and had a trend of correlation with lower plasma VEGF-A levels [7]. This alteration might lead to loss of potential AP-4 binding site which might affect mRNA structure [8]; besides, the T allele has been found to be associated with a reduced uptake of 18F-fluorodeoxyglucose which used for detection and staging of BC [9]. In contrast, the wild CC genotype was associated with high VEGF production [7, 8] and increased risk of BC [6, 7]. Association of this functional single nucleotide polymorphism (SNP) with BC risk has been reported in several studies [10]. However, the results remain conflicting as other investigations did not reproduce these findings [10].

A number of molecules involved in BC appear to be associated with angiogenesis such human epidermal growth factor receptor 2 (HER2/neu), also known as C-erbB2, which is a proto-oncogene and encodes a transmembrane tyrosine kinase receptor. In adult normal tissues, HER2 exists in single copy, with a lower or rare level of expression [11]. This proto-oncogene is amplified and overexpressed in 20–25 % of human BC [12–15]. In vitro transfection studies indicated that overexpression of HER2/neu is associated with increased expression of VEGF in human BC cells at both the RNA and protein levels [16, 17].

Cancer antigen 15.3 (CA 15.3), also called Mucin-1, is encoded by the MUC-1 gene. The CA 15.3 is largely used

for diagnosis and follow-up of patients with BC [18]. Approximately 80 % of the metastatic BC patients and less than 10 % of early BC patients have high CA 15.3 serum levels [19, 20].

A previous investigation demonstrated that MUC1 expression and angiogenesis are correlated in BC [21]. Moreover, a recent study showed that MUC1 expression promotes angiogenesis in human BC in vivo and in vitro [22].

We have recently reported that –1154G, –2578A and –460C alleles of three VEGF-A polymorphisms seem to have a protective effect against BC in Moroccan women [23]. To explore further the role played by potentially functional VEGF polymorphism in Moroccan population, we investigated the association of a 3'UTR region polymorphism of VEGF, +936C/T, with BC susceptibility and two tumor markers involved in BC, HER2/neu and CA 15.3. No association studies of VEGF +936C>T polymorphism with BC have been reported in Morocco until now.

## Subjects and methods

### Study population

This investigation used the population of subjects defined in a recent Moroccan BC study [23]. The overall study design was described previously in detail [23] but is also summarized briefly here.

Two groups were enrolled in this case–control study between September 2012 and July 2013. Cases group (mean age of 46.3 years) was composed 70 patients with histologically confirmed BC. They were recruited in the medical oncology and gynecology departments of the Military Training Hospital Mohammed V of Rabat and obstetrics gynecology service of Maternity Souissi of Rabat. The status of tumor markers was recorded (Table 1).

The control group, composed by 70 healthy volunteer women (mean age 44.26 years) and matched to cases by age (2-year interval), had no previous or concurrent malignant disease.

The study was approved by the Ethical committee of the faculty of medicine and pharmacy of Rabat. All samples were obtained after informed consent, according to the Declaration of Helsinki.

### DNA extraction and genotyping assays

DNA was extracted from whole blood using PureLink™ Genomic DNA Kits (Invitrogen, USA) following the manufacturer's protocol.

Genotyping analyses for the +936C/T polymorphism (rs 3025039) were performed using the TaqMan SNP

**Table 1** Tumor markers expression

Cases characteristics	Cases (n = 70)
Age at diagnosis	mean 46.26; range (23–76)
HER2/neu status <sup>a</sup> (n = 56)	Negative (n = 28): 50.0 % Borderline (n = 4): 7.1 % Positive (n = 24): 42.9 %
CA 15.3 status <sup>b</sup> (n = 36)	Negative (n = 29): 80.6 % Positive (n = 7): 19.4 %

<sup>a</sup> HER2 Human epidermal growth factor receptor 2: results of immunohistochemistry test (0 or 1+: negative, 2+: borderline, 3+: positive)

<sup>b</sup> Ca 15.3 cancer antigen 15.3: negative (normal value <30 U/ml), positive (overexpression >30 U/ml)

Genotyping assays (C\_16198794\_10, Applied Biosystems) according to previously described method [23].

Statistical analysis methods

Statistical analyses were performed using SNPStats [24] and SPSS software version 13.0 (SPSS Inc., Chicago, IL).

The test for Hardy–Weinberg equilibrium, allele and genotype frequencies and the analysis of association with a response were performed using SNPStats.

The  $\chi^2$  test and Fisher’s exact test were used to detect the association of tumor markers expression (HER2/neu and CA 15.3) with +936C/T polymorphism.

The value of  $p < 0.05$  was considered to be statistically significant.

**Results**

Allelic and genotypic distribution

The allelic and genotypic frequencies for the +936C/T polymorphism among healthy group are in Hardy- Weinberg equilibrium ( $p$  value >0.05).

The distribution of +936C/T genotypes and allelic frequencies is given in Table 2.

Minor allele frequency among controls was T (0.06).

Homozygote for the allele T of +936C/T is not present in any of the groups, while the +936 CT heterozygote did not show any association with BC risk compared with common genotype carriers [ $p = 0.8$ ; OR 0.87, 95 % CI (0.32–2.42)].

+936C/T VEGF polymorphism and tumor markers

The associations of +936C/T polymorphism with tumor markers expression in patient group are listed in Table 3.

A significant association was observed between the +936 C/T and the (HER2/neu) status ( $p = 0.009$ ). None of the CT genotype carriers overexpress HER2/neu, while 50 % of CC genotype carriers overexpress this tumor marker.

However, no association was observed between the +936 C/T SNP and the CA 15.3 expression ( $p = 0.090$ ).

**Discussion**

The aim of this investigation was to determine the association of the +936C/T VEGF-A polymorphism with BC risk and tumor markers expression.

The allelic frequencies of the +936C/T SNP among Moroccan control group (0.06) were less than Bahrainis (0.126) [25] and Caucasians rate (0.176) (<http://www.ncbi.nlm.nih.gov/snp>), but comparable with sub-Saharan African (0.068) (<http://www.ncbi.nlm.nih.gov/snp>).

Analysis of +936T/T genotype demonstrated that none of the patients or controls had this genotype. For Turkish BC women, Eroğlu et al. [26] did not find this genotype in their case–control study, while in the contrary, it was found among Austrian, Spanish, Chinese and American populations [3, 27–29]. These discrepancies in the results might

**Table 2** Genotypic and allelic frequencies of +936C/T polymorphism and breast cancer risk

Genotype/allele	Cases (n = 70)	Controls (n = 70)	OR (95 % CI)	(95 % CI)	p value
+936C/T					
C/C	61 (87.1 %)	62 (88.6 %)	1.00	Reference	
C/T	9 (12.9 %)	8 (11.4 %)	0.87	(0.32–2.42)	0.8
T/T	0	0	–	–	
C allele	131 (0.94)	132 (0.94)			
T allele <sup>a</sup>	9 (0.06)	8 (0.06)			

Values were statistically significant if  $p$  value was <0.05

OR odds ratio, CI confidence interval

<sup>a</sup> Minor allele

**Table 3** Distribution of +936 C/T polymorphism in breast cancer patients according to tumor markers expression

Clinicopathological characteristics	+936 C/T			<i>p</i> value
	CC	CT	TT	
<i>HER2/neu</i> status				
Negative ( <i>n</i> = 28)	20	8	–	<b>0.009</b>
Bordline ( <i>n</i> = 4)	4	–	–	
Positive ( <i>n</i> = 24)	24	–	–	
CA 15.3				
Negative ( <i>n</i> = 29)	28	1	–	0.090*
Positive ( <i>n</i> = 7)	5	2	–	

Significant values (*p* value <0.05) are in bold

\* Fisher's exact test

be due to the small size population taken in the Turkish and our studies.

The +936 CT genotype was also analyzed in the present study, and no association with BC risk was found (*p* = 0.5), as has been reported for larger white BC Caucasian group (*p* = 0.41) [30] using the same detection method. In contrast to the current work, CT genotype was more frequent in a small size of Turkish patients and was associated with BC susceptibility (*p* = 0.001) [26]. Interestingly, association of combined CT+TT genotypes had protective effect against BC (*p* = 0.014) among Spanish population [27] and were associated with reduced risk of BC (*p* = 0.042) in Polish BRCA1 mutation carriers [6]. A recent meta-analysis suggests that the VEGF +936C/T polymorphism is significantly associated with BC development and the VEGF 936T allele carriers may be associated with decreased BC risk [31].

We can presume that inter-individual variability in addition to variations of allele frequencies within different ethnic groups may be responsible for +936C/T SNP and their association with BC. These results may be explained by the low penetrance of this SNP in BC susceptibility.

It was interesting to observe that after stratification of patients according to the status of tumor markers, association of +936C/T SNP and HER2/neu expression has reached the level of significance (*p* = 0.009). In contrast, we failed to find any association with the +936C/T SNP and the other tumor marker, CA 15.3 (*p* = 0.090).

Konecny et al. [32] demonstrated that HER2/neu overexpression is associated with expression of two of the most abundantly expressed VEGF isoforms in BC (VEGF<sub>121–206</sub> and VEGF<sub>165–206</sub>), and suggested that VEGF may in part mediate the aggressive phenotype of BC that overexpress HER2/neu.

In our study, none of +936CT genotype carriers overexpress HER2/neu. Considering that alteration of C to T at position 936 of VEGF gene is correlated with lower plasma

levels in BC [7] and that HER2/neu overexpression is associated with increased expression of VEGF [16, 17], we supposed that the T allele of +936C/T SNP had an association with the non-overexpression of HER2/neu.

This case–control study failed to find any association between +936C/T VEGF polymorphism and BC susceptibility in Moroccan population. In contrast, for the first time, the +936C/T SNP was found to be associated with HER2/neu expression.

In conclusion, further large-scale studies in Moroccan population are necessary to clarify the association of +936C/T polymorphism with BC risk. Moreover, the importance of angiogenesis in BC development requires the interest to conduct studies on the mechanism of action of the VEGF gene in BC and its association with tumor markers expression.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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