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RESEARCH ARTICLE

Association of *VEGF* and *VEGFR1* polymorphisms with breast cancer risk in North Indians

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Abstract The aim of present study was to evaluate the relationship between vascular endothelial growth factor (*VEGF*) −2578C/A, −2549I/D, −460T/C and −7C/T and *VEGFR1* −710C/T polymorphisms with risk to breast cancer in North Indians. A total of 204 sporadic breast cancer patients and 204 controls were recruited for this case-control study. Significantly increased frequency of II genotype of −2549I/D polymorphism was observed in patients as compared to control individuals (odds ratio (OR)=2.76, 95 % confidence interval (CI), 1.55–4.92; p=0.0005). *VEGF* −2578AA genotype (OR=2.87; 95 % CI, 1.61–5.10; p=0.0003) and A allele (OR=1.65, 95 % CI, 1.25–2.18; p=0.0004) were found to be associated with increased risk for breast cancer. Individuals carrying CC genotype (OR=2.23, 95 % CI, 1.25–3.97) and C allele (OR=1.42, 95 % CI, 1.07–1.87) of *VEGF* −460T/C polymorphism were at higher risk of breast cancer. There was no significant difference in genotype and allele distribution of *VEGF* −7C/T and *VEGFR*1 −710C/T polymorphisms between cases and control individuals (p>0.05). Linkage disequilibrium analysis showed a strong linkage between *VEGF* −2549I/D and −2578C/A polymorphisms (Lewontin's D'=0.99; r^2=0.97), −2549I/D and −460T/C (D'=0.94; r^2=0.84), and −2578C/A and −460T/C polymorphisms (D'=0.93; r^2=0.83). In the present study, we concluded that *VEGF* −2549I/D, −2578C/A and −460T/C polymorphisms are associated with risk to breast cancer in Punjab, North India.

Keywords Breast cancer · Polymorphism · *VEGF* · *VEGFR*1

Abbreviations

VEGF	Vascular endothelial growth factor
SNP	Single-nucleotide polymorphism
UTR	Untranslated region
HWE	Hardy-Weinberg equilibrium
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
ARMS	Amplification refractory mutation system
OR	Odds ratio
CI	Confidence interval

Introduction

Breast cancer is a heterogeneous disease encompassing multiple subgroups with different molecular signatures, prognosis and responses to therapies and involves lymphangiogenesis [1, 2]. In solid tumours, angiogenic switch is a critical step and is mediated by production of excess of pro-angiogenic molecules over anti-angiogenic factors [3–5]. Vascular endothelial growth factor (VEGF), a potent angiogenic cytokine, plays a pivotal role from tumour proliferation to inflammatory and ischemic processes [6, 7]. It is also a survival factor for endothelial cells during physiological and tumour angiogenesis with vasodilatation, vascular permeability and anti-apoptosis functions [7–9].

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The human *VEGF* (OMIM 192240) mapped to 6p21.1 is highly polymorphic with multiple polymorphisms in the promoter 5′ untranslated region (5′-UTR) and 3′ UTR [10–12]. The single-nucleotide polymorphisms (SNPs) in the promoter and 5′ UTR have been reported to regulate VEGF expression via alternative initiation of transcription and internal initiation of translation [13, 14]. Some of the variants of *VEGF* including −460T/C, −1154G/A, −2549I/D, −2578C/A and +405C/G in the promoter or 5′-UTR and +936C/T in the 3′-UTR have been reported to be associated with variation in VEGF protein production [11, 12, 15–18]. Overexpression of VEGF has been reported in breast cancer [19, 20], and its higher level in tumour tissue has been associated with both increased microvessel density and breast cancer recurrence [21–24]

VEGF −2578C/A polymorphism (rs699947) has been shown to functionally affect VEGF messenger RNA (mRNA) levels [25]. Prognostic importance of *VEGF* −2578C/A polymorphism has been documented in several cancer types including breast [26–28], hepatocellular [29], nasopharyngeal [30], colorectal [31] and colon [32]. Several studies have reported the association of −2549I/D polymorphism (rs35569394) with susceptibility to diseases like bladder cancer [33], renal cell carcinoma [34], diabetic nephropathy [35], diabetic retinopathy [36], Bechet's disease [37, 38], giant cell arteritis [39], Kawasaki disease [40], prostate cancer [41] and end-stage renal disease [42]. The correlation of 18-bp deletion at position 2549 has been reported with 1.95-fold increased transcriptional activity compared with those containing the insert [35]. A number of molecular epidemiological studies have been conducted to examine the association between *VEGF* −460T/C polymorphism (rs833061) and cancer susceptibility with disparate results [43–52]. The association of TT genotype of *VEGF* −460T/C has been reported with poor tumour differentiation in gastric cancer patients from Oman [50].

VEGF participates in the stimulation of both migration and survival of malignant cells by distinct signalling pathways [53]. VEGF and its receptors VEGFR1, VEGFR2 and VEGFR3 are essential in vascular development and maintenance of the adult vasculature [54]. VEGFR1 is one of the important receptors of VEGF angiogenesis signalling and has a relevant role in process of normal vessel formation [55]. It has been documented that breast cancer patients with high sVEGFR1/VEGF-A ratio have a markedly favourable prognosis as compared to patients with low ratio [56]. There is only one published study on *VEGFR1* −710C/T polymorphism in Spanish breast cancer patients showing significant association of *VEGFR1* −710CT + TT genotype with reduced breast cancer risk [57].

VEGF is an important target in anti-cancer therapy, and different *VEGF* polymorphisms have been separately reported to regulate VEGF expression. Therefore, the present study was an attempt to evaluate the relationship between four polymorphisms in *VEGF* (−2578C/A, −2549I/D, −460T/C, −7C/T) and one in *VEGFR1* (−710C/T) with risk to breast cancer in North Indian breast cancer patients. In the Punjab state of North India, the cancer incidence is reportedly increasing [58]. In spite of an increasing incidence of breast cancer in Amritsar city of Punjab state in North West part of India, there is no reported study on *VEGF* polymorphisms in breast cancer from this population.

Methods

Study design and selection of subjects

In the present case-control study, 408 subjects consisting of 204 sporadic breast cancer patients (6 males and 198 females) and 204 unrelated healthy, gender- and age-matched control individuals (6 males and 198 females) were investigated. The breast cancer patients were recruited from Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Amritsar, Punjab. The classification of tumour stages was performed by pathologists as per American Joint Committee on Cancer's TNM staging system [59]. The patients included were those who had not received chemotherapy, radiotherapy or blood transfusion before surgery. The control subjects were recruited from the same geographical area as that of patients. The controls had no history of any cancer or other chronic disease for the last three generations and were not on regular medications for at least 2 years from the date of sampling. The demographic characteristics, detailed family history, reproductive history and disease history of all the subjects were recorded on the pretested structured questionnaire. After informed consent, 5 ml venous blood was collected from each subject in 0.5 M EDTA. The study was approved by ethical committee of Guru Nanak Dev University, Amritsar, Punjab, India.

Analysis of VEGF (−2578C/A, −2549I/D, −460T/C, −7C/T) and VEGFR1 −710C/T polymorphisms

Genomic DNA was extracted from peripheral blood leucocytes by the standard phenol chloroform method [60]. *VEGF* −2549I/D, −7C/T, −2578C/A and −460T/C and *VEGFR1* −710C/T polymorphisms were analyzed using direct PCR, ARMS PCR and PCR-RFLP method. The details of the screening condition have been described in Table 1. To ensure quality control, genotyping was performed without knowledge of case/control status.

Statistical analyses

The power calculations were done using Quanto software, version 1.0 (available from: http://hydra.usc.edu/gxe) using minor allele frequency data from HapMap (http://www.hapmap.org/).

Table 1 Details of reaction conditions and restriction enzymes used to investigate *VEGF* and *VEGFR1* polymorphisms

Polymorphism (RefSNP)	Chromosome position	Location	Genotyping	Annealing temperature	Restriction enzyme	Allele	Fragment size (bp)	Primer references
VEGF −2578C/A (rs699947)	43768652	Promoter	PCR-RFLP	59°	*Bgl*II	C A	459 247, 212	[75]
VEGF −2549I/D (rs35569394)	43768681 to 43768698	Promoter	Direct PCR	55°	–	D I	211 229	[36]
VEGF −460T/C or −1498T/C (rs833061)	43769749	Promoter	PCR-RFLP	59°	*Bst*UI	T C	175 155, 20	[12]
VEGF −7C/T (rs25648)	43771240	5′ UTR	ARMS PCR	60°	–	Control C and T	425 183	[85]
VEGFR1 −710C/T	28495805	Promoter	PCR-RFLP	65°	*Nla*III	C T	665 520, 145	[57]

The present study was designed to have a statistical power of over 80 % for detection of an association of SNPs and breast cancer at significance level of 0.05. The characteristics of patients and controls were compared using *t* test for continuous variables and chi-square test (χ^2) for categorical variables. The allele frequencies were tested for the Hardy-Weinberg equilibrium (HWE) for both patients and controls using the chi-square test. This test was also used to evaluate the differences in the *VEGF* genotypes and allele frequencies between the patient and control groups. Odds ratio (OR) and its

Table 2 Demographic and clinical profiles of study subjects

Parameters	Patients *n* (%)	Controls *n* (%)	*p* value
Gender			
Males	6 (2.94)	6 (2.94)	1
Females	198 (97.06)	198 (97.06)	
Age (years)			
≤40	53 (25.98)	53 (25.98)	1
>40	151 (74.02)	151 (74.02)	
Mean age	49.70±12.17	49.68±12.19	0.99
Range	25–85	25–85	
Menstrual status			
Premenopausal	85 (42.93)	92 (46.46)	0.48
Postmenopausal	113 (57.07)	106 (53.54)	
Reproductive history			
Mean age at menarche	14.82±1.58	14.68±1.49	0.36
Mean age at first child birth	22.53±4.06	22.71±3.54	0.64
Number of full-term pregnancies	3.13±1.30	3.05±1.28	0.54
Type of cancer			
Infiltrating ductal carcinoma	192 (94.12)		
Infiltrating lobular cancer	4 (1.96)		
Others	8 (3.92)		
TNM stage			
Males (*n*=6)			
I	2 (33.33)		
II	4 (66.67)		
Females (*n*=198)			
I	32 (16.16)		
II	98 (49.50)		
III	50 (25.25)		
IV	18 (9.09)		

95 % confidence interval (CI) were used to assess the association between genotypes and alleles with the breast cancer risk. Chi-square analysis was also performed for the correlation of genotype and allele frequencies with various parameters including age, gender, menstrual status and pathological stage of the cancer. Probability value <0.05 was considered statistically significant. The analysis was done using SPSS (version 16, SPSS Inc, Chicago, IL, USA). Lewontin's standardized disequilibrium coefficient (D') among the two SNPs was estimated using Haploview version 4.2 [61].

Results

Characteristics of subjects

In the present case-control study, 204 sporadic breast cancer patients (198 females and 6 males) and 204 healthy unrelated normal individuals (198 females and 6 males) were analyzed. The characteristics of patients and controls are listed in Table 2. The mean age of breast cancer patients and controls was 49.70±12.17 and 49.68±12.19 years, respectively. There was no significant difference in age, gender, menstrual status, mean age at menarche, mean age at first child birth and number of full-term pregnancies between patients and controls ($p>0.05$). Among the female patients, 32 had stage I, 98 had stage II, 50 had stage III, and 18 had stage IV tumour. In male patients, two patients had stage I and four patients had stage II cancer.

Genotype frequencies of VEGF, VEGFR1 polymorphisms and breast cancer risk

The genotype and allele frequencies of *VEGF* polymorphisms in the breast cancer patients and control individuals are shown in Table 3. The genotype distributions of *VEGF* −2578C/A, −2549I/D and −460T/C polymorphisms were in Hardy-Weinberg equilibrium (HWE) in the patients and control groups ($p>0.05$).

The frequencies of CC, CA and AA genotypes for *VEGF* −2578C/A polymorphism were 26.96 vs 37.25 %, 45.59 vs 49.51 % and 27.45 vs 13.24 % in patients and controls, respectively. The frequency of AA genotype was significantly higher in patients as compared to control individuals (27.45 vs 13.24 %; $p=0.0003$) and showed 2.87-fold increased risk to breast cancer (OR=2.87; 95 % CI, 1.61–5.10). Significantly higher frequency of −2578A allele was observed in breast cancer patients ($p=0.0004$), and greater than 1.5-fold risk to breast cancer was associated with −2578A allele (OR=1.65, 95 % CI, 1.25–2.18) (Table 3). Genetic model analysis revealed significantly higher risk for breast cancer (OR=1.61, 95 % CI, 1.06–2.45; $p=0.026$ in dominant model; OR=2.48, 95 % CI, 1.49–4.12; $p=0.0004$ in recessive model) (Table 4).

Table 3 Distribution of genotype and allele frequencies of *VEGF* and *VEGFR1* polymorphisms in sporadic breast cancer patients and controls

Polymorphism (RefSNP)	Genotype frequency					Allele frequency				
	Genotypes	Patients *n*(%)	Controls *n*(%)	OR (95 % CI)	*p* value	Alleles	Cases *n*=408	Controls *n*=408	OR (95 % CI)	*p* value
VEGF −2578C/A (rs699947)	CC	55 (26.96)	76 (37.25)	Reference		C	203 (49.75)	253 (62.01)	Reference	
	CA	93 (45.59)	101 (49.51)	1.27 (0.81–1.99)	0.29	A	205 (50.25)	155 (37.99)	1.65 (1.25–2.18)	**0.0004**
	AA	56 (27.45)	27 (13.24)	2.87 (1.61–5.10)	**0.0003**					
VEGF −2549I/D (rs35569394)	DD	55 (26.96)	72 (35.29)	Reference		D	202 (49.51)	249 (61.03)	Reference	
	ID	92 (45.10)	105 (51.47)	1.15 (0.73–1.80)	0.55	I	206 (50.49)	159 (38.97)	1.60 (1.21–2.11)	**0.0009**
	II	57 (27.94)	27 (13.24)	2.76 (1.55–4.92)	**0.0005**					
VEGF −460T/C (rs833061)	TT	61 (29.90)	72 (35.29)	Reference		T	214 (52.45)	249 (61.03)	Reference	
	TC	92 (45.10)	105 (51.47)	1.03 (0.67–1.61)	0.881	C	194 (47.55)	159 (38.97)	1.42 (1.07–1.87)	**0.012**
	CC	51 (25)	27 (13.24)	2.23 (1.25–3.97)	**0.006**					
VEGF −7C/T (rs25648)	CC	128 (62.75)	138 (67.65)	Reference		C	332 (81.37)	342 (83.82)	Reference	
	CT	76 (37.25)	66 (32.35)	1.24 (0.82–1.87)	0.299	T	76 (18.63)	66 (16.18)	1.19 (0.82–1.70)	0.356
	TT	–	–							
VEGFR1 −710C/T	CC	197 (96.57)	196 (96.08)	Reference		C	401 (98.28)	400 (98.04)	Reference	0.794
	CT	7 (3.43)	8 (3.92)	0.87 (0.31–2.45)	0.792	T	7 (1.72)	8 (1.96)	0.87 (0.31–2.43)	
	TT	–	–	–	–					

p values statistically significant (<0.05) are displayed in bold. HWE (*VEGF* −2578C/A): patients ($p=0.208$), controls ($p=0.468$), both ($p=0.347$). HWE (*VEGF* −2549I/D): patients ($p=0.162$), controls ($p=0.241$), both ($p=0.189$). HWE (*VEGF* −460T/C): patients ($p=0.171$), controls ($p=0.241$), both ($p=0.197$)

OR odds ratio, *CI* confidence interval

Table 4 Analyses of *VEGF* −2578C/A, −2549I/D and −460T/C polymorphisms using different genetic models

Polymorphism	Model	Genotypes	Patients *n* (%)	Controls *n* (%)	OR (95 % CI)	*p* value
−2578C/A	Codominant	CC	55 (26.96)	76 (37.25)	Reference	
		CA	93 (45.59)	101 (49.51)	1.27 (0.81–1.99)	0.29
		AA	56 (27.45)	27 (13.24)	2.87 (1.61–5.10)	**0.0003**
	Dominant	CC	55 (26.96)	76 (37.25)	Reference	
		CA + AA	149 (73.04)	128 (62.75)	1.61 (1.06–2.45)	**0.026**
	Recessive	CC + CA	148 (72.55)	177 (86.76)	Reference	
		AA	56 (27.45)	27 (13.24)	2.48 (1.49–4.12)	**0.0004**
	Overdominant	CC + AA	111 (54.41)	103 (50.49)	Reference	
		CA	93 (45.59)	101 (49.51)	0.85 (0.58–1.26)	0.43
−2549I/D	Codominant	DD	55 (26.96)	72 (35.29)	Reference	
		ID	92 (45.10)	105 (51.47)	1.15 (0.73–1.80)	0.55
		II	57 (27.94)	27 (13.24)	2.76 (1.55–4.92)	**0.0005**
	Dominant	DD	55 (26.96)	72 (35.29)	Reference	
		ID + II	152 (74.51)	132 (64.71)	1.48 (0.97–2.25)	0.069
	Recessive	DD + ID	147 (72.06)	177 (86.76)	Reference	
		II	57 (27.94)	27 (13.24)	2.54 (1.53–4.22)	**0.0002**
	Overdominant	DD + II	112 (54.90)	99 (48.53)	Reference	
		ID	92 (45.10)	105 (51.47)	0.77 (0.52–1.14)	0.20
−460T/C	Codominant	TT	61 (29.90)	72 (35.29)	Reference	
		TC	92 (45.10)	105 (51.47)	1.03 (0.67–1.61)	0.881
		CC	51 (25)	27 (13.24)	2.23 (1.25–3.97)	**0.006**
	Dominant	TT	61 (29.90)	72 (35.29)	Reference	
		TC + CC	143 (70.10)	132 (64.71)	1.28 (0.84–1.94	0.245
	Recessive	TT + TC	153 (75)	177 (86.76)	Reference	
		CC	51 (25)	27 (13.24)	2.19 (1.31–3.65)	**0.002**
	Overdominant	TT + CC	112 (54.90)	99 (48.53)	Reference	
		TC	92 (45.10)	105 (51.47)	0.77 (0.52–1.14)	0.19

p values statistically significant (<0.05) are displayed in bold

OR odds ratio, *CI* confidence interval

For *VEGF* −2549I/D polymorphism, the frequencies of DD, ID and II genotypes were 26.96 vs 35.29 %, 45.10 vs 51.47 % and 27.94 vs 13.24 %, in patients and controls, respectively. There was a significant increase in the frequency of II genotype in patients as compared to control individuals (p=0.0005), and 2.76-fold risk to breast cancer was associated with II genotype (OR=2.76, 95 % CI, 1.55–4.92). The frequency of −2549I allele in patients and controls was 50.49 vs 38.97 %, respectively, and presence of I allele revealed greater than 1.5-fold risk to breast cancer (OR=1.60, 95 % CI, 1.21–2.11) (Table 3). In the recessive genetic model, II genotype was associated with higher breast cancer risk as compared to combined DD + ID genotype (OR=2.54, 95 % CI, 1.53–4.22; p=0.0002) (Table 4).

For *VEGF* −460T/C polymorphism, the frequency of TT, TC and CC genotypes was 29.90 vs 35.29 %, 45.10 vs 51.47 % and 25 vs 13.24 % in cases and controls, respectively. A significant increased risk for breast cancer was observed with CC genotype (OR=2.23, 95 % CI, 1.25–3.97; p=0.006) and C allele (OR=1.42, 95 % CI, 1.07–1.87; p=0.012) of −460T/C polymorphism (Table 3). *VEGF* −460CC genotype was associated with increased breast cancer risk as compared to TT + TC genotype in recessive genetic model (OR=2.19, 95 % CI, 1.31–3.65; p=0.002) (Table 4). There was no significant difference in genotype and allele frequencies of *VEGF* −7C/T and *VEGFR1* −710C/T polymorphisms between cases and controls (p>0.05) (Table 3).

Additionally, we stratified our study subjects to investigate the relationship of the studied polymorphisms with various parameters including gender, age, menstrual status and tumour stage. We observed significant difference in genotype distribution of *VEGF* −2578C/A (p=0.006), −2549I/D (p=0.006), −460T/C (p=0.032) and −7C/T (p=0.026) polymorphisms in breast cancer patients aged ≤40 years and cases aged >40 years (Table 5). Lewontin's standardized disequilibrium coefficient (D') was calculated as a measure for linkage disequilibrium between the studied *VEGF* polymorphisms (Fig. 1). A strong

Table 5 Association of *VEGF* and *VEGFR1* polymorphisms with demographic and clinical characteristics of patients

	VEGF −2578C/A			*VEGF* −2549I/D			*VEGF* −460T/C			*VEGF* −7C/T			*VEGFR1* −710C/T		
	CC *n* (%)	CA + AA *n* (%)	*p* value	DD *n* (%)	ID + II *n* (%)	*p* value	TT *n* (%)	TC + CC *n* (%)	*p* value	CC *n* (%)	CT *n* (%)	*p* value	CC *n* (%)	CT *n* (%)	*p* value
Gender															
Males	–	6 (100)	NC	–	6 (100)	NC	–	6 (100)	NC	5 (83.33)	1 (16.67)	0.29	6 (100)	–	NC
Females	55 (27.78)	143 (72.22)		55 (27.78)	143 (72.22)		61 (30.81)	137 (69.19)		123 (62.12)	75 (37.88)		191 (96.46)	7 (3.54)	
Age															
≤40	22 (41.51)	31 (58.49)	**0.006**	22 (41.51)	31 (58.49)	**0.006**	22 (41.51)	31 (58.49)	**0.032**	40 (75.47)	13 (24.53)	**0.026**	50 (94.34)	3 (5.66)	0.3
>40	33 (21.85)	118 (78.15)		33 (21.85)	118 (78.15)		39 (25.83)	112 (74.17)		88 (58.28)	63 (41.72)		147 (97.35)	4 (2.65)	
Menstrual status															
Premenopausal	27 (31.76)	58 (68.24)	0.277	27 (31.76)	58 (68.24)	0.277	27 (31.76)	58 (68.24)	0.800	56 (65.88)	29 (34.12)	0.344	81 (95.29)	4 (4.71)	0.439
Postmenopausal	28 (24.78)	85 (75.22)		28 (24.78)	85 (75.22)		34 (30.09)	79 (69.91)		67 (59.29)	46 (40.71)		110 (97.34)	3 (2.66)	
Stage															
I and II	38 (27.94)	98 (72.06)	0.655	38 (27.94)	98 (72.06)	0.655	44 (32.35)	92 (67.65)	0.280	88 (64.71)	48 (35.29)	0.413	130 (95.59)	6 (4.41)	0.277
III and IV	17 (25)	51 (75)		17 (25)	51 (75)		17 (25)	51 (75)		40 (58.82)	28 (41.77)		67 (98.53)	1 (1.47)	

p values statistically significant (<0.05) are displayed in bold

NC not calculated

linkage was observed between *VEGF* −2549I/D and −2578C/A polymorphisms (Lewontin's D'=0.99; correlation coefficient, r^2=0.97), −2549I/D and −460T/C (D'=0.94; r^2=0.84) and −2578C/A and −460T/C polymorphisms (D'=0.93; r^2=0.83). We also analyzed the distributions of combined genotypes and observed that genotype combinations *VEGF* −2549II/−2578AA (p=0.0006), −2549II/−460CC (p=0.003), −2549II/−7CC (p=0.002), −2549II/−7CT (p=0.009), −2549II/*VEGFR1* −710CC (p=0.001), −2578AA/−460CC (p=0.002), −2578CA/−460TT (p=0.013), −2578AA/*VEGFR1* −710CC (p=0.0007), −2578AA/−7CC (p=0.0009), −2578AA/−7CT (p=0.009), −460CC/−7CC (p=0.010), −460CC/−7CT (p=0.037) and −460CC/*VEGFR1* −710CC (p=0.015) were more common among breast cancer patients as compared to control individuals. However, after Bonferroni correction of multiple variables, significant association to increased breast cancer risk remained with *VEGF* −2549II/−2578AA (p_c=0.0024), −2549II/−460CC (p_c=0.024), 2549II/−7CC (p_c=0.012), 2549II/*VEGFR1* −710CC (p_c=0.006), −2578AA/−460CC (p_c=0.018), −2578AA/*VEGFR1* −710CC (p_c=0.0042) and −2578AA/−7CC (p_c=0.0054) genotype combinations (Table 6).

Discussion

Angiogenesis not only is essential for tumour growth but also plays a critical role in the invasion and metastasis. It is regulated by many growth factors, among which *VEGF* is one of the most important activators of tumour-associated angiogenesis [62]. The human *VEGF* is highly polymorphic, and there is considerable variation between individuals in VEGF expression [18]. VEGF plasma levels are highly predictive for tumour growth and survival rate of breast cancer patients [4, 22]. Elevated serum VEGF levels have been reported in several cancers including breast cancer [63–65]. In our previous study, we reported significantly higher serum VEGF levels in breast cancer patients as compared to controls [66]. In vitro and in vivo data suggested that genetic variability affects the activity and expression of VEGF [16, 18, 67]. A number of functional polymorphisms in the *VEGF* have been reported and have been associated with increased risk for several tumours [68].

In the present study, we observed significant association of *VEGF* −2549I/D, −2578C/A and −460T/C polymorphisms with the risk to breast cancer. For −2549I/D polymorphism, we observed significant association of II genotype (p=0.0005) and I allele (p=0.0009) with the increased risk to breast cancer. *VEGF* −2549II genotype has been correlated with higher VEGF protein production in lipopolysaccharide-stimulated peripheral blood mononuclear cells [37, 39]. Significant association of −2549D allele has been reported with the reduced risk for bladder cancer in North Indians [33]. Contrary to our

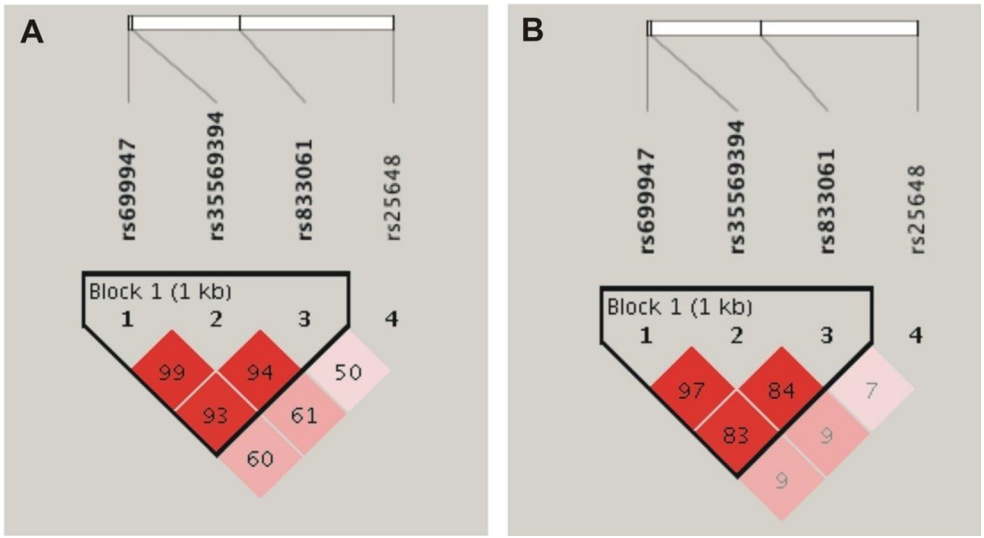

Fig. 1 **a** Linkage disequilibrium (LD) map of *VEGF* polymorphisms based on Lewontin's D'. **b** LD map of *VEGF* polymorphisms based on r^2

findings, significant association of −2549D allele and ID genotype has been reported with renal cell carcinoma [34] and prostate cancer, respectively [41]. No correlation of −2549I/D polymorphism has been observed in Chinese hepatocellular carcinoma [69] and Swedish colorectal cancer cases [70].

For −2578C/A polymorphism, homozygous AA genotype revealed 2.87-fold risk of breast cancer (OR=2.87; 95 % CI, 1.61–5.10). Our results are concordant with few other studies that also showed significant risk association. Association of AA genotype has been reported with higher risk to breast cancer [71], colon cancer [72] and lung cancer [73]. In Italian population, significant reduced risk for the colorectal cancer has been reported with the −2578CC and CA genotypes [51]. We also observed significantly higher frequency of A allele in cases in comparison to controls (50.25 vs 37.99 %, p=0.0004). Association of −2578A allele with increased VEGF expression has been reported in lung cancer cells [74]. Correlation of A allele has been reported with an increased risk to nasopharyngeal carcinoma [75] and thyroid cancer [76]. In North Indian population, significant association of CA genotype has been reported with the increased risk for bladder cancer [33]. Contrary to our findings, association of C allele has been reported with increased risk for invasive breast [77] and nasopharyngeal carcinoma [30]. A case-control study including patients with familial breast cancer from Germany and Poland and patients with sporadic breast cancer from Sweden failed to find a relation between −2578C>A polymorphism and risk of breast cancer [26]. However, −2578C/A polymorphism has not been associated with different cancers including skin [78], gastric [79, 80], prostate [47], colorectal [31, 70, 81] and epithelial ovarian cancers [82] and renal carcinoma [83].

For *VEGF* −460T/C polymorphism, we observed significant association of CC genotype (p=0.006) and C allele (p=0.012) with increased risk of breast cancer. Similarly, an association of CC genotype has been documented with colorectal cancer in Italian population [51]. Another study from India has also documented significant association of −1498C allele with type 1 diabetic retinopathy [73]. In smoker oesophageal adenocarcinoma cases, increased cancer risk has been reported with −460CT and combined CT + CC genotype [49]. No association of *VEGF* −460T/C polymorphism has been reported in lung [43], breast [44, 46], ovarian, cervical and endometrial [84], colon [45], prostate [47, 48], pancreatic [52] and renal cell carcinoma [83].

There was no significant difference in genotype and allele distribution of *VEGF* −7C/T polymorphism in breast cancer patients and controls (p>0.05). Similarly, no correlation of *VEGF* −7C/T polymorphism has been documented in Austrian breast cancer patients [46], Caucasian prostate cancer patients [47] and North Indian bladder cancer patients [33]. Significant association of C allele of −7C/T polymorphism has been reported with neuropathy in British-Caucasian type 1 diabetic subjects [85].

VEGFR1 is one of the important receptors of VEGF angiogenesis signalling and has a relevant role in process of normal vessel formation [55]. In the present study, we did not observe any association between *VEGFR1* −710C/T polymorphism and breast cancer risk. Significant association of combined CT and TT genotype has been reported with reduced breast cancer risk in Spanish population [57].

We observed a strong linkage between *VEGF* −2549I/D and −2578C/A polymorphisms (D'=0.99; r^2=0.97), −2549I/D and −460T/C (D'=0.94; r^2=0.84) and −2578C/A and −460T/C polymorphisms (D'=0.93; r^2=0.83). Linkage disequilibrium between promoter polymorphisms −2549I/D and −2578C/A of *VEGF* has also been reported in Polish, German, Swedish breast cancer

Table 6 Interaction between *VEGF* and *VEGFR1* polymorphisms in breast cancer patients and healthy controls

VEGF −2549I/D and −2578C/A

Genotype combination	Patients *n* (%)	Controls *n* (%)	OR (95 % CI)	*p* value
DD-CC	55 (26.96)	72 (35.29)	Reference	
ID-CA	92 (45.10)	101 (49.51)	1.19 (0.76–1.87)	0.444
II-AA	56 (27.45)	27 (13.24)	2.71 (1.52–4.84)	**0.0006**[a]
II-CA	1 (0.49)	4 (1.96)	0.33 (0.04–3.01)	0.301

[a]Bonferroni-corrected *p* value (p_c)=**0.0024**

VEGF −2549I/D and −460T/C

Genotype combination	Patients *n* (%)	Controls *n* (%)	OR (95 % CI)	*p* value
DD-TT	52 (25.49)	69 (33.82)	Reference	
DD-TC	2 (0.98)	3 (1.47)	0.88 (0.14–5.49)	0.895
DD-CC	1 (0.49)	1 (0.49)	1.33 (0.08–21.71)	0.842
ID-TT	7 (3.43)	3 (1.47)	3.10 (0.76–12.55)	0.099
ID-TC	85 (41.67)	101 (49.51)	1.12 (0.70–1.77)	0.639
II-TT	2 (0.98)	–	–	–
II-CC	49 (24.02)	27 (13.24)	2.41 (1.33–4.35)	**0.003**[a]
II-TC	6 (2.94)	–	–	–

[a]Bonferroni-corrected *p* value (p_c)=**0.024**

VEGF −2549I/D and −7C/T

Genotype combination	Patients *n* (%)	Controls *n* (%)	OR (95 % CI)	*p* value
DD-CC	45 (22.06)	63 (30.88)	Reference	
ID-CC	54 (26.47)	63 (30.88)	1.2 (0.71–2.03)	0.498
ID-CT	38 (18.63)	43 (21.08)	1.24 (0.69–2.21)	0.472
II-CT	28 (13.72)	15 (7.36)	2.61 (1.25–5.45)	**0.009**[a]
II-CC	29 (14.22)	12 (5.88)	3.38 (1.56–7.33)	**0.002**[b]
DD-CT	10 (4.90)	8 (3.92)	1.75 (0.64–4.78)	0.271

[a]Bonferroni-corrected *p* value (p_c)=0.054
[b]Bonferroni-corrected *p* value (p_c)=**0.012**

VEGF −2549I/D and *VEGFR1* −710C/T

Genotype combination	Patients *n* (%)	Controls *n* (%)	OR (95 % CI)	*p* value
DD-CC	54 (26.47)	67 (32.84)	Reference	
ID-CC	88 (43.14)	103 (50.49)	1.06 (0.67–1.67)	0.803
II-CC	55 (26.96)	26 (12.75)	2.62 (1.46–4.73)	**0.001**[a]
II-CT	2 (0.98)	1 (0.49)	2.48 (0.22–28.10)	0.449
ID-CT	4 (1.96)	2 (0.98)	2.48 (0.44–14.06)	0.29
DD-CT	1 (0.49)	5 (2.45)	0.25 (0.03–2.19)	0.177

[a]Bonferroni-corrected *p* value (p_c)=**0.006**

VEGF −2578C/A and −460T/C

Genotype combination	Patients *n* (%)	Controls *n* (%)	OR (95 % CI)	*p* value
CC-TT	52 (25.49)	71 (34.80)	Reference	
CA-TC	85 (41.67)	100 (49.02)	1.16 (0.73–1.84)	0.526
AA-CC	49 (24.02)	26 (12.75)	2.57 (1.42–4.67)	**0.002**[a]
CC-CC	2 (0.98)	1 (0.49)	2.73 (0.24–30.93)	0.399
CC-TC	1 (0.49)	4 (1.96)	0.34 (0.04–3.14)	0.322
AA-TC	5 (2.45)	–	–	–
CA-CC	1 (0.49)	–	–	–
CA-TT	7 (3.43)	1 (0.49)	9.56 (1.14–80.08)	**0.013**[b]
AA-TT	2 (0.98)	1 (0.49)	2.73 (0.24–30.93)	0.399

[a]Bonferroni-corrected *p* value (p_c)=**0.018**
[b]Bonferroni-corrected *p* value (p_c)=0.117

VEGF −2578C/A and *VEGFR1* −710C/T

Table 6 (continued)

Genotype combination	Patients*n* (%)	Controls*n* (%)	OR (95% CI)	*p* value
CC-CC	54 (26.47)	71 (34.80)	Reference	
CA-CC	89 (43.63)	99 (48.53)	1.18 (0.75–1.86)	0.471
AA-CC	54 (26.47)	26 (12.75)	2.73 (1.52–4.91)	**0.0007**[a]
AA-CT	2 (0.98)	1 (0.49)	2.63 (0.23–29.76)	0.418
CA-CT	4 (1.96)	2 (0.98)	2.63 (0.46–14.89)	0.258
CC-CT	1 (0.49)	5 (2.45)	0.26 (0.03–2.31)	0.198

[a]Bonferroni-corrected *p* value (p_c)=**0.0042**

VEGF −2578C/A and −7C/T

Genotype combination	Patients*n* (%)	Controls*n* (%)	OR (95% CI)	*p* value
CC-CC	45 (22.06)	66 (32.35)	Reference	
CA-CC	54 (26.47)	60 (29.41)	1.32 (0.78–2.24)	0.302
CA-CT	39 (19.12)	41 (20.10)	1.39 (0.78–2.49)	0.259
AA-CT	27 (13.23)	15 (7.35)	2.64 (1.26–5.51)	**0.009**[a]
CC-CT	9 (4.41)	10 (4.9)	1.32 (0.50–3.51)	0.58
AA-CC	29 (14.22)	12 (5.88)	3.54 (1.64–7.67)	**0.0009**[b]

[a]Bonferroni-corrected *p* value (p_c)=0.054

[b]Bonferroni-corrected *p* value (p_c)=**0.0054**

VEGF −460T/C and −7C/T

Genotype combination	Patients*n* (%)	Controls*n* (%)	OR (95% CI)	*p* value
TT-CC	47 (23.04)	61 (29.90)	Reference	
CC-CC	27 (13.23)	13 (6.37)	2.70 (1.26–5.78)	**0.010**[a]
TC-CC	54 (26.47)	64 (31.37)	1.09 (0.65–1.85)	0.735
TC-CT	38 (18.62)	42 (20.59)	1.17 (0.66–2.10)	0.588
CC-CT	24 (11.76)	14 (6.86)	2.22 (1.04–4.76)	**0.037**[b]
TT-CT	14 (6.86)	10 (4.90)	1.82 (0.74–4.45)	0.188

[a]Bonferroni-corrected *p* value (p_c)=0.06

[b]Bonferroni-corrected *p* value (p_c)=0.222

VEGF −460T/C and *VEGFR1* −710C/T

Genotype combination	Patients*n* (%)	Controls*n* (%)	OR (95% CI)	*p* value
TT-CC	61 (29.90)	67 (32.84)	Reference	
TC-CC	88 (43.14)	103 (50.49)	0.94 (0.60–1.47)	0.781
CC-CC	49 (24.02)	26 (12.75)	2.07 (1.15–3.73)	**0.015**[a]
TC-CT	4 (1.96)	2 (0.98)	2.20 (0.39–12.42)	0.362
TT-CT	–	5 (2.45)		
CC-CT	2 (0.98)	1 (0.49)	2.20 (0.19–24.84)	0.515

[a]Bonferroni-corrected *p* value (p_c)=0.09

VEGF −7C/T and *VEGFR1* −710C/T

Genotype combination	Patients*n* (%)	Controls*n* (%)	OR (95% CI)	*p* value
CC-CC	122 (59.80)	131 (64.22)	Reference	
CT-CC	75 (36.76)	65 (31.86)	1.24 (0.82–1.88)	0.31
CC-CT	6 (2.94)	7 (3.43)	0.92 (0.30–2.81)	0.884
CT-CT	1 (0.49)	1 (0.49)	1.07 (0.07–17.36)	0.960

p values statistically significant (<0.05) are displayed in bold

OR odds ratio, *CI* confidence interval

patients [26] and Swedish colorectal cancer patients [70]. In the present study, we observed no significant correlation of *VEGF* polymorphisms with the advancing stage of breast cancer. Association of AA genotype of −2578C/A polymorphism has been reported with aggressiveness of tumour in gastric [79] and hepatocellular cancers [29].

The functional property of −2578C/A polymorphism has been shown to affect mRNA levels [86]. In metastatic breast cancer, comparison of the effect of paclitaxel with paclitaxel and bevacizumab combination showed that patients with −2578AA genotype had longer median overall survival as compared to −2578CA + CC genotype in the paclitaxel and bevacizumab combination [27]. Therefore, in the present study, we concluded that *VEGF* −2549I/D, −2578C/A and −460T/C polymorphisms are associated with risk to breast cancer in Punjab, North India. SNPs in the *VEGF* polymorphisms might influence the delivery of chemotherapy to the cancer cells and might consequently hold predictive information in relation to response [7, 87–89]. A follow-up investigation of the subjects of the present study is ongoing and will provide further information on role of *VEGF* polymorphisms on metastasis risk and survival of breast cancer patients. VEGF is an important target in anti-cancer therapy, and findings about SNPs influencing VEGF-targeted therapies will help physicians to tailor therapy in individual manner.

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Conflicts of interest None

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