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Research article

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Role of tumour necrosis factor gene polymorphisms (-308 and -238) in breast cancer susceptibility and severity

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Abstract

Introduction Genetic polymorphisms in the promoter region of the tumour necrosis factor (TNF) gene can regulate gene expression and have been associated with inflammatory and malignant conditions. We have investigated two polymorphisms in the promoter of the TNF gene (-308 G>A and -238 G>A) for their role in breast cancer susceptibility and severity by means of an allelic association study.

Methods Using a case–control study design, breast cancer patients (n = 709) and appropriate age-matched and sex-matched controls obtained from the Breast Screening Unit (n = 498) were genotyped for these TNF polymorphisms, using a high-throughput allelic discrimination method.

Results Allele frequencies for both polymorphisms were similar in both breast cancer cases and controls. However, the -308 polymorphism was found to be associated with vascular invasion in breast tumours (P = 0.024). Comparison with other standard prognostic indices did not show any association for either genotype.

Conclusions We demonstrated no association between the -308G>A polymorphism and the -238G>A polymorphism in the promoter region of TNF and susceptibility to breast cancer, in a large North European population. However, the -308 G>A polymorphism was found to be associated with the presence of vascular invasion in breast tumours.

Keywords: breast cancer, genetic polymorphisms, tumour necrosis factor, vascular invasion

Introduction

The role of genetic factors in the epidemiology and pathogenesis of both sporadic breast cancer and familial breast cancer are now well established [1,2]. Only a small minority (~5%) of patients with breast cancer develop the disease as a result of inheritance of germline mutations in dominant, highly penetrant susceptibility genes such as BRCA1 and BRCA2. However, polymorphisms in genes involved in the complex mechanisms of carcinogenesis may confer low penetrant susceptibility to breast cancer in a significant proportion of the remainder of the patients [2-4].

The multifunctional cytokine, tumour necrosis factor (TNF), is involved in the promotion of inflammatory responses and plays a critical role in the pathogenesis of inflammatory, autoimmune and malignant diseases [5]. Initially proposed to have anti-carcinogenic effects [6,7], TNF was later shown to be tumourigenic in both *in vitro* studies [8] and *in vivo* studies [9-11]. High plasma TNF levels in cancer patients are associated with a poor disease outcome [12,13]. TNF is also a key angiogenic molecule that may promote angiogenesis directly by stimulating endothelial cell proliferation and indirectly by modulating expression of other proangiogenic factors [14]. Moreover, TNF is known to induce expression of adhesion molecules thought to be involved in the increased motility and invasive/metastatic behaviour of tumour cells [15].

Single nucleotide polymorphisms at -308 and -238 of the promoter region of the TNF gene have been commonly studied [16]. The -308 polymorphism is a G → A substitution and reportedly affects gene expression, the rare A

FAM = 6-carboxy-fluorescein; PCR = polymerase chain reaction; TET = 6-carboxy-4,7,2',7'-tetrachlorofluorescein; TNF = tumour necrosis factor.

allele resulting in higher TNF production *in vitro* [17,18]. This influence on gene transcription is also supported by gene reporter assays [19]. For the -238 polymorphism, the common G allele has been shown to be associated with high TNF production [20].

Studies in Tunisian populations have suggested that the -308 polymorphism may be associated with breast cancer [21,22]. However, there are conflicting data from a Korean study [23]. The -238 polymorphism has previously been found to have an apparently protective role against a range of tumours [24]. The aim of the current study, therefore, was to investigate the role of these two polymorphisms (-308 G>A and -238 G>A) in breast cancer susceptibility and severity, in a large Northern European population.

Methods

Patients and controls

Seven hundred and nine pathologically confirmed breast cancer patients were recruited from outpatient clinics at the Royal Hallamshire Hospital, Sheffield, UK. Four hundred and ninety-eight controls were recruited from women attending the Sheffield Breast Screening Service. The study was restricted to white Caucasians, as there were insufficient individuals from other ethnic groups for meaningful analysis. The South Sheffield Research Ethics Committee approved the study (reference number SS98/137), and informed written consent was obtained from all participants.

DNA extraction and genotyping

Genomic DNA was extracted from EDTA-preserved peripheral venous blood from all individuals, as described previously [25]. Genotyping of the polymorphisms was performed by the 5' nuclease PCR method, using the ABI/PE Biosystems Taqman™ system (PE Biosystems, Foster City, CA, USA), essentially as described by di Giovine and colleagues [25]. Using specific primer and probe sequences (Table 1), PCR amplification was carried out separately for the two polymorphisms. The final volume for PCR was 25 μl.

The final concentrations of the constituents for the TNF -308 polymorphism were 0.8 ng/μl genomic DNA template, 300 nM each primer, 50 nM 6-carboxy-fluorescein (FAM)-labelled probe, 125 nM 6-carboxy-4,7,2',7'-tetrachlorofluorescein (TET)-labelled probe and 1 × (12.5 μl) Universal PCR mastermix (PE Biosystems) containing optimised buffer components and Rox reference dye. The final concentrations of the constituents for the TNF -238 polymorphism were 0.8 ng/μl genomic DNA template, 900 nM each primer, 50 nM FAM-labelled probe, 75 nM TET-labelled probe and 1 × (12.5 μl) Universal PCR mastermix.

The PCR amplification cycles (for both polymorphisms) were 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 58°C for 1 min. Levels of FAM and TET fluorescence were determined, and the allelic discrimination was carried out using the ABI 7200 Sequence Detector (PE Biosystems).

Quality control of genotyping data was verified by repeat testing of 15% of randomly selected individuals for each of the polymorphisms tested (12 samples from each batch of 72). There were no discrepancies encountered on these analyses.

Statistical methods

All data were entered into a Microsoft Access database and exported to SPSS (version 10.0 for Windows; SPSS UK Ltd., Surrey, UK) for statistical analyses. Genotype data were available for 708 breast cancer cases (705 cases and 708 cases for the -308 and the -238 genotypes, respectively) and for 498 breast screening controls (498 controls and 495 controls for the -308 and the -238 genotypes, respectively). Odds ratios and 95% confidence intervals were determined for the genotype comparisons.

The age at diagnosis/recruitment was compared between cases and controls using the two-sample Mann–Whitney U test. A family history of breast cancer (defined as the presence of at least one first-degree or second-degree relative with breast cancer) was compared between cases

Table 1

Primer and probe sequences for the two polymorphisms

	Tumour necrosis factor (-308)	Tumour necrosis factor (-238)
Forward primer	5'-GGC CAC TGA CTG ATT TGT GTG T-3'	5'-GCA TCA AGG ATA CCC CTC ACA-3'
Reverse primer	5'-CAA AAG AAA TGG AGG CAA TAG GTT-3'	5'-ATC AGT CAG TGG CCC AGA AGA-3'
Probe FAM	FAM AAC CCC GTC CTC ATG CCC C TAMRA	FAM TCC TCC CTG CTC TGA TTC CGA TAMRA
Probe TET	TET ACC CCG TCC CCA TGC CC TAMRA	TET CCT CCC TGC TCC GAT TCC G TAMRA

FAM, 6-carboxy-fluorescein; TET, 6-carboxy-4,7,2',7'-tetrachlorofluorescein.

Table 2

Genotype frequencies of the two tumour necrosis factor (TNF) polymorphisms in breast cancer patients and controls

Genotype	TNF-α-308G>A polymorphism		TNF-α-238G>A polymorphism	
	Breast cancer ($n = 705$)	Screening controls ($n = 498$)	Breast cancer ($n = 708$)	Screening controls ($n = 495$)
GG	475 (67.4%)	313 (62.9%)	621 (87.7%)	434 (87.7%)
GA	208 (29.5%)	167 (33.5%)	84 (11.9%)	59 (11.9%)
AA	22 (3.1%)	18 (3.6%)	3 (0.4%)	2 (0.4%)

and controls using the chi-square test. Comparison of genotypes relative to pathological prognostic indicators was performed using the chi-square test and Fisher's exact test (for smaller numbers on subgroup analysis). All tests were two sided.

Results

There were no significant differences between the breast cancer cases and the mammography screening controls for age at diagnosis/recruitment, with a median age of 57 years for both groups ($P = 0.637$). However, the age range of the controls was narrower (45–77 years for controls versus 28–88 years for cases) since the majority of controls were within the age range of women routinely invited for screening (50–64 years). Breast cancer patients, as expected, were more likely to have a positive family history (32% versus 22.3%, respectively; $P < 0.001$).

The genotype frequencies of the two polymorphisms are presented in Table 2. The genotype frequencies of the -308 and -238 polymorphisms in the control population are in Hardy–Weinberg equilibrium ($P = 0.758$ and $P = 1.0$, respectively).

There was no association between either the -308 polymorphism (odds ratio [95% confidence interval] = 0.82 [0.64–1.04]) or the -238 polymorphism (odds ratio [95% confidence interval] = 1 [0.70–1.41]) and breast cancer. Also, the rare alleles of the two polymorphisms were not associated with breast cancer in subgroups of individuals with a family history of breast cancer (odds ratio for -308 polymorphism [95% confidence interval] = 0.79 [0.49–1.26]; odds ratio for -238 polymorphism [95% confidence intervals] = 1.06 [0.53–2.14]).

Table 3 presents the distribution of genotypes for both the -308 and the -238 polymorphisms in breast cancer patients with different histological features indicative of prognosis. A significant association between the TNF -308AA genotype and vascular invasion was observed (Table 3). Comparison of TNF -308AA versus TNF -308GG for the presence of vascular invasion yielded an odds ratio of 1.69 (95% confidence interval = 1.07–2.7) and the chi-squared test for the trend was significant ($P = 0.023$), indicating a dose effect for carriage of the rare (A) allele. Analysis of all other subgroups as presented in Table 3 using allele carriage rates demonstrated similar results to those obtained with individual genotype frequencies (data not shown).

Discussion

We studied a large cohort of 709 breast cancer patients and 498 unrelated controls to assess the role of TNF polymorphisms in breast cancer. We sought to ensure that this study had sufficient power to identify any clinically relevant genotype–phenotype associations. We found no evidence of association between the two polymorphisms in the TNF promoter region and breast cancer susceptibility. This is in contrast to a previous study in a Tunisian population that demonstrated a positive association between the -308 polymorphism and breast cancer susceptibility with a significant relative risk of 3.2 [21]. The same investigators subsequently confirmed this observation in a larger cohort of breast cancer patients ($n = 243$) and controls ($n = 174$), as well as showing a relationship with clinical outcome [22]. A study of the -308 polymorphism in a Korean population comprising 95 breast cancer cases and 190 controls, however, showed no such association [23].

The current study has a greater power than any of the preceding studies to detect small associations between these polymorphisms and breast cancer: a power of 93% to detect an odds ratio of 1.5 (with a type I error of 0.05) for the -308G>A polymorphism. In addition, previous studies did not ensure age matching and sex matching between cases and controls. However, inherent differences between populations may account for the conflicting results obtained in studies of the predisposition of the TNF genotype to cancer. Conventional case–control gene association studies have been known to produce false-positive results partly because of population stratification as well as poor study design [26].

Our control cohort, identified from a breast screening population, is sex matched and ethnicity matched to the cases. This is a population in which the disease under investigation typically occurs, and thus this control group may be

Table 3

Genotype frequencies of tumour necrosis factor (TNF) polymorphisms in relation to pathological indices of breast cancer severity

Pathological feature	Number of patients (%)			*P* value
	GG	GA	AA	
TNF -238 polymorphism				
Nodal status				
Negative	387 (88.8)	46 (10.6)	3 (0.7)	
Positive	171 (85.5)	29 (14.5)	0 (0)	0.20[a]
Grade				
Grade 1/2	363 (88.1)	47 (11.4)	2 (0.5)	
Grade 3	189 (87.1)	27 (12.4)	1 (0.5)	0.87[a]
Vascular invasion				
Negative	417 (88.3)	53 (11.2)	2 (0.4)	
Positive	105 (89.0)	13 (11.0)	0 (0)	1.0[a]
TNF -308 polymorphism				
Nodal status				
Negative	299 (68.9)	123 (28.3)	12 (2.8)	
Positive	129 (64.8)	62 (31.2)	8 (4.0)	0.50[b]
Grade				
Grade 1/2	286 (69.6)	112 (27.3)	13 (3.2)	
Grade 3	140 (65.1)	68 (31.6)	7 (3.3)	0.50[b]
Vascular invasion				
Negative	326 (69.2)	132 (28.0)	13 (2.8)	
Positive	70 (60.3)	38 (32.8)	8 (6.9)	0.044[b]

[a] Fisher's exact test. [b] Chi-square test.

considered appropriate for an association study [26]. Genotype frequencies of the polymorphisms were also compared with well-established tumour severity indices (tumour grade, nodal status and vascular invasion). An association was demonstrated between the TNF -308 polymorphism and the presence of vascular invasion in tumours. This observation suggests that carriage of -308A may predispose to more aggressive disease, possibly as a result of increased levels of TNF protein.

A positive association found on subgroup analysis, as in this case, should be regarded with caution, as the likelihood of finding a positive result by chance is higher. Correcting for multiple testing is commonly undertaken for subgroup analyses (e.g. Bonferroni correction). Such statistical tests are inappropriate when the variables under investigation are highly correlated, however, as is the case with the prognostic indicators for breast cancer [27]. It is also interesting to note that the genotype distributions across all the remaining histological variants were remarkably consistent (Table 3). Furthermore, there is a biological plausibility to this observation, in view of the role played by TNF in the promotion of tumour angiogenesis, which is a requirement for tumour proliferation [28].

Although the exact mechanisms underlying vascular invasion remain unexplained, a positive correlation has been demonstrated between blood vessel density and vascular invasion in tumours [29]. The hypothesis that TNF, produced in the tumour microenvironment, may promote cancer development and dissemination is supported by evidence from a range of animal experiments [30]. Ovarian cancer xenografts treated with TNF showed evidence of increased peritoneal adhesion and solid tumour formation [31], and overexpression of TNF confers metastatic properties on transplantable tumours in nude mice [32-34].

Although many genetic polymorphisms have been studied for their association with breast cancer, only a few have been shown to be consistently associated with increased breast cancer risk [2,4]. Further studies are required to establish the significance of the positive association

between the -308 polymorphism and vascular invasion, including the study of other polymorphisms in angiogenic genes, which are thought to be important in solid cancers [35].

We recently investigated the role of polymorphisms in key angiogenic genes including vascular endothelial growth factor and endostatin, and we found an association between an endostatin polymorphism and invasiveness in breast cancer [36]. As further polymorphisms are being detected and investigated in several different classes of genes, including those involved in carcinogen and steroid metabolism, in angiogenesis, in DNA repair and in apoptosis, the search for low penetrance genes that may influence breast cancer susceptibility and severity in the general population continues.

Conclusions

We have not demonstrated an association between TNF polymorphisms at positions -238 and -308 and breast cancer. However, an association was found between the rare allele at position -308 and the presence of vascular invasion in breast tumours. This finding suggests a possible role for the TNF protein in the process of vascular invasion, and studies of the functional consequences of variations within the TNF gene in breast cancer patients are warranted.

Competing interests

None declared.

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References

1. Greene MH: **Genetics of breast cancer.** *Mayo Clin Proc* 1997, **72:**54-65.
2. Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF: **A systematic review of genetic polymorphisms and breast cancer risk.** *Cancer Epidemiol Biomarkers Prev* 1999, **8:**843-854.
3. Coughlin SS, Piper M: **Genetic polymorphisms and risk of breast cancer.** *Cancer Epidemiol Biomarkers Prev* 1999, **8:**1023-1032.
4. Weber BL, Nathanson KL: **Low penetrance genes associated with increased risk for breast cancer.** *Eur J Cancer* 2000, **36:**1193-1199.
5. Bazzoni F, Beutler B: **The tumor necrosis factor ligand and receptor families.** *N Engl J Med* 1996, **334:**1717-1725.
6. Beutler B, Cerami A: **The biology of cachectin/TNF – a primary mediator of the host response.** *Annu Rev Immunol* 1989, **7:**625-655.
7. Jaattela M: **Biologic activities and mechanisms of action of tumor necrosis factor-alpha/cachectin.** *Lab Invest* 1991, **64:**724-742.
8. Komori A, Yatsunami J, Suganuma M, Okabe S, Abe S, Sakai A, Sasaki K, Fujiki H: **Tumor necrosis factor acts as a tumor promoter in BALB/3T3 cell transformation.** *Cancer Res* 1993, **53:**1982-1985.
9. Fujiki H, Suganuma M: **Tumor necrosis factor-alpha, a new tumor promoter, engendered by biochemical studies of okadaic acid.** *J Biochem (Tokyo)* 1994, **115:**1-5.
10. Fujiki H, Suganuma M, Komori A, Yatsunami J, Okabe S, Ohta T, Sueoka E: **A new tumor promotion pathway and its inhibitors.** *Cancer Detect Prev* 1994, **18:**1-7.
11. Suganuma M, Okabe S, Marino MW, Sakai A, Sueoka E, Fujiki H: **Essential role of tumor necrosis factor alpha (TNF-alpha) in tumor promotion as revealed by TNF-alpha-deficient mice.** *Cancer Res* 1999, **59:**4516-4518.
12. Warzocha K, Salles G, Bienvenu J, Bastion Y, Dumontet C, Renard N, Neidhardt-Berard EM, Coiffier B: **Tumor necrosis factor ligand-receptor system can predict treatment outcome in lymphoma patients.** *J Clin Oncol* 1997, **15:**499-508.
13. Nakashima J, Tachibana M, Ueno M, Miyajima A, Baba S, Murai M: **Association between tumor necrosis factor in serum and cachexia in patients with prostate cancer.** *Clin Cancer Res* 1998, **4:**1743-1748.
14. Leek RD, Landers R, Fox SB, Ng F, Harris AL, Lewis CE: **Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma.** *Br J Cancer* 1998, **77:**2246-2251.
15. Ioculano M, Altavilla D, Squadrito F, Canale P, Squadrito G, Saitta A, Campo GM, Caputi AP: **Tumour necrosis factor mediates E-selectin production and leukocyte accumulation in myocardial ischaemia-reperfusion injury.** *Pharmacol Res* 1995, **31:**281-288.
16. Hajeer AH, Hutchinson IV: **Influence of TNFalpha gene polymorphisms on TNFalpha production and disease.** *Hum Immunol* 2001, **62:**1191-1199.
17. Bouma G, Xia B, Crusius JB, Bioque G, Koutroubakis I, Von Blomberg BM, Meuwissen SG, Pena AS: **Distribution of four polymorphisms in the tumour necrosis factor (TNF) genes in patients with inflammatory bowel disease (IBD).** *Clin Exp Immunol* 1996, **103:**391-396.
18. Louis E, Franchimont D, Piron A, Gevaert Y, Schaaf-Lafontaine N, Roland S, Mahieu P, Malaise M, De Groote D, Louis R, Belaiche J: **Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans.** *Clin Exp Immunol* 1998, **113:**401-406.
19. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW: **Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation.** *Proc Natl Acad Sci USA* 1997, **94:**3195-3199.
20. Huizinga TW, Westendorp RG, Bollen EL, Keijsers V, Brinkman BM, Langermans JA, Breedveld FC, Verweij CL, van de Gaer L, Dams L, Crusius JB, Garcia-Gonzalez A, van Oosten BW, Polman CH, Pena AS: **TNF-alpha promoter polymorphisms, production and susceptibility to multiple sclerosis in different groups of patients.** *J Neuroimmunol* 1997, **72:**149-153.
21. Chouchane L, Ahmed SB, Baccouche S, Remadi S: **Polymorphism in the tumor necrosis factor-alpha promotor region and in the heat shock protein 70 genes associated with malignant tumors.** *Cancer* 1997, **80:**1489-1496.
22. Mestiri S, Bouaouina N, Ahmed SB, Khedhaier A, Jrad BB, Remadi S, Chouchane L: **Genetic variation in the tumor necrosis factor-alpha promoter region and in the stress protein hsp70-2: susceptibility and prognostic implications in breast carcinoma.** *Cancer* 2001, **91:**672-678.
23. Park KS, Mok JW, Ko HE, Tokunaga K, Lee MH: **Polymorphisms of tumour necrosis factors A and B in breast cancer.** *Eur J Immunogenet* 2002, **29:**7-10.
24. Jang WH, Yang YI, Yea SS, Lee YJ, Chun JH, Kim HI, Kim MS, Paik KH: **The -238 tumor necrosis factor-alpha promoter polymorphism is associated with decreased susceptibility to cancers.** *Cancer Lett* 2001, **166:**41-46.
25. di Giovine FS, Camp NJ, Cox A, Chaudhary AG, Sorrell JA, Crane A, Duff GW: *Detection and Population Analysis of IL-1 and TNF Gene Polymorphisms* Oxford: Oxford University Press; 2000.
26. Daly AK, Day CP: **Candidate gene case–control association studies: advantages and potential pitfalls.** *Br J Clin Pharmacol* 2001, **52:**489-499.
27. Perneger TV: **What's wrong with Bonferroni adjustments.** *Br Med J* 1998, **316:**1236-1238.

28. Yoshida S, Ono M, Shono T, Izumi H, Ishibashi T, Suzuki H, Kuwano M: **Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis.** *Mol Cell Biol* 1997, **17:**4015-4023.
29. Philp EA, Stephenson TJ, Reed MW: **Prognostic significance of angiogenesis in transitional cell carcinoma of the human urinary bladder.** *Br J Urol* 1996, **77:**352-357.
30. Balkwill F: **Tumor necrosis factor or tumor promoting factor?** *Cytokine Growth Factor Rev* 2002, **13:**135-141.
31. Malik ST, Griffin DB, Fiers W, Balkwill FR: **Paradoxical effects of tumour necrosis factor in experimental ovarian cancer.** *Int J Cancer* 1989, **44:**918-925.
32. Malik ST, Naylor MS, East N, Oliff A, Balkwill FR: **Cells secreting tumour necrosis factor show enhanced metastasis in nude mice.** *Eur J Cancer* 1990, **26:**1031-1034.
33. Okahara H, Yagita H, Miyake K, Okumura K: **Involvement of very late activation antigen 4 (VLA-4) and vascular cell adhesion molecule 1 (VCAM-1) in tumor necrosis factor alpha enhancement of experimental metastasis.** *Cancer Res* 1994, **54:**3233-3236.
34. Orosz P, Kruger A, Hubbe M, Ruschoff J, Von Hoegen P, Mannel DN: **Promotion of experimental liver metastasis by tumor necrosis factor.** *Int J Cancer* 1995, **60:**867-871.
35. Balasubramanian SP, Brown NJ, Reed MW: **Role of genetic polymorphisms in tumour angiogenesis.** *Br J Cancer* 2002, **87:**1057-1065.
36. Balasubramanian SP, Cox A, Wilson AG, Brown NJ, Reed MW: **Endostatin polymorphism in breast cancer.** *Eur J Surg Oncol* 2002, **28:**786 [Abstract].