

TNF alpha promoter polymorphisms analysis in benign and malignant breast lesions

Sirotković-Skerlev, Maja; Čačev, Tamara; Križanac, Šimun; Kulić, Ana; Pavelić, Krešimir; Kapitanović, Sanja

Source / Izvornik: **Experimental and Molecular Pathology, 2007, 83, 54 - 58**

Journal article, Accepted version

Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

<https://doi.org/10.1016/j.yexmp.2006.11.004>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:105:469921>

Rights / Prava: [In copyright](#) / [Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2024-09-28**



Repository / Repozitorij:

[Dr Med - University of Zagreb School of Medicine
Digital Repository](#)





Središnja medicinska knjižnica

Sirotković-Skerlev, M., Čačev, T., Križanac, S., Kulić, A., Pavelić, K., Kapitanović, S.
(2007) *TNF alpha promoter polymorphisms analysis in benign and malignant breast lesions*. *Experimental and Molecular Pathology*, [Epub ahead of print].

<http://www.elsevier.com/locate/yexmp>

<http://www.sciencedirect.com/science/journal/00144800>

<http://medlib.mef.hr/211>

University of Zagreb Medical School Repository

<http://medlib.mef.hr/>

TNF alpha promoter polymorphisms analysis in benign and malignant breast lesions

Maja Sirotkovic-Skerlev¹, Tamara Cacev², Simun Krizanac³, Ana Kulić¹, Kresimir Pavelic², Sanja Kapitanovic²

¹Department of Pathophysiology, Zagreb University Hospital and Zagreb University Medical School, Zagreb, Croatia

²Division of Molecular Medicine, Rudjer Boskovic Institute, Zagreb, Croatia

³Department of Clinical and Experimental Pathology, Clinical Hospital Dubrava, Zagreb, Croatia

Address of correspondence:

Maja Sirotkovic-Skerlev, M.D., Ph.D.

Department of Pathophysiology

Zagreb University Hospital and

Zagreb University Medical School

Kispaticeva 12

HR- 10000 Zagreb

Croatia

Phone: + 385 91 466 77 04

Fax: + 385 1 2421 969

E-mail: majas@irb.hr

ABSTRACT

Polymorphisms in genes involved in the complex mechanisms of carcinogenesis may affect the susceptibility to cancer. The multifunctional cytokine, tumor necrosis factor alpha (TNF alpha) has an important role in the pathogenesis of inflammatory, autoimmune and malignant diseases. It has a large spectrum of activities, including both antitumorigenic and protumorigenic. In recent years, several TNF alpha promoter polymorphisms have been identified and related to the expression level of cytokine and to the susceptibility to solid tumors. The aim of our study was to investigate the frequency of three TNF alpha promoter polymorphisms (-1031, -308 and -238) in benign (fibrocystic changes) and malignant (invasive carcinoma) breast lesions. Using "real-time" PCR SNP analysis these polymorphisms were determined in 76 patients with benign and 158 patients with malignant breast lesions. The high expression genotypes at any of the three SNP polymorphisms were more frequent in invasive breast carcinoma (in 81 of 158 examined, 51,3%) than in fibrocystic changes (in 33 of 76 examined, 43,4%). The combined frequency of high production genotypes (-1031 T/C and C/C, -308 G/A and A/A and -238 G/A and A/A) was higher in patients with invasive breast carcinoma than in those with fibrocystic changes. However, these results were not statistically significant. Further studies on a larger group of patients are needed to evaluate the significance of potential differences in TNF alpha genotypes in different breast lesions.

Keywords: breast lesions, TNF alpha promoter polymorphisms

INTRODUCTION

The etiology of breast cancer is extremely complex and while not yet elucidated, appears to involve numerous genetic, endocrine, and external environmental factors. Breast cancer is the most common non-cutaneous malignancy in women and is second only to lung cancer in mortality rates (Tsongalis and Ricci, 2003). The increased incidence of breast cancer in the last 20 years is coincident with a large improvement in early detection due to the implementation of mammography as the standard for surveillance (Tabar et al., 2001). Despite the mass screening efforts and some advances in adjuvant therapy, the overall mortality rate for breast cancer has not yet changed significantly to reflect the rather dramatic shift to earlier stage detection (Olsen and Gotzsche, 2001). A better understanding of tumor cell biology and development of novel therapeutic drugs are expected to make major advances in improving breast cancer outcomes. The role of genetic factors in epidemiology and pathogenesis of both sporadic breast cancer and familial breast cancer are now well established. 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 (Greene, 1997; Coughlin and Piper, 1999). The multifunctional cytokine, tumor necrosis factor alpha (TNF alpha), is involved in the promotion of inflammatory responses and plays a critical role in the pathogenesis of inflammatory, autoimmune and malignant diseases (Bazzoni and Beutler, 1996). Initially proposed to have anti-carcinogenic effects (Jaattela, 1991), TNF alpha was later shown to be tumorigenic in both *in vitro* (Komori et al., 1993) and *in vivo* studies (Fujiki and Suganuma, 1994). High plasma TNF alpha levels in cancer patients are associated with a poor disease outcome (Warzocha et al., 1997; and Nakashima et al., 1998). TNF alpha 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 (Leek et al., 1998). Several TNF alpha promoter polymorphisms have been identified and have been implicated in the regulation of TNF alpha transcription (Kroeger et al., 1997; Wilson et al., 1997). Single nucleotide polymorphisms at -308 and -238 of the promoter region of the TNF alpha gene have been commonly studied. The -308 polymorphism is a G → A substitution and reportedly affects gene expression, the rare A allele resulting in higher TNF alpha production *in vitro* (Bouma et al., 1996). For the -238 polymorphisms, the rare A allele has been shown to be associated with high

TNF alpha production (Grove et al., 1997). The -1031 polymorphism is a T → C substitution and the rare C allele has been shown to be associated with high TNF alpha production (Higuchi et al., 1998).

The aim of our study was to investigate the frequency of these three polymorphisms in breast lesions, fibrocystic changes and invasive breast carcinoma.

MATERIAL AND METHODS

Patients and tissue specimens

This retrospective study was carried out using specimens of 76 benign (fibrocystic changes without proliferative breast disease) and 158 malignant (invasive carcinoma) breast lesions. All specimens were obtained from the Croatian Human Tumor Bank (Spaventi et al., 1993). All specimens were obtained through routine surgery; the diagnoses were established by standard diagnostic procedures and confirmed histopathologically. The study included 76 women with benign breast lesions with age range between 19 and 75 years (mean age 45.1 years) and 158 women with malignant breast lesions with age range between 23 and 82 years (mean age 59.2 years).

Fresh samples of resected tissue were snap-frozen in liquid nitrogen and stored in the Human Tumor Bank at -80°C until further use. Before inclusion in the study, each specimen was verified by a histopathologist (S.K.).

DNA isolation

DNA isolation from frozen-tissue sections was performed using digestion buffer (50mM Tris, pH 8.5, 1 mM EDTA, 0.5% Tween 20) and proteinase K (2 mg/ml, Sigma).

"Real-time" PCR analysis of TNF alpha SNP promoter polymorphisms

"Real-time" PCR analysis for three TNF α SNP promoter polymorphisms was performed using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, USA) and predeveloped TaqMan assay reagents, C_7514871_10 (rs1799964) for TNF alpha -1031 C/T SNP, C_7514879_10 (rs1800629) for TNF alpha -308 A/G SNP and C_2215707_10 (rs361525) for TNF alpha -238 A/G SNP. The PCR reaction was carried out according to the manufacturer's protocol.

Statistical analysis

The data were analyzed using χ^2 statistics. Difference was considered significant when P value was less than 0.05.

RESULTS

Genetic polymorphisms of TNF α were determined in 76 patients with fibrocystic changes and 158 with invasive breast carcinoma. Using “real-time” PCR SNP analysis we detected TNF alpha -1031, -308 and -238 genotypes. The genotype frequencies of the three polymorphisms are presented in Table 1. Genotypes frequencies were distributed in accordance with Hardy-Weinberg equilibrium.

The high expression genotypes at any of the three SNP polymorphisms were more frequent in invasive breast carcinoma (in 81 of 158 examined, 51,3%) than in fibrocystic changes (in 33 of 76 examined, 43,4%), but not statistically significant (Figure 1).

The combined frequency of high production genotypes (-1031 T/C and C/C, -308 G/A and A/A and -238 G/A and A/A) was higher in patients with invasive breast carcinoma than in those with fibrocystic changes but not statistically significant (Table 1, Figure 2).

The haplotype analysis revealed no statistically significant difference in haplotype distribution between two groups of breast lesions (Table 2).

DISCUSSION

TNF alpha has a large spectrum of activity including both protumorigenic and antitumorigenic activity (Naylor et al., 1990; Balkwill, 2002). It has been shown that dysregulation and overproduction of TNF alpha could be involved in cancer development and progression (Mocellin et al., 2005). Blood levels of TNF alpha are significantly higher in patients with solid tumors including breast cancer (Ardizzoia et al., 1992; Anderson, 2004). Several mechanisms of pro-tumor activities of TNF alpha in breast carcinoma have been suggested: induction of promalignant chemokines, matrix metalloproteinases, endothelial adhesion molecules, angiogenic mediators and reactive oxygen intermediates (Ben-Baruch, 2003).

In recent years, several TNF alpha promoter polymorphisms have been identified and their association with susceptibility to solid tumors as well as with high TNF alpha expression has been shown. Single nucleotide polymorphisms at -308 and -238 of the promoter region of the TNF alpha gene have been commonly studied. Many studies have shown that inheritance of the TNF alpha-308 A allele is associated with increased production of TNF

alpha (Baseggio et al., 2001; Sallakci et al., 2005; Jeong et al., 2004). Several authors have directly demonstrated markedly higher transcription of TNF alpha *in vitro* in association with -308A allele (Abraham et al., 1999; Kroeger et al., 1997; Kroeger et al., 2000, Wilson et al., 1997), but some other studies didn't support these findings (Brinkman et al., 1996, Stuber et al., 1996, Uglialoro et al., 1998). These differences might be related to cell-specific factors and the differences in the stimuli that have been used (Suriano et al., 2005). For the -238 polymorphism, the rare A allele has been shown to be associated with high TNF alpha production (Grove et al., 1997). But data on the association between the -238 polymorphism and the TNF alpha production are not equivocal. Some authors have found no association between -238 polymorphism and TNF alpha production (Paciot et al., 1995; Kaijzel et al., 1998). The -1031 polymorphism is a T → C substitution and the rare C allele has been shown to be associated with high TNF alpha production (Higuchi et al., 1998). The aim of our study was to investigate the frequency of -1031, -308 and -238 TNF alpha promoter polymorphisms in breast lesions: fibrocystic changes and invasive breast carcinoma.

It was shown that TNF alpha -308 A/A genotype may play an important role in the tumorigenesis of breast carcinoma (Mestiri et al., 2001). Azmy and coworkers (2004) demonstrated no association between the -308 and -238 TNF alpha polymorphism and susceptibility to breast cancer in a North European population. However, the -308 polymorphism was found to be associated with the presence of vascular invasion in breast tumors. Park and coworkers (2002) did not find the association between -1031, -863, -857 and -308 SNPs in TNF alpha promoter and susceptibility to breast cancer. Smith and coworkers (2004) found a non-significant trend for association between the TNF alpha -308 GG genotype and breast cancer compared to controls. To our knowledge, our study is one of the first reports showing the frequency of TNF alpha promoter polymorphisms in fibrocystic breast changes compared with their frequencies in invasive breast carcinoma.

In this study, using "real-time" PCR SNP analysis, genetic polymorphisms of TNF alpha were determined in 51 patients with fibrocystic changes and 82 with invasive breast carcinoma. The high expression genotypes at any of the three SNP polymorphisms were more frequent in invasive breast carcinoma (in 35 of 82 examined, 42.9%) than in fibrocystic changes (in 17 of 51 examined, 33.3%), but not statistically significant. Samples with two high expression genotypes were also more frequent in invasive breast carcinoma than in samples with fibrocystic changes but not statistically significant. The combined frequency of high production genotypes (-1031 T/C and C/C, -308

G/A and A/A and -238 G/A and A/A) was higher in patients with invasive breast carcinoma than in those with fibrocystic changes but not statistically significant.

Previous breast biopsy showing benign conditions is considered as a possible risk factor for breast cancer. However, only a few types of benign breast lesions have significant premalignant potential (Allred and Mohsin., 2000). The magnitude of the risk depends a great deal on the histological category of the benign breast lesion, degree of proliferative changes and the atypia presence in the biopsy material being the most important factors (Schnitt, 2003; Bilous et al., 2005). Women with proliferative lesions without atypia have 1.5 to 2 fold increase in risk (Page and Dupont., 1990), while women with atypical hyperplasia have fourfold to fivefold increase in breast cancer risk (Fitzgibbons et al., 1998). Several clinical factors modify the risk associated with these lesions such as time elapsed since biopsy, menopausal status, and family history of breast cancer (Schnitt, 2003). Benign breast lesions included in this study were of the non-proliferative type. The consensus view is that women with nonproliferative fibrocystic changes are not at significantly increased risk of developing breast cancer (Richie and Swanson, 2003; Dupont and Page, 1985; Fitzgibbons et al., 1998).

Our results show a difference between TNF alpha genotypes associated with fibrocystic changes and those found in invasive breast carcinoma but this difference was not statistically significant. Further studies on a larger group of patients are needed to evaluate the significance of potential differences in TNF alpha genotypes in different breast lesions.

REFERENCES

- Abraham, L.J., Kroeger, K.M. 1999. Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *J. Leukoc. Biol.* 66, 562-566.
- Allred, D.C., Mohsin, S.K., 2000. Biological features of premalignant disease in the human breast. *J. Mammary Gland Biol. Neoplasia* 5, 351-364.
- Anderson, G.M., Nakada, M.T., DeWitte, M., 2004. Tumor necrosis factor-alpha in the pathogenesis and treatment of cancer. *Curr. Opin.Pharmacol.* 4, 314-320.
- Ardizzoia, A., Lissoni, P., Brivio, F., Tisi, E., Perego, M.S., Grassi, M.G., Pittalis, S., Crispino, S., Barni, S., Tancini, G., 1992. Tumor necrosis factor in solid tumors: increased blood levels in the metastatic disease. *J. Biol.Regul. Homeost. Agents* 6, 103-107.
- Azmy, I.A.F., Balasubramanian, S.P., Wilson, A.G., Stephenson, T.J., Cox, A., Brown, N.J., Reed, M.W.R., 2004. Role of tumour necrosis factor gene polymorphisms (-308 and -238) in breast cancer susceptibility and severity. *Breast Cancer Res.* 6, 395-400.
- Balkwill, F., 2002. Tumor necrosis factor or tumor promoting factor? *Cytokine Growth Factor Rev.* 13, 135-141.
- Baseggio, L., Bienvenu, J., Charlot, C., Picollet, J., Felman, P., Coiffier, B., Salles, G. 2001. Higher LPS-stimulated TNF-alpha mRNA levels in peripheral blood mononuclear cells from non-Hodgkin's lymphoma patients. *Exp. Hematol.* 293, 330-338.
- Bazzoni, F., Beutler, B.,1996. The tumor necrosis factor ligand and receptor families. *N. Engl. J. Med.* 334, 1717-1725.
- Ben-Baruch, A., 2003. Host microenvironment in breast cancer development: inflammatory cells, cytokines and chemokines in breast cancer progression: reciprocal tumor-microenvironment interactions. *Breast Cancer Res.* 5, 31-36.
- Bilous, M., Brennan, M., French, J., Boyages, J., 2005. Making sense of breast pathology. *Aust. Fam. Physician* 34, 581-586.
- Bouma, G., Xia, B., Crusius, J.B., Bioque, G., Koutroubakis, I., Von Blumberg, B.M., Meuwissen, S.G., Pena, A.S., 1996. Distribution of four polymorphisms in the tumour necrosis factor (TNF) genes in patients with inflammatory bowel disease (IBD). *Clin. Exp. Immunol.* 103, 391-396.

Brinkman, B.M., Huizinga, T.W., Breedveld, F.C., Werweij, C.L., 1996. Allele-specific quantification of TNFA transcripts in rheumatoid arthritis. *Hum. Genet.* 97, 813-818.

Coughlin, S.S., Piper, M., 1999. Genetic polymorphisms and risk of breast cancer. *Cancer Epidemiol. Biomarkers Prev.* 8, 843-854.

Dupont, W.D., Page, D.L., 1985. Risk factors for breast cancer in woman with proliferative breast disease. *N. Engl. J. Med.* 312, 146-151.

Fitzgibbons, P.L., Henson, D.E., Hutter, R.V., 1998. Benign breast changes and the risk for subsequent breast cancer: an update of the 1985 consensus statement. Cancer Committee of the College of American Pathologists. *Arch. Pathol. Lab. Med.* 122, 1048-1050.

Fujiki, H., Suganuma, M., 1994. Tumor necrosis factor-alpha, a new tumor promoter, engendered by biochemical studies of okadaic acid. *J. Biochem. (Tokyo)* 115, 1-5.

Greene, M.H., 1997. Genetics of breast cancer. *Mayo Clin. Proc.* 72, 54-65.

Grove, J., Daly, A.K., Bassendine, M.F., Day, C.P., 1997. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. *Hepatology* 26, 232-233.

Higuchi, T., Seki, N., Kamizono, S., Yamada, A., Kimura, A., Kato, H., Itoh, K., 1998. Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. *Tissue Antigens* 51, 605-612.

Jaattela, M., 1991. Biologic activities and mechanisms of action of tumor necrosis factor-alpha/cachectin. *Lab. Invest.* 64, 724-742.

Jeong, P., Kim, E.J., Kim, E.G., Byun, S.S., Kim, C.S., Kim, W.J. 2004. Association of bladder tumors and GA genotype of -308 nucleotide in tumor necrosis factor-alpha. *Urology.* 64,1052-1056.

Kaijzel, E.L., van Krugten, M.V., Brinkman, B.M., Huizinga, T.W., van der Straaten, T., Hazes, J.M., Ziegler-Heitbrock, H.W., Nedospasov, S.A., Breedveld, F.C., Verweij, C.L. 1998. Functional analysis of a human tumor necrosis factor alpha (TNF-alpha) promoter polymorphism related to joint damage in rheumatoid arthritis. *Mol. Med.* 4, 724-733.

Komori, A., Yatsunami, J., Suganuma, M., Okabe, S., Abe, S., Sakai, A., Sasaki, K., Fujiki, H., 1993. Tumor necrosis factor acts as a tumor promoter in BALB/3T3 cell transformations. *Cancer Res.* 53, 1982-1985.

Kroeger, K.M., Carville, K.S., Abraham, L.J. 1997. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Mol. Immunol.* 34, 391-399.

Kroeger, K.M., Steer, J.H., Joyce, D.A., Abraham, L.J. 2000. Effects of stimulus and cell type on the expression of the -308 tumour necrosis factor promoter polymorphism. *Cytokine*. 12, 110-119.

Leek, R.D., Landers, R., Fox, S.B., Ng, F., Harris, A.L., Lewis, C.E., 1998. Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. *Br. J. Cancer* 77, 2246-2251.

Mestiri, S., Bouaouina, N., Ahmed, S.B., Khedhaier, A., Jrad, B.B., Remadi, S., Chouchane, L., 2001. Genetic variation in the tumor necrosis factor- α promoter region and in the stress protein hsp70-2. *Cancer* 91, 672-678.

Mocellin, S., Rossi, C.R., Pilati, P., Nitti, D., 2005. Tumor necrosis factor, cancer and anticancer therapy. *Cytokine Growth Factor Rev.* 16, 35-53.

Nakashima, J., Tachibana, M., Ueno, M., Miyajima, A., Baba, S., Murai, M., 1998. Association between tumor necrosis factor in serum and cachexia in patients with prostate cancer. *Clin. Cancer Res.* 4, 1743-1748.

Naylor, M.S., Malik, S.T., Stamp, G.W., Jobling, T., Balkwill, F.R., 1990. In situ detection of tumour necrosis factor in human ovarian cancer specimens. *Eur. J. Cancer* 26, 1027-1030.

Olsen, O., Gotzsche, P.C., 2001. Cochrane review on screening for breast cancer with mammography. *Lancet* 358, 1340-2.

Page, D.L., Dupont, W.D., 1990. Anatomic markers of human premalignancy and risk of breast cancer. *Cancer* 66 (6 Suppl), 1326-1335.

Park, K.S., Mok, J.W., Ko, H.E., Tokunaga, K., Lee, M.H., 2002. Polymorphisms of tumour necrosis factors A and B in breast cancer. *J. Immunogenetics* 29, 7-10

Pociot, F., D'Alfonso, S., Compasso, S., Scorza, R., Richiardi, P.M. 1995. Functional analysis of a new polymorphism in the human TNF alpha gene promoter. *Scand J Immunol.* 42, 501-504.

Richie, R.C., Swanson, J.O., 2003. Breast cancer: a review of the literature. *J. Insur. Med.* 35, 85-101.

Sallakci, N., Akcurin, G., Koksoy, S., Kardelen, F., Uguz, A., Coskun, M., Ertug, H., Yegin, O. 2005. TNF-alpha G-308A polymorphism is associated with rheumatic fever and correlates with increased TNF-alpha production. *J. Autoimmun.* 25,150-154.

Schnitt, S.J., 2003. Benign breast disease and breast cancer risk: morphology and beyond. *Am. J. Surg. Pathol.* 27, 836-841.

Smith, K.C., Bateman, A.C., Fussell, H.M., Howell, W.M., 2004. Cytokine gene polymorphisms and breast cancer susceptibility and prognosis. *Eur. J. Immunogenet.* 31, 167-73.

Spaventi, R., Pecur, L., Pavelic, K., Pavelic, Z.P., Spaventi, S., Stambrook, P.J., 1994. Human Tumor Bank in Croatia: a possible model for small bank as a part of the future European Tumor Bank Network. *Eur. J. Cancer* 30A, 419.

Stuber, F., Udalova, I.A., Book, M., Drutskava, L.N., Kuprash, D.V., Turetskava, R.L., Schade, F.U., Nedospasov, S.A. 1995-1996. -308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. *J. Inflamm.* 46, 42-50.

Suriano, A.R., Sanford, A.N., Kim, N., Sanford, A.N., Oh, M., Kennedy, S., Henderson, M.J., Dietzmann, K., Sullivan, K.E. 2005. GCF2/LRRFIP1 represses tumor necrosis factor alpha expression. *Mol. Cell. Biol.* 20, 9073-9081.

Tabar, L., Itak, B., Chen, H.H., Yen, M.F., Duffy, S.W., Smith, R.A., 2001. Beyond randomized controlled trials: organized mammographic screening substantially reduces breast cancer mortality. *Cancer* 91, 1724-1731.

Tsongalis, G.J., Ricci, A., 2003. Breast cancer as a model of realistic challenges in pharmacogenomics. *Clin. Biochem.* 36, 89-94.

Ugialoro, A.M., Turbay, D., Pesavento, P.A., Delgado, J.C., McKenzie, F.E., Gribben, J.G., Hartl, D., Yunis, E.J., Goldfeld, A.E. 1998. Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor-alpha gene promoter. *Tissue Antigens.* 52, 359-367.

Warzocha, K., Salles, G., Bienvenu, J., Bastion, Y., Dumontet, C., Renard, N., Neidhardt-Berard, E.M., Coiffier, B., 1997. Tumor necrosis factor ligand-receptor system can predict treatment outcome in lymphoma patients. *J. Clin. Oncol.* 15, 499-508.

Wilson, A.G., Symons, J.A., McDowell, T.L., McDevitt, H.O., Duff, G.V. 1997. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc. Natl. Acad. Sci. U S A.* 94, 3195-3199.

FIGURE LEGENDS

Figure 1. Frequency of common and high expression genotypes in fibrocystic changes and invasive breast cancer.

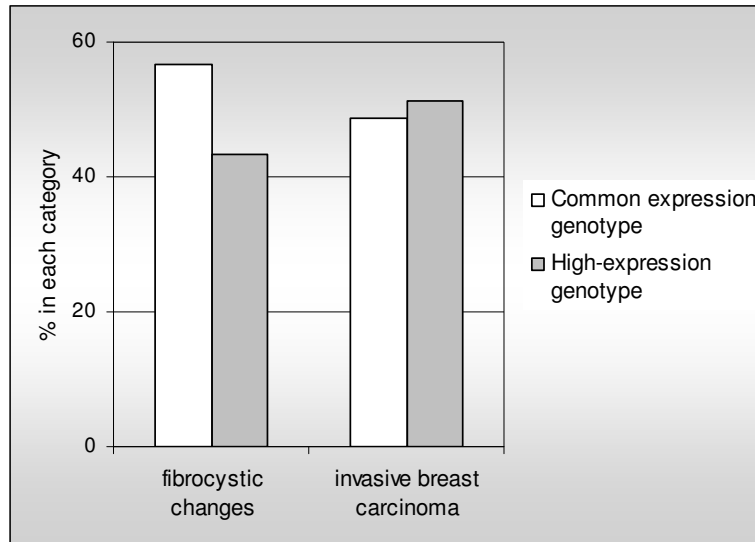
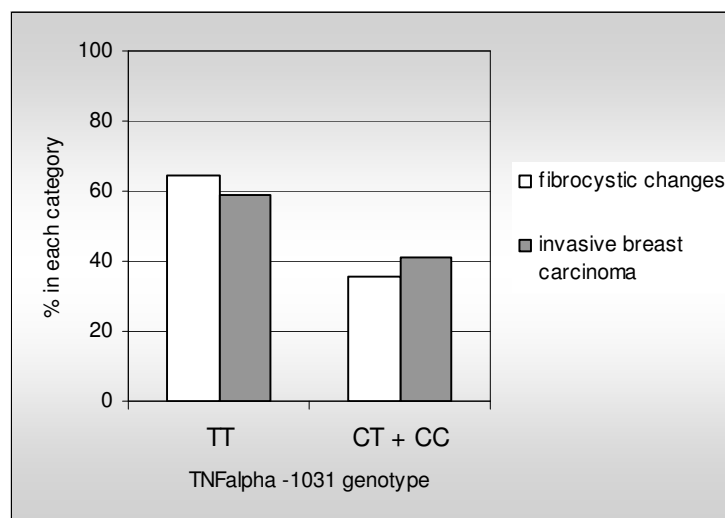
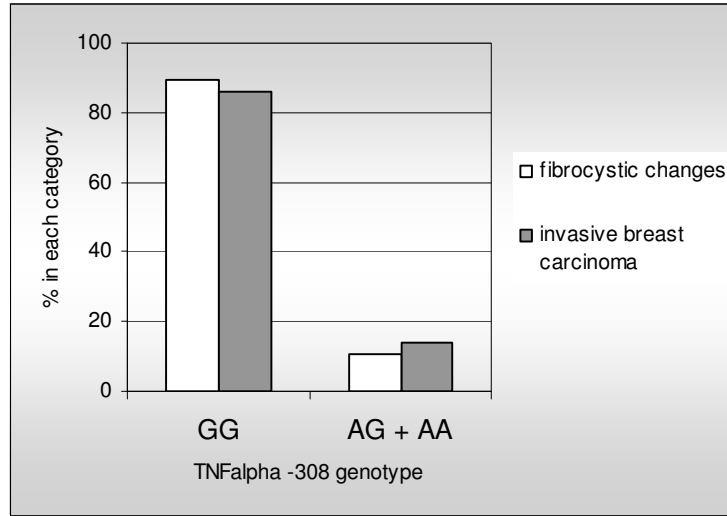


Figure 2. Frequency of TNFalpha promoter polymorphisms in fibrocystic changes and invasive breast cancer. A. -1031 polymorphism; B. -308 polymorphism; C. -238 polymorphism.



B



C

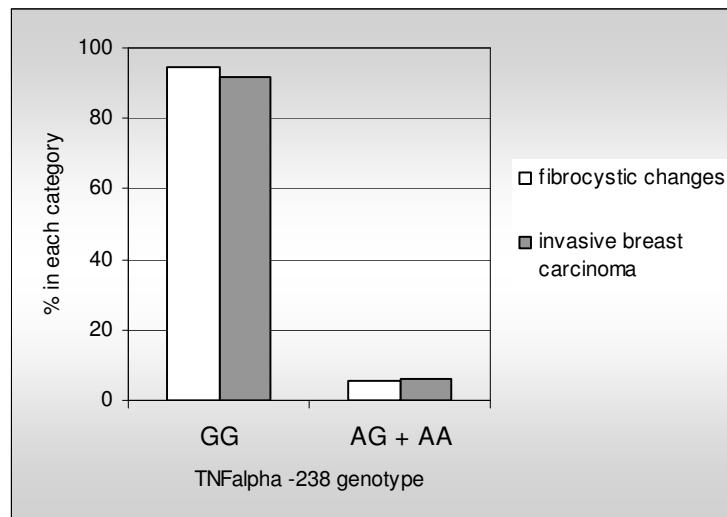


Table 1. Genotype and allele frequencies for each TNF α SNP promoter polymorphism in patients with benign breast lesions (fibrocystic changes) and invasive breast cancer

		% Patient (n)	% Patient (n)	P
		Fibrocystic changes	Breast cancer	
<i>TNFα-1031</i>	T/T	64.5 (49)	58.9 (93)	
	T/C	31.6 (24)	35.4 (56)	
	C/C	3.9 (3)	5.7 (9)	0.671
	T/C + C/C	35.5 (27)	41.1 (65)	0.410
	T allele	80.3 (122)	76.6 (242)	
	C allele	19.7 (30)	23.4 (74)	0.370
<i>TNFα-308</i>	G/G	89.5 (68)	86.1 (136)	
	G/A	10.5 (8)	13.9 (22)	
	A/A	0 (0)	0 (0)	0.467
	G/A + A/A	10.5 (8)	13.9 (22)	0.467
	G allele	94.7 (144)	93.0 (294)	
	A allele	5.3 (8)	7.0 (22)	0.482
<i>TNFα-238</i>	G/G	94.7 (72)	93.7 (148)	
	G/A	5.3 (4)	5.7 (9)	
	A/A	0 (0)	0.6 (1)	0.777
	G/A + A/A	5.3 (4)	6.3 (10)	0.747
	G allele	97.4 (148)	96.5 (305)	
	A allele	2.6 (4)	3.5 (11)	0.625

Table 2. Haplotype frequencies for three TNF α SNP promoter polymorphism in patients with benign breast lesions (fibrocystic changes) and invasive breast cancer

TNF α haplotype	% Patient (n)	% Patient (n)
-1031/-308/-238	Fibrocystic changes	Breast cancer
TT GG GG	56.7 (43)	48,7 (77)
CT GG GG	25.0 (19)	27.2 (43)
TT AG GG	7.9 (6)	10.1 (16)
CT GG AG	3.9 (3)	5.1 (8)
CC GG GG	2.6 (2)	3.8 (6)
CT AG GG	2.6 (2)	3.2 (5)
CC GG AG	1.3 (1)	0.63 (1)
CC GG AA		0.63 (1)
CC AG GG		0.63 (1)