

**Aim of the Study:** TRAIL-mediated signalling has emerged as an extensively studied biological mechanism reported to differentially induce apoptosis in cancer cells. However, overwhelmingly increasing experimentally verified data is shedding light on resistance against TRAIL-induced apoptosis in cancer cells. Moreover, genetic and epigenetic mutations also exert effects on the functionality of TRAIL and its receptors. In this study we investigated the association between breast cancer and polymorphisms in tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) in a Pakistani Population.

**Material and methods:** Genotyping for TRAIL gene 1595 C/T polymorphism was done for 363 breast cancer patients and 193 age- and sex-matched healthy controls. DNA was extracted using standard organic methods. PCR-RFLP analysis was done for C/T polymorphism at position 1595 in exon 5 of the TRAIL gene using site-specific primers and restriction enzyme. The results were statistically evaluated by SPSS14.

**Results:** In this study, CC homozygotes were 46.3% in patients and 49.7% in controls,  $p = 0.729$  with OR value 0.8705 (95% CI: 0.6137–1.2348). CT was statistically insignificant,  $p = 0.837$  with OR value 0.9242 (95% CI: 0.6494–1.3154). However, the minor allele or risk allele genotype TT had a higher percentage among breast cancer patients (12.1%) than in the control group (6.7%). Since there was a statistically insignificant difference ( $p = 0.212$ , OR value 1.9098 with 95% CI 1.0019 to 3.6406) of TT genotype between the two groups, the contrastingly higher percentage of TT genotype in breast cancer patients seems to be a risk factor for the disease. Moreover, the frequency of minor allele T was also found to be higher in the patients (0.329) than in the controls (0.285).

**Conclusions:** The TRAIL gene 1595 C/T SNP has a contradictory role in cancer development in different populations. In our population group although the percentage of homozygous risk allele TT was higher in patients it was statistically non-significant. The raised T allele and TT genotype in patients may suggest its association with breast cancer in the Pakistani population.

**Key words:** TRAIL, cancer, signaling, apoptosis.

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# Association between breast cancer and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) gene 1595C/T SNP in a Pakistani population

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## Introduction

Breast cancer is a genomically complex disease. Research over the years has shown that specific genetic, epigenetic factors, suppression of tumour suppressors, overexpression of oncogenes, loss of apoptosis, and increased cell survival underlie breast carcinogenesis. TRAIL has emerged as one of the most extensively studied molecules in oncology because of its ability to selectively induce apoptosis in cancer cells but leave normal cells intact [1, 2]. Increasingly detailed information is deepening our understanding about TRAIL-mediated signalling and it is now evident that TRAIL signals through death receptors that belong to the tumour necrosis factor receptor superfamily. Structurally death receptors contain cytoplasmic death domain (DD). Death-inducing signalling complex consisting of FADD and Pro-caspase-8 is formed at the death receptor. cFLIP negatively regulates TRAIL-induced signalling by interfering with the activation of caspase-8. Caspase-8 activates its downstream effector caspase-3, thus functionalising extrinsic pathway. Intrinsic pathway is activated via Caspase-8-mediated processing of Bid into truncated Bid. tBid moves into mitochondrion to promote release of cytochrome c, SMAC/DIABLO, Omi/Htra. Cytochrome c co-operates with Apaf-1 to form apoptosome, which results in activation of caspase-9 [3].

There is a rapidly increasing list of *in vitro* studies addressing approaches to induce apoptosis in TRAIL-resistant breast cancer cell lines. Wide-ranging intracellular mechanisms have been reported to induce resistance against TRAIL in cancer cells. Using natural and synthetic agents, burgeoning evidence is substantiating the fact that targeting of negative regulators of TRAIL-induced signalling can improve rates of apoptosis [4].

It is relevant to mention that previously in breast cancer patients and controls, no differences in the distribution of TRAIL genotypes and frequencies of the alleles were observed [5]. There is direct evidence suggesting a relationship between G1525A and C1595T gene polymorphisms and susceptibility to gastric cancer in the Chinese Han population [6].

## Material and methods

In this study 363 breast cancer patients and 193 age- and sex-matched healthy controls participated. The breast cancer patients were ascertained from tertiary care hospitals of Pakistan. 5-ml blood samples were collected in ACD Vacutainers (BD Franklin Lakes NJ, USA) from each breast cancer

patient along with clinical and pathological features, and healthy controls, after informed consent was obtained. Genomic DNA was extracted from whole blood.

The genotyping of TRAIL gene 1595 C>T SNP was done through PCR-RFLP. The 391bp region harbouring the SNP was amplified in PCR using forward primer 5'-TGAGCACTA-CAGCAAACATGA-3' and reverse primer 5'-GCACCACTAAAA-GATCGCAGT-3'. The PCR reaction mixture contained 2 µl of 20 ng/µl genomic DNA, 2 µl of 10X buffer (NH<sub>4</sub>Cl) (Fermentas, Lithuania), 1.2 µl of 25 mM MgCl<sub>2</sub> (Fermentas, Lithuania), 1 µl of 2 mM dNTPs, 0.1 µl of 5 U/µl *Taq* DNA polymerase (Fermentas, Lithuania), and 0.2 µl of 20 µM of forward and reverse primer in a final reaction volume of 20 µl. The PCR reaction was performed as follows: initial denaturation at 95°C for 4 minutes, 35 cycles each comprising of 94°C for 40 seconds, 55°C for 40 seconds, and 72°C for 40 seconds. The PCR product was run on agarose gel in elec-

trophoresis and analysed in a Syngene gel documentation system (Syngene, Canada).

The 391bp product was digested with restriction enzyme *RsaI* (Fermentas, Lithuania) at 37°C for two hours. *RsaI* enzyme cleaved the restriction site of amplified variants. The digested product was resolved on 2.5% agarose gel in electrophoresis and the fragments were visualised in the gel documentation unit. The C allele showed 332 and 59bp bands, and T allele was observed as three fragments of 186, 146, and 59bp. Statistical analysis was performed using SPSS version 20.0. Hardy Weinberg equilibrium,  $\chi^2$  test, and odds ratio were calculated.  $p < 0.05$  was considered statically significant.

## Results

The genotyping for TRAIL gene 1595 C/T polymorphism was done for 363 Breast cancer patients and 193 age- and sex-matched healthy controls. The clinical parameters of the breast cancer patients are shown in Table 1. All of the genotypes for the polymorphism were in Hardy-Weinberg equilibrium. The homozygous CC genotype of major allele was 46.3% in the patients and 49.7% in the controls which was statistically insignificant difference  $p = 0.729$  with OR value 0.8705 (95% CI: 0.6137–1.2348). Heterozygous CT genotypes also showed no gross variation between the two groups, i.e. 41.6% in the breast cancer patients and 43.5% in healthy controls. More importantly, TRAIL 1595 C/T genotype and allele frequencies between larynx cancer patients and controls were not statistically significant ( $p > 0.05$ ) (Personal Communication Ilhan Yaylim). We have previously shown that there was no significant difference in major allele C genotype between prostate cancer patients and controls,  $p$  value  $> 0.05$ . A similar statistically non-significant difference was observed for T allele genotype in prostate cancer patients and control groups. However, surprisingly, heterozygous genotype CT was significantly higher,  $p$  value 0.053 ( $\sim 0.05$ ), in prostate cancer patients as compared to controls [7].

In this study, CC homozygotes were 46.3% in patients and 49.7% in controls,  $p = 0.729$  with OR value 0.8705 (95% CI: 0.6137–1.2348), as shown in Table 2. CT was statistically insignificant  $p = 0.837$  with OR value 0.9242 (95% CI: 0.6494–1.3154). However, the minor allele or risk allele genotype TT had a higher percentage among breast cancer patients (12.1%) than in the control group (6.7%). Since there is a statistically insignificant difference ( $p = 0.212$ , OR

**Table 1.** Clinical parameters of the breast cancer patients. ILCA: Infiltrating Lobular Carcinoma, IDCA: Infiltrating Ductal Carcinoma, ITCA: Infiltrating Tubular Carcinoma. Types of breast cancer and stages of the patients are mentioned

Clinical parameters		Respective values and incidence percentage
Age		Mean ( $\pm$ SD) = 46 $\pm$ 9.2
BMI patients		Mean ( $\pm$ SD) = 30.38 $\pm$ 10.2
Marital status		100%
Type of breast cancer	IDCA	95.2%
	ILCA	2.1%
	ITCA	2.7%
Stage of breast cancer	I	11.6%
	II	58.9%
	III	29.5%
	IV	Nil
Position of breast cancer	Right	47.3%
	Left	51.9%
	Both	0.8%
Pre-/post-menopause breast cancer	Pre-menopause	63%
	Post-menopause	37%

**Table 2.** Genotype distribution of TRAIL gene 1595 C/T polymorphism

Subject Groups	TRAIL gene 1595 C/T SNP genotypes		
	CC	CT	TT
Patients $n = 363$	168 (46.3%)	151 (41.6%)	44 (12.1%)
Controls $n = 193$	96 (49.7%)	84 (43.5%)	13 (6.7%)
$p$ value	0.729	0.837	0.212
OR	0.8705	0.9242	1.9098
95% CI	95% CI: 0.6137–1.2348	95% CI: 0.6494–1.3154	95% CI: 1.0019–3.6406

value 1.9098 with 95% CI: 1.0019–3.6406) of TT genotype between the two groups, the contrastingly higher percentage of TT genotype in breast cancer patients seems to be a risk factor for the disease, as shown in Table 2. Moreover, the frequency of minor allele T was also found to be higher in the patients (0.329) than in controls (0.285), as shown in Table 3.

## Discussion

A substantial amount of information has been added to the TRAIL receptor-mediated signalling. There are diametrically opposed evidences related to the role of SNPs in TRAIL and DR4 as a risk for cancer. In a Turkish population heterozygous TRAIL CT polymorphism in exon 5 was present in 8.3% of tumour stage III–IV, but the findings cannot be extrapolated. Detailed analysis revealed that there were no differences in the TRAIL genotypes distribution and frequencies of the alleles breast cancer patients and controls [5]. In a recently reported study, it was revealed that TRAIL G1525A and C1595T polymorphisms associated considerably with gastric cancer susceptibility in a Chinese Han population [6].

In a previous study, no association between the genotype and clinicopathological characteristics of gastric carcinoma patients was found [8]. On a similar note, another recent report revealed that there was no significant association between DR 4 gene polymorphisms and lung cancer in a Turkish population [9]. However, a previous study indicated a considerable association between DR4 Ala228Glu polymorphism and bladder cancer. Intriguingly, the tumorigenic effect appeared to be more pronounced in individuals exposed to smoking [10]. It has also previously been shown that the frequency of homozygosity for both alleles significantly increased in the primary non-small cell lung cancer samples [11].

*The authors declare no conflict of interest.*

## References

- Twomey JD, Kim SR, Zhao L, Bozza WP, Zhang B. Spatial dynamics of TRAIL death receptors in cancer cells. *Drug Resist Updat* 2015; 19: 13-21.
- Stuckey DW, Shah K. TRAIL on trial: preclinical advances in cancer therapy. *Trends Mol Med* 2013; 19: 685-94.
- Dimberg LY, Anderson CK, Camidge R, Behbakht K, Thorburn A, Ford HL. On the TRAIL to successful cancer therapy? Predicting and counteracting resistance against TRAIL-based therapeutics. *Oncogene* 2013; 32: 1341-50.
- den Hollander MW, Gietema JA, de Jong S, Walenkamp AM, Reyners AK, Oldenhuis CN, de Vries EG. Translating TRAIL-receptor targeting agents to the clinic. *Cancer Lett*. 2013; 332: 194-201.
- Yildiz Y, Yaylim-Eraltan I, Arikan S, Ergen HA, Küçüçük S, Isbir T. Is there any correlation between TNF-related apoptosis-inducing ligand (TRAIL) genetic variants and breast cancer? *Arch Med Sci* 2010; 6: 932-6.
- Wang C, Xu S, Yi F, Wang X, Lei Y, Huang S, Zhou R, Xia B. Tumour necrosis factor-related apoptosis inducing ligand gene polymorphisms are correlated with gastric cancer in central China. *Pharm Res* 2015; 32: 762-8.
- Farooqi AA, Mansoor Q, Fayyaz S, Ismail M. Penetrance and phenotypic effects of C/T polymorphism at 1595 position in exon 5 of

**Table 3.** TRAIL gene 1595 C/T allele frequency between patients and controls

Alleles	Patients (n = 363)	Controls (n = 193)
C allele	0.67	0.715
T allele	0.329	0.285

the TRAIL gene among prostate cancer patients in Pakistani population. *J Exp Ther Oncol* 2015; 11: 1-3.

- Kuraoka K, Matsumura S, Sanada Y, Nakachi K, Imai K, Eguchi H, Matsusaki K, Oue N, Nakayama H, Yasui W. A single nucleotide polymorphism in the extracellular domain of TRAIL receptor DR4 at nucleotide 626 in gastric cancer patients in Japan. *Oncol Rep* 2005; 14: 465-70.
- Taştemir-Korkmaz D, Demirhan O, Kuleci S, Hastürk S. There is no significant association between death receptor 4 (DR4) gene polymorphisms and lung cancer in Turkish population. *Pathol Oncol Res* 2013; 19: 779-84.
- Mittal RD, Srivastava P, Mittal T, Verma A, Jaiswal PK, Singh V, Mandal RK, Mandhani A. Association of death receptor 4, Caspase 3 and 5 gene polymorphism with increased risk to bladder cancer in North Indians. *Eur J Surg Oncol* 2011; 37: 727-33.
- Fisher MJ, Virmani AK, Wu L, Aplenc R, Harper JC, Powell SM, Rebeck TR, Sidransky D, Gazdar AF, El-Deiry WS. Nucleotide substitution in the ectodomain of trail receptor DR4 is associated with lung cancer and head and neck cancer. *Clin Cancer Res* 2001; 7: 1688-97.

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