

# Re-Interpretation of the Single Nucleotide Polymorphism in the Extracellular Domain of Tumor Necrosis Factor Related Apoptosis Inducing Ligand Receptor at Nucleotide 626 among Ovarian Cancer Patients in Pakistani Population

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## ABSTRACT

**Objective:** The study is focused on the SNP in the extracellular domain of TRAIL receptor DR4 at nucleotide 626 in ovarian cancer patients diagnosed in local population in Pakistan.

**Methods:** 16 ovarian cancer patients and 23 control subjects participated in this study. 5ml venous blood was taken from participants with informed consent. DNA was extracted using standard organic methods. PCR-RFLP analysis was done for 626 C→G polymorphism in DR4 gene using site specific primers and restriction enzyme. The results were statistically evaluated in SPSS14.

**Results:** 16 ovarian cancer and 23 control samples were genotyped for C→G polymorphism in DR4 gene. The GG genotype was significantly lower in ovarian cancer patients and controls than the CC and CG in cancer patients and controls.

**Conclusion:** A detailed analysis in future studies and different cancers and cancer subtypes will provide a better understanding of the impact of SNPs in DR4 gene on its transcriptional status.

**Keywords:** Polymorphism, tumor necrosis factor, ovarian cancer

## INTRODUCTION

Overwhelmingly increasing preclinical experimentation has revolutionized the way basic and clinical scientists have perceived ovarian cancer. The advances in analytical tools have, along with the considerable advancement of cancer genomics, generated an increasingly complex understanding of TRAIL mediated signaling in ovarian cancer cells. TRAIL is a proapoptotic protein ligand in the TNF superfamily. Cellular studies indicate that TRAIL induces apoptosis through the TRAIL-R1 (DR4) and TRAIL-R2 (DR5) and it is a well established piece of information that DcR1 nor DcR2 are unable to induce apoptosis either due to absence of a cytoplasmic domain (DcR1) or because of a truncated death domain (DcR2) Farooqi et al, 2011; Farooqi et al, 2011. Increasing sophisticated information provides evidence that C→G single nucleotide polymorphism exists in exon 4 of the DR4 gene and results in substitution of an arginine for threonine.

## MATERIALS AND METHODS

This work was approved by the ethical review committee of the Institute of Biomedical and Genetic

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Engineering, Islamabad. Ovarian cancer patients were ascertained from primary care hospitals of Pakistan. 5ml blood sample of each cancer patients along with age and sex matched controls were collected with informed consent.

The genomic DNA of the cancer patients and controls was extracted using standard organic methods. The RFLP-PCR method was used to amplify the polymorphic region of DR4 exon 4 to study variant alleles. The PCR primers used were forward, 5'-ATCCTCTGGGAAGTCTGTGG-3', and reverse, 5'-GGGGACAGGCAGATGGAC-3'. The PCR was carried out in a final volume of 20µl containing 1X PCR buffer without Mg<sup>2+</sup>, 1mM MgCl<sub>2</sub>, 1.5mM dNTPs, 1U Taq DNA polymerase, 10 µM forward and reverse primers and 40ng genomic DNA. The PCR products were subjected to digestion with DraIII restriction enzyme. The genotyping results were statistically evaluated using SPSS version 14.

## RESULTS

Table 1: RFLP results of DR4 gene in ovarian cancer patients

	GG	CC	CG
DR4 Genotypes	1(6.25%)	3(18.75%)	12(75%)

Table 2: RFLP results of DR4 gene in controls

	GG	CC	CG
DR4 Genotypes	4(17.3%)	10(43.4%)	9(39.1%)

Sixteen ovarian cancer and 23 control samples were genotyped for C→G polymorphism in DR4 gene. The GG genotype was significantly lower in ovarian cancer patients and controls than the CC and CG in cancer patients and controls.

## DISCUSSION

There is a systematic and considerable increase in genetic and laboratory studies that suggest that TRAIL mediated pathway is vital to induce apoptosis in prostate cancer cells. There are confirmatory and contradictory reports that address relationship of carcinogenesis and SNP in the extracellular domain of TRAIL receptor DR4 at nucleotide 626.

It has previously been shown that alterations of the DR4 gene did not lead to clinically relevant ovarian cancer predisposition Horak et al, 2005. On the contrary another contemporary study suggested that DR4 polymorphism is associated with environmental exposure and bladder cancer risk Hazra et al, 2003; Wang et al, 2009. Thr209Arg polymorphism did not show any significance with regard to breast cancer risk Frank et al, 2005. On a similar note, Thr/Arg SNP in the extracellular domain of DR4 was also not found to be connected with the gastric carcinogenesis Kuraoka et al, 2005.

We plan to confirm these data in larger studies and determine the mechanism by which variant genotypes of pro-apoptotic genes influence cancer risk.

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