

RESEARCH COMMUNICATION

No Association Between the Trp53 Codon 72 Polymorphism and Head and Neck Cancer: A Case-Control Study in a South Indian Population

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Abstract

Genetic polymorphisms are important for predispositions to several human cancers. In the tumour suppressor Trp53 gene, a codon 72 polymorphism is frequent in the form of a single nucleotide polymorphism that leads to substitution of an arginine for a proline. In the present study, we analysed the association of Trp53 codon 72 polymorphisms with head and neck cancer through a case-control study approach with PCR-RFLP of DNA from blood of 47 clinically confirmed patients and 52 healthy controls. The Pro (Trp53^{72P}) and Arg (Trp53^{72R}) allele frequencies in the healthy controls were 0.44 and 0.56, and not significantly different from those in the cancer patients at 0.56 and 0.44. The genotype distribution in the controls was 32.7% Arg/Arg, 46.2% Arg/Pro and 21.2% Pro/Pro and in the cancer patients 17.0% Arg/Arg, 53.2% Arg/Pro and 29.8% Pro/Pro. No significant difference in the distribution of genotypes between head and neck cancer patients and healthy controls ($P=0.18$, χ^2 test) was observed. We conclude no association of Trp53 codon 72 polymorphism was observed with head and neck cancer susceptibility.

Keywords: Trp53 codon 72 polymorphism - cancer of head and neck - India

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Introduction

The squamous cell carcinoma of the head and neck may occur in the oral cavity, oropharynx, hypopharynx and larynx, it is among the five most common cancers and accounts for >500,000 new cases every year worldwide (Pisani et al., 1999). In India, it accounts for 23% of all cancers in males and of 6% in females (ICMR, 1992). This excessively higher incidence may be due to use of tobacco in various forms, alcohol consumption, low socioeconomic condition related to poor hygiene, poor diet and rampant viral infections (Franceschi et al., 2000). A causal association between this squamous cell carcinoma of the head and neck and exposure to tobacco and alcohol is well established (Lewin et al., 1998).

Since the 18th century, it has been recognized that, exposure to environmental chemicals plays a major role in the etiology of human cancers. The development of cancer is not only due to endogenous or exogenous carcinogens but also their interactions with genes whose products are involved in the detoxification of carcinogens, repair of DNA damage and control of cell signaling and cell cycle. Genetic predisposition due to polymorphisms and mutations in such low penetrance genes facilitate the development of sporadic cancers upon appropriate exposure (Kotnis et al., 2005). Single nucleotide polymorphisms (SNPs) are minor genetic variations in the genome that play an important role in

promoting susceptibility to disease and in the response to various carcinogens (Hemminki and Shields, 2002). Altered function of Trp53 gene due to SNPs may affect the gene-environment and gene-gene interaction, thereby increasing the risk of the development of sporadic cancers. The importance of the Trp53 tumor suppressor gene in the process of carcinogenesis is well established (Hussain and Harris, 1998).

The Trp53 codon 72 polymorphism is the most common in the general population and arises from a single-base-pair polymorphism where CCC encodes proline or CGC encodes arginine. These two alleles generate three genotypes, Arg/Arg (Trp53^{72R}), Pro/Pro (Trp53^{72P}) and Arg/Pro (Trp53^{72R}/Trp53^{72P}). The two polymorphic variants of wild type Trp53 have been shown to have different biochemical properties like differential binding to components of the transcriptional machinery (Thomas et al., 1999), inducing cell death (Dumont et al., 2003; Sullivan et al., 2004), cell-cycle arrest (Pim and Banks, 2004) and besides these functions Trp53 regulates the various DNA-repair processes (Sengupta and Harris, 2005). We have investigated the frequency of this Trp53 codon 72 polymorphism in cancer of head and neck and their association with risk of this cancer.

Patients and Methods

Blood was obtained from 47 clinically confirmed

patients with cancer of head and neck and from 52 controls. The age of the cancer patients range from 45-70, age of the control subjects range from 42-79. The cancer patients included in the study were from various territory cancer care hospitals at Coimbatore and Erode districts of Tamilnadu State, South India. The control subjects were from general population employed in various professions, living in the same geographical area. The purpose of the study was explained to the participants, all participants gave their written consent prior to inclusion in the study.

Trp53 codon 72 polymorphism determination

DNA was extracted from the peripheral blood cells using standard procedure, involving SDS / Proteinase K digestion followed by ethanol precipitation. The primers were commercially purchased [Ist base, Singapore] and the primer sequences were verified through UCSC In-silico PCR [<http://genome-mirror.duhs.duke.edu/cgi-bin/hgPcr>] to eradicate the possibility of amplification of any non-specific DNA sequences. Purified genomic DNA isolated from the cancer patients and controls was amplified by PCR for exon 4 codon 72 of Trp53 gene. A total of 100-200ng of genomic DNA was amplified through PCR containing 1 μ M of each forward 5' - TTG CCG TCC CAAGCAATG GAT GA - 3' and reverse 5' - TCT GGG AAG GGA CAG AAG ATG AC - 3' primers in a final reaction volume of 50 μ l contained 10 mM of Tris-HCl, 50 mM of KCl, 2 mM MgCl₂, 0.2 mM of each dNTPs (Fermentas, Germany) and 1.25 U of *Taq* DNA polymerase (Fermentas, Germany). The PCR amplification involved an initial denaturation at 94°C for 4 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 1 min and a final cycle of extension at 72°C for 5 min. The polymorphisms were identified by digesting the PCR products (199 bp long) with 5 U of Bsh12361 (Fermentas, Germany) for 4-16 hours and the digestion product was resolved on an 8% polyacrylamide gel electrophoresis for 2:15 hours at 65V/cm in 1X TBE buffer. Detection of bands was performed by the silver staining method. An undigested PCR product (199 bp) was representing the homozygous Trp53^{72P}, two fragments of 113-bp and 86-bp representing homozygous Trp53^{72R}, three fragments of 199-bp, 113-bp and 86-bp representing heterozygous Trp53^{72R}/Trp53^{72P} for codon 72.

Statistical analysis

Chi-square analysis (χ^2) was used to test the association of the genotypes and alleles in cancer patients and controls. The odds ratio (OR) and their confidence intervals (CI) were calculated to estimate the strength of the association of polymorphism genotype alleles in patients and controls (Martin Bland and Douglas, 2000).

Table 1. Genotype Distribution and Allele Frequency of Trp53 Genotypes in Head and Neck Cancer Patients and Controls

Genotypes	Controls n=52 (%)	Breast cancer patients n=47 (%)	P (χ^2)	OR (95% CI) P
Arg/Arg	17 (32.69)	8 (17.02)		Reference
Arg/Pro	24 (46.15)	25 (53.19)	0.18 (3.37)	2.21 (0.80-6.07) 0.11
Pro/Pro	11 (21.15)	14 (29.78)		1.22 (0.46-3.21) 0.68
Pro allele frequency	0.44	0.56	0.47 (0.512)	0.81 (0.46-1.42)

Significance level P<0.01

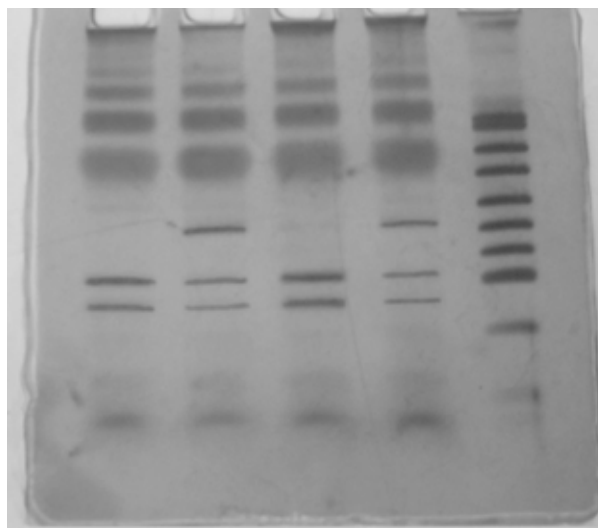


Figure 1. Digestion of the Amplified Fragments with the Bsh12361. From left to right: Lanes 1 and 3: Arg/Arg; 2 and 4: Arg/Pro; 5 pUC 19/MspI Digest marker

Results

The mean age of the control subjects and cancer patients was 56.7 \pm 9.00 and 56.9 \pm 7.12 respectively. The mean height of the controls was 159.4 \pm 9.12, whereas cancer patients have shown 163.6 \pm 7.80. The mean weight of the controls was 53.3 \pm 9.15, cancer patients showed 51.3 \pm 8.92. The number of subjects who used tobacco in controls was 19 and 34 in cancer patients. Alcohol use was seen among 8 controls and 14 cancer patients. No family history of cancer was seen among cancer patients. Types, stages and sites of cancer observed among the patients were, squamous cell carcinoma, primary stage squamous cell carcinoma, buccal mucosa squamous cell carcinoma, adenoid cystic carcinoma, invasive squamous cell carcinoma, infiltrating squamous cell carcinoma, secondary deposit of squamous cell carcinoma, squamous cell carcinoma moderately differentiated, submandibular node cancer supraglottis and posterior tongue cancer.

Smoking was significantly associated with cancer ($\chi^2 = 12.72$, P=0.0003), but not alcohol use ($\chi^2 = 2.96$, P=0.08). The frequency of the genotypes Trp53^{72R}, Trp53^{72R}/Trp53^{72P} and Trp53^{72P} of the controls was 32.7%, 46.2% and 21.2% respectively. Of the cancer patients 17.0%, 53.2% and 29.8% had Trp53^{72R}, Trp53^{72R}/Trp53^{72P} and Trp53^{72P} respectively. The allele frequency of both groups fitted in the Hardy-Weinberg equilibrium with allele frequencies of 0.56 (controls) and 0.44 (cancer) for Trp53^{72R}-coding alleles and 0.44 (controls) and 0.56 (cancer) for Trp53^{72P}-coding alleles. Allele frequency did not differ significantly between the cancer patients and healthy controls ($\chi^2 = 0.512$, P=0.47). Overall, there was no

significant difference in the genotype distribution between controls and cancer patients. ($P=0.18$, $\chi^2=3.37$). Further, combined analysis of Trp53^{72R}/Trp53^{72P} and Trp53^{72P}/Trp53^{72P} genotypes ($P=0.685$, $\chi^2=0.165$, $OR=1.22$, 95% $CI=0.46-3.21$) and Trp53^{72R}/Trp53^{72R} and Trp53^{72R}/Trp53^{72P} genotypes ($\chi^2=2.424$, $P=0.119$, $OR=1.22$, 95% $CI=0.464-3.21$) revealed no significant association of this polymorphism with head and neck cancer (Table 1; Figure 1).

Discussion

It is well-known that the role of tobacco and alcohol in the etio-pathogenesis of head and neck cancer (Brennan et al., 1995; Lewin et al., 1998; Zhang et al., 2000; Khandekar et al., 2006; Freedman et al., 2007). In our study, we found that the tobacco use was significantly associated with the incidence of head and neck cancer ($P=0.0003$, $\chi^2=12.72$), in addition, an increased risk ($OR=4.54$, 95% $CI=1.93$ to 10.65) of tobacco use with head and neck cancer was seen, but an insignificant association was found for alcohol use with head and neck cancer incidence ($P=0.08$, $\chi^2=2.96$, $OR=2.33$, 95% $CI=0.87-6.21$).

Variations in the function of genes responsible for DNA repair mechanisms and cell-cycle control is an attractive mechanism for explaining any inter-individual variation in cancer susceptibility (Matakidou et al., 2003). In the Trp53 gene, a common genetic variant at codon 72 has been extensively studied for its association with cancer risk, but the findings have ranged from conflicting (Suspitsin et al., 2003) to conclusive (Hildesheim et al., 1998; Rosenthal et al., 1998; Soultzis et al., 2002; Gemignani et al., 2004). The polymorphism is balanced, varies with latitude and race, and is maintained at different allelic frequencies across the population (Sjalander et al., 1996). The distribution of allele Trp53^{72P} in different world populations is, Swedish Saamis (0.17), Finns (0.24), Swedes (0.29) and Caucasians (0.21) in which a lower frequency of Trp53^{72P} allele was found (Sjalander et al., 1995; Sjalander et al., 1996). A higher frequency of Trp53^{72P} allele was observed in African-Americans (0.63) (Jin et al., 1995). In Western Europe (France, Sweden, Norway), North America (USA), Central and South America (Mexico, Costa-Rica, Peru) and Japan, the most common allele is Trp53^{72R}, with frequencies ranging from 0.60 to 0.83 (IARC-TP53 Database, 2010). In Asian populations, the distribution of the heterozygous form (Trp53^{72R}/Trp53^{72P}) was more common than the homozygous genotypes and this distribution pattern was different from Caucasian populations which showed higher Trp53^{72R} and lower Trp53^{72P} homozygous genotypes (Shen et al., 2002).

The two polymorphic variants (Trp53^{72R} and Trp53^{72P}) of the wild type Trp53 have been shown to have different biochemical properties such as, (i) the Trp53^{72P} variant was a more active transcriptional activator than the Trp53^{72R} variant (Thomas et al., 1999), (ii) the Trp53^{72R} variant is more efficient in inducing cell death than the Trp53^{72P} variant in some cell types (Dumont et al., 2003), (iii) the Trp53^{73P} variant was shown to induce cell-cycle arrest better than the Trp53^{72R} variant (Pim and Banks,

2004). These data suggested that both the polymorphic variants of Trp53 might have involved for selectively regulating specific cellular functions and the functional differences between the two forms of Trp53 suggest that their expression status may thus influence cancer risk.

The association of Trp53 codon 72 polymorphism with head and neck cancer in different ethnic backgrounds remains uncertain. In our study, we compared the genotype and allele frequencies of the Trp53 codon 72 polymorphism between head and neck cancer patients and healthy controls in South Indian population. We found no significant difference in the distribution of genotypes, and they were similarly represented in cancer patients and healthy controls ($P=0.18$, $\chi^2=3.37$). Even though the distribution of genotypes were not significantly different, an increased frequency of Trp53^{72P} alleles in cancer patients ($P=0.47$, $\chi^2=0.512$) over the controls was observed.

The genotype frequencies observed in our study is consistent with the previous observations in oral cancer patients of Indian population (Tandle et al., 2001; Mitra et al., 2005). However, the presence of higher number of the Trp53^{72P}/Trp53^{72P} genotype and the Trp53^{72P} allele in the cancer patients made it reasonable to suspect the susceptibility of Trp53^{72P} allele to head and neck cancer. Hiyama et al., (2008) reviewed 20 epidemiological studies and suggested that individuals with Trp53^{73P} genotype showed a higher risk for head and neck cancer than individuals with Trp53^{72R} genotype in 15 of 20 studies. Two studies showed a significantly higher risk for head and neck cancer in Trp53^{72P} homozygotes than in Trp53^{72R} homozygotes.

By contrast, recent evidences (Siddique and Sabapathy, 2006) confirmed that, Trp53^{72P} allele reduces genomic instability better than the Trp53^{72R} allele in many respects. First, the Trp53^{72P} variant transcriptionally activates Trp53-dependent target genes involved in DNA-repair better than the Trp53^{72R} form. Consequently, cells expressing Trp53^{72P} form are able to repair DNA-damage much more efficiently than the Trp53^{72R}-expressing cells (preferentially by inducing Trp53 dependent DNA-repair target gene promoters (Trp53R2)) in the Trp53 dependent DNA-repair process, which may influence cancer risk. Second, the efficiency of the unscheduled DNA synthesis, that is DNA synthesis owing to repair replication in non-S-phase cells, revealed that, Trp53^{72P}-expressing cells consistently and reproducibly incorporated significantly more thymidine (³H) (at both UV doses 25 and 50 J/m² at 32°C) compared to Trp53^{72R}-expressing cells, which indicate that NER occurred much more efficiently in Trp53^{72P}-expressing cells compared to Trp53^{72R}-expressing cells. Third, cyclobutane pyrimidine dimers (CPDs) are the predominant product of photo damage in DNA after exposure of cells to UV light. CPDs are recognized and removed by NER, and defects in this process often lead to predisposition to cancer (Hanawalt et al., 2003). Trp53^{72P}-expressing cells remove CPDs more rapidly than the Trp53^{72R}-expressing cells. Fourth, Trp53^{72R}-expressing cells are less able to remove the micronuclei (acentric chromatids or chromosome fragments) which are induced by radiation or other DNA damaging agent, suggesting

that Trp53^{72R} might be less potent in reducing genomic instability, and perhaps cancer predisposition. In this context, it can be assumed that the Trp53^{72P} allele might not be a predisposing allele to cancer. In our study, the Trp53^{72P} allele showed a higher prevalence in the cancer group (0.56), it might be due to the natural selection. A few investigations, examined the Trp53 codon 72 polymorphism in Indian population and reported that the Trp53^{72P} allele frequency in different parts of India vary from 0.45-0.56 (Tandle et al., 2001; Katiyar et al., 2003; Mitra et al., 2003). Hence, we report that the presence of higher number of Trp53^{72P} allele (0.56) in cancer patients is not associated with susceptibility to cancer of the head and neck; it might be due to the natural selection.

Siddique et al., (2005) indicated that healthy Asian (Chinese) heterozygote individuals (Trp53^{72R}/Trp53^{72P}) tend to preferentially express the Trp53^{72P} allele at the RNA level. By contrast, Trp53^{72R} allele was preferentially expressed in most heterozygote breast cancer patients (73.4%), suggesting that the Trp53^{72R} allele is selectively activated and the Trp53^{72P} allele is silenced in heterozygote cancers. It indicated that the Trp53^{72R} was associated with cancer predisposition (Sjalander et al., 1996; Bergamaschin et al., 2003), although the Trp53^{72R} form might be capable of inducing apoptosis better than the Trp53^{72P} form (this apoptotic effect might be of cell type specific), it might not be efficient in preventing cancer formation. Taken together, suggest that the Trp53^{72P} polymorph of Trp53 has a selective advantage over Trp53^{72R} and the Trp53^{72P} form might be more efficient in other Trp53-related functions in inhibiting malignancy.

In conclusion, from the genotypic analysis of our study, an equal distribution of the genotypes and no over-representation of either Trp53^{72P} or Trp53^{72R} genotypes in the head and neck cancer patients as compared to the normal healthy controls were seen. Hence, we support the hypothesis that either Trp53^{72P} or Trp53^{72R} variant is not associated with predisposition to cancer of the head and neck in South Indian population. To add more strength to the conclusion of our study, several recent investigations found no association of Trp53 codon 72 polymorphic variants with head and neck cancer (Hamel et al., 2000; McWilliams et al., 2000; Summersgill et al., 2000; Shen et al., 2002; Kietthubthew et al., 2003), including one study from Indian population (Tandle et al., 2001). Even though, our study found higher frequency of Trp53^{72P} allele in cancer patients, it is not associated with head and neck cancer. However, for a more definitive and appropriate conclusion, the study should be performed with larger sample size, in the mean time, the expression status of the Trp53 polymorphs should be determined.

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