Study of p53 codon 72 polymorphism in various ethnic groups of North India

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Various studies have been done to investigate the status of p53 at codon 72 for arginine (Arg) and proline (Pro) alleles in different ethnic populations and also the association of this codon 72 polymorphism with various tumours, including human papillomavirus (HPV)-related uterine cervical carcinoma. The Arg allele is susceptible to degradation by the E6 protein of HPV. However, no literature is available regarding allele frequencies from the different ethnic groups in India. We investigated the status of p53 at codon 72 for Arg/Pro allele polymorphism in different ethnic groups (Ladakhi, Kashmiri, Punjabi, Rajasthani Gujjar and Rajasthani Rajput) in North India. It is known that the incidence of HPV-related cervical carcinoma in the Kashmiri population is very low. The *Bst*U1 polymorphism at codon 72 was studied by PCR–RFLP. The only group, which showed significantly increased frequency of the Arg/Arg allele, was from Ladakh. This could be because of their Oriental descent and relative geographical isolation. There is no literature or cancer registry data on the status of cervical carcinoma in the Ladakhi population. The low prevalence of HPV-related cervical cancer in the Kashmiri population did not seem to affect or be related to the frequency of the presumed susceptibility allele (Arg allele).

THE tumour suppressor gene *p53* is crucial for host defence against genomic mutations that might give rise to many types of tumours. Somatic mutations of *p53* that alter the DNA binding and transactivation function of the p53 protein are known to be involved in diverse types of cancers. The wild type of p53 protein exists as two variants: Arg and Pro at codon 72. This Arg/Pro polymorphism at codon 72 of the wild type *p53* gene is shown to be associated with various cancers. Birgander *et al.*[1] have demonstrated an increase in the frequency of Pro/Pro genotype in nasopharyngeal carcinoma patients in South China. Kawajiri *et al.*[2] showed that genotype Pro/Pro has a 1.7-fold higher risk of smoking-induced lung cancer. Likewise Wang *et al.*[3] demonstrated that Pro allele of p53 increased the risk of lung cancer among female Taiwanese and suggested that codon 72 polymorphism may play a role in cancer susceptibility and prognosis in specific classes of lung cancer patients. To-Figueras *et al.*[4] suggested that Pro allele of p53 germline polymorphism may increase the risk of the GSTM1 (Glutathione-S-Transferase M1) null genotype among smokers, which has been reported to be a risk factor for developing lung cancer. A similar study by Birgander *et al.*[5] in Swedish lung cancer patients showed no significant association between lung cancer and Pro/Pro genotype. However, some association was observed when the Pro/Pro alleles were associated with the 16 bp 1 allele of intron 3 of p53. Storey *et al.*[6] have demonstrated that the p53 with Arg at codon 72 is more susceptible to degradation by the E6 protein of Human papilloma virus (HPV) and the allele encoding arginine at the codon 72 of the *p53* gene represents a significant risk factor in the development of HPV-related cervical carcinoma. The E6 oncoprotein from HPV binds to the p53 protein and directs the degradation of the cellular p53 protein through the ubiquitin pathway. In a study from India, Saranath *et al.*[7] have shown that there is 2.5-fold increased risk of HPV-related cervical carcinoma attributable to homozygous p53 Arg. However, several other related studies have shown no significant association of p53 arginine at codon 72 with an increased risk of HPV associated cervical carcinoma. Helland *et al.*[8], Josefsson *et al.*[9] and Hildesheim *et al.*[10] in their study on the frequency of Arg allele in HPV-related cervical carcinoma patients found no significant difference from that of control. Similarly, Rosenthal *et al.*[11] and Sonoda *et al.*[12] demonstrated no increased risk of cervical cancer in individuals homozygous for the Arg variant of codon 72 of the *p53* gene.

There are various studies on p53 codon 72 polymorphism in different ethnic groups[13–15] which showed a wide range of variation. Weston *et al.*[16] in a study of codon 72 polymorphism in American lung cancer patients, found a significant difference between American blacks and whites with respect to the frequencies of Pro and Arg allele. Beckman *et al.*[14] have revealed striking ethnic differences and correlation between the arginine allele frequency and latitude suggesting that the polymorphism may be maintained by natural selection related to climatic factors. However, no literature is available regarding allele frequencies from the different ethnic groups in India. Observations by different studies on p53 codon 72 polymorphism among ethnic groups prompted us to look at the status of codon 72 polymorphism in the Indian population.

We investigated the status of p53 at codon 72 for Arg/Pro allele polymorphism in different ethnic groups (Ladakhi, Kashmiri, Punjabi, Rajasthani Gujjar and Rajasthani Rajput) in North India by PCR amplification of p53 exon 4 and digestion with *Bst*U1 restriction enzyme (PCR–RFLP). HPV-related cervical cancer is

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Table 1. Frequency of p53 codon 72 polymorphism in different ethnic groups of North India

Ethnic group	Total	p53 exon 4 genotype			Arg allele frequency	Fischer exact *P* value of p53 alleles (Arg/Pro) of Kashmiri population versus others	Fischer exact *P* value of p53 alleles (Arg/Pro) of Ladakhi population versus others
		Arg/Arg	Arg/Pro	Pro/Pro			
Ladakhi	36	20	12	4	0.722	0.003	
Kashmiri	53	12	29	12	0.5		0.003
Punjabi	34	4	21	9	0.426	0.262	0.00013
Rajasthani Rajput	39	5	25	9	0.449	0.254	0.00016
Rajasthani Gujjar	39	6	24	9	0.461	0.436	0.00053

Figure 1. Gel electrophoresis of PCR product after digestion with *Bst*U1. Lane M, 100 bp ladder; lane 1, heterozyote (Arg/Pro); lane 2, homozygote (Arg/Arg), and lane 3, homozygote (Pro/Pro).

far less frequent in the Kashmiri population[17] compared to the rest of India and the Kashmiris were taken to be representative of a population with a low prevalence of HPV infection. The Ladakhi population is geographically isolated from the rest of the country and, unlike the rest of the groups studied, is of Oriental (Mongolian) descent. The two caste groups from Rajasthan, Rajput and Gujjar though from the same geographical location generally, do not intermarry.

Finger-prick blood samples from unrelated random populations of both sexes of all the ethnic groups were collected on Whatman filter paper. DNA extraction was carried out using the standard phenol–chloroform method. The detection of p53 codon 72 polymorphism was carried out using PCR–RFLP technique. A 309 bp fragment from exon 4 of p53 containing codon 72 *Bst*U1 polymorphism site was amplified using the following exon 4 primers:

Forward primer 5′TTC ACC CAT CTA CAG TCC 3′
Reverse primer 5′CTC AGG GCA ACT GAC CGT 3′

Amplification was carried out as described by Chattopadhyay *et al.*[18]. The amplified fragments were purified and digested with *Bst*U1 restriction enzyme (New England Biolab, USA) at 60°C overnight. After digestion, the fragments were electrophoresed on 2% agarose gel and visualized by UV light after ethidium bromide staining. The Pro allele was 309 bp, while the Arg allele was restricted into two fragments of 175 and 134 bp.

A representative PCR–RFLP pattern is depicted in Figure 1. The results on genotype distribution are presented in Table 1. There was no significant difference in the distribution of Arg/Pro allele in male and female populations of the ethnic groups studied. The frequency of Arg/Arg allele in Ladakhi samples was significantly different from other North Indian ethnic groups. Fischer exact *P* value for Arg/Arg homozygotes in the Ladakhi sample was statistically significant compared to other populations ($P = 0.0014$ with Punjabi samples, $P = 0.0025$ with Rajasthani Gujjar, $P = 0.0009$ with Rajasthani Rajput samples, and $P = 0.012$ with Kashmiri samples). Though the sample size of each group is small, the interpretations are statistically valid. Except for the Ladakhi population, the frequencies of homozygotes for the Arg allele in all the ethnic groups (Kashmiri, Punjabi, Rajasthani Gujjar and Rajasthani Rajput) were not statistically different from each other (Table 1). We noted similar allelic frequency in the Kashmiri population as compared to the other populations in the North Indian plains, in spite of very low incidence of HPV-related uterine cervical carcinoma in the Kashmiri population[17]. Therefore it can be concluded that a population with a low prevalence of HPV-induced cervical cancer does not show any change in the frequency of the Arg allele.

There are various factors (e.g. early marriage, low socio-economic status, cigarette smoking, immune-suppressed individuals, etc.) known to be involved in the development of cervical cancer[19]. Wahi *et al.*[20] have also found low incidence of HPV-related cervical carcinoma in Muslims in Agra. Male circumcision is probably responsible for the low prevalence of cervical cancer in Muslim females.

There is no literature or cancer registry data on the incidence of HPV-related cervical carcinoma, specifically in the Ladakhi population. The significant difference in prevalence of Arg allele at the codon 72 in the same population could be because of racial differences and geographical isolation.

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Real time wheat yield assessment using technology trend and crop simulation model with minimal data set

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The issue of real time assessment of the direction and quantum of variability in wheat yields is addressed. A simple technology trend model in conjunction with crop simulation model (CERES-Wheat in DSSAT environment) was used for early wheat yield prediction at six locations representing the six major wheat-growing states, which contribute about 93% of national wheat production. A three-step approach, viz. (a) prediction of technological trend-based yields, (b) quantification of weather-induced yield variability using Crop Simulation Model (CSM), and (c) final yield prediction combining the previous two steps (a) and (b), was applied. A simulation model when run on a common set of soil properties, genetic coefficients and agronomic practices, is supposed to capture inter-annual yield variability due to year-to-year varying weather conditions. Deviation in observed wheat yield from its technology trend and deviation in simulated wheat yield from its trend/average showed positive relationship ($r = 0.57$, $P > 0.05$). An overall RMSE of 0.158 t ha^{-1} (5.619%) with R^2 0.97 was found against mean wheat yield of 2.815 t ha^{-1}. Real time weather data up to February and normal onward were used, for early wheat yield assessment at six locations. The study has significance in issuing an early 'national wheat' production forecast using in-season weather data up to February and normal weather data for the rest of the period.

FOODGRAIN production in India crossed 200 Mt mark in year 1999–2000, in comparison to 50.82 Mt in 1950–51. In spite of technological advancement in agriculture, large year-to-year variations in production continue, which is related to fluctuations in monsoon at a gross level[1]. Wheat, the second most important foodgrain crop, contributes about 37% to the total foodgrain production in India. Punjab, Haryana, Uttar Pradesh, Madhya Pradesh and Bihar contribute about 93% of wheat production in India. Wheat production fluctuated between a high of 75.6 Mt in 1999–2000 and 62 Mt in 1995–96 in India, while state-level fluctuations in wheat production ranged from 12.52 to 14.5 Mt (1995–96, 1998–99), 6.56 to 8.46 Mt (1995–96, 1999–00), 5.25 to 6.88 Mt (1995-96, 1998–99), 21.63 to 24.05 Mt (1995–

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