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SHORT - COMMUNICATION

p53 Codon 72 Polymorphism and Risk of Cervical Cancer

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ABSTRACT

Storey et al. (1998) implicated the proline/argine polymorphism of the codon 72 of the tumor-suppressor gene p53 in the development of cervical cancer (CC) with the observation that the p53 protein is more efficiently inactivated by the E6 oncoprotein of human papillomavirus in p53 arginine as compared with its proline isoform. These authors further noted that in the United Kingdom, individuals homozygous for the arginine allele were several times more susceptible to HPV-associated tumorigenesis that proline/arginine heterozygotes. Subsequent studies in different countries failed to unanimously confirm this association. Motivated by the high incidence of CC in Chile, we undertook a case control study obtaining the following frequencies for genotypes PP, AP and AA in 60 ICC cases and 53 carefully selected controls: 0.067, 0.250, 0.683 and 0.075, 0.453, 0.472 respectively. A significant difference ($X^2 = 3.19$ $p < 0.02$) and an odds ratio of 2.62 supported Storey et al (1998)'s results. In addition, rejecting previous hypotheses about the world distribution of the p53 codon 72 polymorphism, we conclude that this distribution most likely represents ancient human dispersal routes. Several methodological and biological explanations for the results obtained in previous negative association studies are briefly discussed.

INTRODUCTION

Cervical cancer (CC) together with other anogenital tract cancers account for almost 12% of all carcinomas in women, representing the second most frequent group of gynecological malignancies in the world (Ferlay *et al.* 2001). Epidemiological and virological studies have shown that high risk human papillomavirus (HPV) infections (particularly those caused by genotypes 16 and 18) are one of the most important factors identified and probably the main cause for cervical cancer (Muñoz *et al.* 2000; zur Hausen 2002). However the fact that not all women infected with these viruses develop cancer indicates that HPV is not a sufficient cause for the malignant transformation.

In fact, the proline/argine polymorphism of the codon 72 of the tumor-suppressor gene p53 has been implicated in the development of cervical cancer (CC) in a study by Storey *et al.* (1998) with the observation that the p53 protein is more efficiently inactivated by the E6 oncoprotein of human papillomavirus in p53 arginine as compared with its proline isoform. Consequently, these authors noted that, in the United Kingdom, individuals homozygous for the arginine allele were about seven times more susceptible to HPV-associated tumorigenesis than proline/arginine heterozygotes. Subsequent studies in several countries (Rosenthal *et al.*, 1998; Lanham *et al.*, 1998; Minaguchi *et al.*, 1998; Hayes *et al.*, 1998; Helland *et al.*, 1998; Josefsson *et al.*, 1998; Hildesheim *et*

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al., 1998; Ngan *et al.*, 1999; Tachezy *et al.*, 1999; Dybikowska *et al.*, 2000; Klug *et al.*, 2001; Suárez-Rincon *et al.*, 2002) failed to confirm this association, leading to the suspicion that in the process of HPV-associated tumorigenesis due to genotype differences at the codon 72 of p53, other factors in addition to these isoforms of p53 are important as risk factors for the development of cervical cancer.

Motivated by the recent publication of three studies (Andersson *et al.*, 2001; Pegoraro *et al.*, 2002; Saranath *et al.*, 2002) that confirmed original Storey *et al.* (1998) results, as well as by the fact that cervical cancer has a high incidence in Chile, and finally by the idea that the investigation of the differential effects of the p53 polymorphism in different populations could further contribute to unraveling the complexities of its function, we undertook a case control study of the p53 polymorphism and cervical carcinoma in the population of Santiago. To our knowledge, there is no data on this subject in the Chilean population.

SUBJECTS AND METHODS

The Chilean mixed population of Santiago has an average Amerindian admixture of 40% (Rothhammer, 1987). Amerindian admixture and socioeconomic level are associated in Chile, a complication that must be taken into account in case control studies in order to avoid spurious results due to population stratification. Sixty patients belonging to the Chilean mixed populations, the same age group, and socioeconomic level with newly-diagnosed and histologically-confirmed invasive cervical cancer were identified by the Centro de Oncología Preventiva. Fifty-three matched controls were recruited from women without cervical cancer or diseases associated with known risk factors for this malignancy. An informed consent was obtained from all subjects.

Cervical cells and blood samples were collected from cases and controls. Cervical specimens were processed for Papanicolaou stain and cytological diagnosis. DNA was extracted from peripheral leukocytes. First, white blood cells were separated from red blood cells by incubation in lysis buffer, re-suspended in TNE buffer containing 0.5% SDS and 250 ug/L prteinaseK (Boehringer Mannheim) and incubated at 37ºC overnight. Protein was precipitated with NaCl and DNA extracted with phenol/ chloroform and precipitated with cold ethanol. All DNA samples were dissolved in water and stored at -20 °C. The quality of DNA was monitored by OD 260/280 measures.

Genotyping of p53 at codon 72 was carried out with an allele specific PCR amplification procedure. Two pairs of primers with different 3´ terminal bases were used to amplify p53 sequences separately: p53-p1 (GC-CAGAGGCTGCTCCCC) and p53-p2 (CGTGCAAGTCA-CAGACTT) for proline (178 base pairs [bp]) and p53-a1 (TCCCCCTTGCCGTCCCAA) and p53-a2 (CTGGTG-CAGGGGCCACG) for arginine (142 bp). The p53 arginine and proline variants were separately amplified from each sample with primers as described by Storey *et al.* (1998). The PCR reaction was performed in a total volume of 25 uL. The PCR mixture contained 10 mM Tris-Cl, pH 8.3; 50 mM KCl; 1.5 mM MgCl; 0.01% gelatin; 200 pmol of each primer; 0.5U Taq DNA polymerase; and 100 ng DNA. Amplification was performed with denaturation at 95 °C for 5 minutes followed by 30 cycles of 1 minute at 95°C, 1.5 minutes at 72 °C, and a final extension for 15 minutes at 72 °C. The resulting PCR products of each sample were then mixed electrophoresed on 2% agarose gels and visualized under UV with ethidium bromide staining.

RESULTS

DNA amplification using the specific set of primers showed that DNA from all specimens was suitable for PCR analysis. p53 homozygotes for Arg and p53 homozygotes for Pro were observed as specific bands with expected sizes of 141bp and 177bp repectively.

The distribution of p53 codon 72 genotypes in healthy women were as follows: 4 samples were proline

homozygotes (Pro/Pro 7.5%), 25 arginine homozygotes (Arg/Arg 47.2%), and 24 heterozygotes (Arg/Pro 45.3%. Among the 60 cervical cancer patients, 4 were Pro/Pro (6.7%), 41 Arg/Arg (68.3%), and 15 were Arg/Pro (25%).

A statistically significant odds ratio of 2.62 was obtained in our study. Consequently, homozigote women for arginine in Santiago have a 2.6-fold increased risk for cervical cancer.

p53 genotype and allele frequencies, in cancer patient samples from different countries are exhibited as Table I. Significant differences between cases and controls were observed in the UK ($X^2 = 11.19$ $p < 0.001$) (Storey *et al.*, 1998), in Sweden ($X^2 = 1680$ $p< 0.0001$) (Andersson *et al.* (2001), in South Africa ($X^2 = 57.99$ $p < 0.0001$) (Pegoraro *et al.*, 2002), in India ($X^2 = 6.34$ $p < 0.01$) (Saranath *et al.*, 2002), and as already mentioned, in Chile ($X^2 = 5.19$ $p < 0.02$).

The combined results of all studies reveal however that gene frequencies for the A1 (Pro) and A2 (Arg) alleles, as well as genotype frequencies do not differ significantly for cases and controls, leading to a non-significant Mantel-Haenzel odds ratio of 1.10 (95% CI 0.51-2.72).

DISCUSSION

Methodological shortcomings such as the lack of representativeness or genetic heterogeneity of some samples, improper selection of control groups, and technical problems related to genotyping may be partially responsible for some inconclusive results observed in the reviewed studies (Table I). It is worth noting that populations exhibiting lower A2 (Arg) frequencies for numerical reasons manifest on the average higher odds ratios, ($r = -0.52$ $p < 0.01$). Similarly, a significantly lower mean arginine homozygote frequency (0.235) was found in populations in which significant associations between the p53 codon 72 polymorphims and cervical cancer were detected, with respect to populations in which these associations were not found to be significant (0.522). Therefore some negative results may be a consequence of high A2 (Arg) frequencies in control populations and not necessarily a lack of association.

Beckman *et al.* (1994) claimed that the frequency of the A1 (Pro) allele showed a north-south cline and that there was a significant correlation between A2 (Arg)

TABLE I

p53 polymorphism phenotypic and allele frequencies in cervical cancer patients

Country	N	A_1(P)	A_2 (A)	PP	AP	AA	Author
Norway	77	0.279	0.721	0.130	0.300	0.570	Helland *et al.*, 1998
Sweden	488	0.280	0.720	0.080	0.390	0.520	Josefsson *et al.*, 1998
Sweden	111	0.145	0.855	0.000	0.290	0.710	Andersson *et al.*, 2001
UK	50	0.260	0.740	0.060	0.400	0.540	Rosenthal *et al.*, 1998
UK	38	0.276	0.724	0.079	0.395	0.526	Lanham *et al.*, 1998
UK	30	0.150	0.850	0.066	0.166	0.766	Storey *et al.*, 1998
Holland	25	0.180	0.820	0.040	0.280	0.680	Hayes *et al.*, 1998
Poland	44	0.160	0.840	0.023	0.273	0.704	Dybikowska *et al.*, 2000
Czech Republic	71	0.282	0.718	0.085	0.394	0.521	Tachezy *et al.*, 1999
Japan	103	0.354	0.646	0.100	0.500	0.400	Minaguchi *et al.*, 1998
China	102	0.451	0.549	0.206	0.490	0.304	Ngan *et al.*, 1999
India	134	0.460	0.540	0.180	0.560	0.260	Saranath *et al.*, 2002
South Africa	281	0.417	0.583	0.174	0.660	0.340	Pegoraro *et al.*, 2002
USA	187	0.321	0.679	0.097	0.449	0.454	Hildesheim *et al.*, 1998
Costa Rica	49	0.367	0.633	0.143	0.449	0.408	Hildesheim *et al.*, 1998
Perú	119	0.310	0.690	0.118	0.378	0.504	Klug *et al.*, 2001
Chile	60	0.192	0.808	0.067	0.250	0.683	This study

and latitude. Moreover, they suggested that the codon-72 polymorphism was balanced and maintained by natural selection. Using Beckman *et al.*, (1994) data and the information that has become available since the publication of their report (Table II) we computed the correlation between the A2 (Arg) frequencies and latitude and obtained $r = 0.59$ ($p<0.01$), confirming the Beckman *et al.* (1994) results. Nevertheless, if the genotypes in agglomerated control populations (N = 3155) are tested for Hardy-Weinberg proportions, no significant deviations are found, detracting from the argument that the p53 codon 72 polymorphism is balanced.

Furthermore, although the p53 codon 72 polymorphism has been associated with an increased susceptibility to malignant conversion for various neoplastic diseases including lung cancer, (Wang *et al.*, 1999), hepatocellular carcinoma, (Yu *et al.*, 1999), ovarian and endometrical cancer, (Peller *et al.*, 1999), urologic cancer, (Wu *et al.*, 1995) and upper aero-digestive tract cancer, it is unlikely that these associations play a role in the maintenance of the polymorphism, since the onset of most cancers occurs after reproduction. The hypothesis of Beckman *et al.* (1994) that the world distribution of codon 72 polymorphism reflect an adaptation to ultra violet radiation is contradicted by the high A2 (Arg) frequencies found in Central and South America. We suggest that the world distribution of the p53 codon 72 polymorphism reflects ancient human migration routes, more specifically the dispersal of modern *Homo sapiens* out of Africa some 100,000-150,000 years ago.

With respect to the relationship between p53 genotypes and cervical carcinoma, the association is undoubtedly more complex than initially postulated and warrants additional studies in patients with more clearly defined tumor types (e.g., squamous cell versus adenocarcinoma), differences with respect to invasiveness of the cancer and the presence of different strains of HPVs. Studies in ethnic populations with different genetic backgrounds but similar ecogeographic areas may provide interesting new insights on this polymorphism in the future.

TABLE II

p53 polymorphism in different populations

Country	N	A_1(P)	A_2 (A)	PP	AP	AA	Author
Finland	171	0.243	0.757	0.053	0.380	0.567	Beckman *et al.*, 1994
Norway	225	0.258	0.742	0.060	0.400	0.540	Helland *et al.*, 1998
Sweden	626	0.307	0.693	0.111	0.390	0.500	Josefsson *et al.*, 1998
Sweden	188	0.310	0.690	0.090	0.440	0.470	Andersson *et al.*, 2001
UK	246	0.222	0.778	0.069	0.305	0.626	Rosenthal *et al.*, 1998
UK	250	0.246	0.754	0.060	0.372	0.568	Lanham *et al.*, 1998
UK	41	0.342	0.658	0.049	0.585	0.366	Storey *et al.*, 1998
Holland	158	0.240	0.759	0.060	0.370	0.570	Hayes *et al.*, 1998
Poland	52	0.153	0.847	0.038	0.231	0.731	Dybikowska *et al.*, 2000
Czech Republic	172	0.288	0.712	0.110	0.355	0.535	Tachezy *et al.*, 1999
Spain	90	0.320	0.680	0.102	0.435	0.463	Beckman *et al.*, 1994
Japan	110	0.404	0.595	0.180	0.460	0.360	Minaguchi *et al.*,1998
China	68	0.449	0.551	0.120	0.660	0.220	Ngan *et al.*, 1999
India	131	0.505	0.495	0.150	0.710	0.140	Saranath *et al.*, 2002
India	71	0.542	0.458	0.296	0.493	0.211	Beckman *et al.*, 1994
Nigeria	122	0.631	0.369	0.385	0.492	0.123	Beckman *et al.*, 1994
South Africa	340	0.690	0.310	0.470	0.440	0.090	Pegoraro *et al.*, 2002
USA	245	0.282	0.718	0.082	0.400	0.518	Hildesheim *et al.*, 1998
Costa Rica	123	0.309	0.691	0.065	0.488	0.447	Hildesheim *et al.*, 1998
Perú	127	0.303	0.697	0.126	0.354	0.520	Klug *et al.*, 2001
Chile	53	0.302	0.698	0.075	0.453	0.472	This study
Chilean Amerinds	25	0.240	0.760	0.080	0.320	0.600	This study

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