Is the p53 codon 72 polymorphism a key biomarker for cervical cancer development? A meta-analysis review within European populations

HUGO SOUSA[1,2], ALEXANDRA M. SANTOS[1], DANIELA PINTO[1] and RUI MEDEIROS[1,2]

[1]Molecular Oncology Group and Virology Department, Portuguese Institute of Oncology of Porto;
[2]ICBAS, Abel Salazar Institute for the Biomedical Sciences, Porto, Portugal

Received February 27, 2007; Accepted May 21, 2007

Abstract. Human papillomavirus (HPV) is the necessary cause for cervical cancer development, and the interaction of HPV-E6 with p53 is known as the most important event in HPV-associated carcinogenesis. *In vitro* studies have suggested that HPV-E6 interacts more efficiently with the arginine (Arg) p53 variant at position 72 as it appears to be more susceptible to degradation through the ubiquitin proteasome pathway. However, few reports have corroborated this data, and the role of the p53 codon 72 polymorphism in the development of cervical cancer requires further elucidation. We performed a meta-analysis review of all studies published within European populations to summarize the overall risk of this polymorphism considering the influence of the geographical/ethnic location as an important factor in defining a genetic profile and the susceptibility for cervical cancer development. Our analysis revealed that the p53 Arg/Arg genotype does not seem to represent a risk marker for the development of cervical lesions in the majority of the European countries analysed. However, in countries with low incidence rates of cervical cancer, this polymorphism might represent a significant genetic marker.

Introduction

Cervical cancer is the second most common cancer in women worldwide, with 493,000 new cases and 273,000 deaths estimated in 2002 (1). In Europe, data from 2002, revealed a median age standardized rate (ASR) incidence of 12.9/100,000, varying from 4.3 in Finland to 27.4 in Serbia and Montenegro (Fig. 1). Even Western European countries, considered to be developed countries, have a median ASR incidence of 8.7/100,000.

The major cause accepted as necessary for the development of cervical cancer is the infection by certain types of a sexually transmitted agent, *human papillomavirus* (HPV), such as HPV-16 or -18 (group I carcinogens) (2-4). Nevertheless, HPV is not sufficient for cervical carcinogenesis, and several co-factors have been associated with tumoral progression, such as age at first sexual intercourse, number of sexual partners, parity (>3 children), tobacco and alcohol consumption, co-infection with other sexually transmitted agents, as well as immunologic and host genetic factors (2,5-8).

The essential mechanism of HPV carcinogenesis begins with the integration of its DNA into the host cell DNA, leading to constitutive expression of HPV proteins. Only high-risk HPV genotypes are capable of integration. However, when it occurs, it happens to be an incomplete integration, and the absence of the viral gene E2 introduces a phenotype modification on the host cell (9). The HPV viral gene E2 is responsible for the expression of HPV-E2 protein, which controls the expression of both HPV-E6 and HPV-E7 viral proteins that have the ability to promote genetic instability in cells leading to cell cycle regulation and malignant progression (10). HPV-E6 is a viral oncoprotein that cooperates with the cellular ubiquitin protein ligase E6-AP to bind p53 leading to its degradation through ubiquitin-dependent proteolysis (Fig. 2) (11,12).

The tumour suppressor protein p53 has three major functions: cell cycle arrest, DNA repair activation and regulation of apoptosis. p53 acts as 'the guardian of the genome' and when cells have vast or irreparable DNA damage, it activates cell cycle arrest and induces DNA repair or apoptosis, preventing the cells' progression with genetic mutations (13). The functional inactivation of p53 by HPV-E6 seems to be equivalent to any mutation on *TP53* that could affect p53 normal functions (14-17). This fact supports the importance of HPV in cell cycle regulation and malignant progression.

Genetic polymorphisms have been described as having an important role in cancer development (18-22). Matlashewsky *et al* described a polymorphism on exon 4 of

Correspondence to: Dr Hugo Sousa, Grupo de Oncologia Molecular-CI, Laboratórios 4º Piso Instituto Português de Oncologia do Porto FG, EPE, Rua Dr António Bernardino Almeida, 4200-072 Porto, Portugal
E-mail: hugomls@gmail.com
patpriv@ipoporto.min-saude.pt

Key words: cervical cancer, human papillomavirus, HPV-E6, p53 codon 72 polymorphism, geographical location

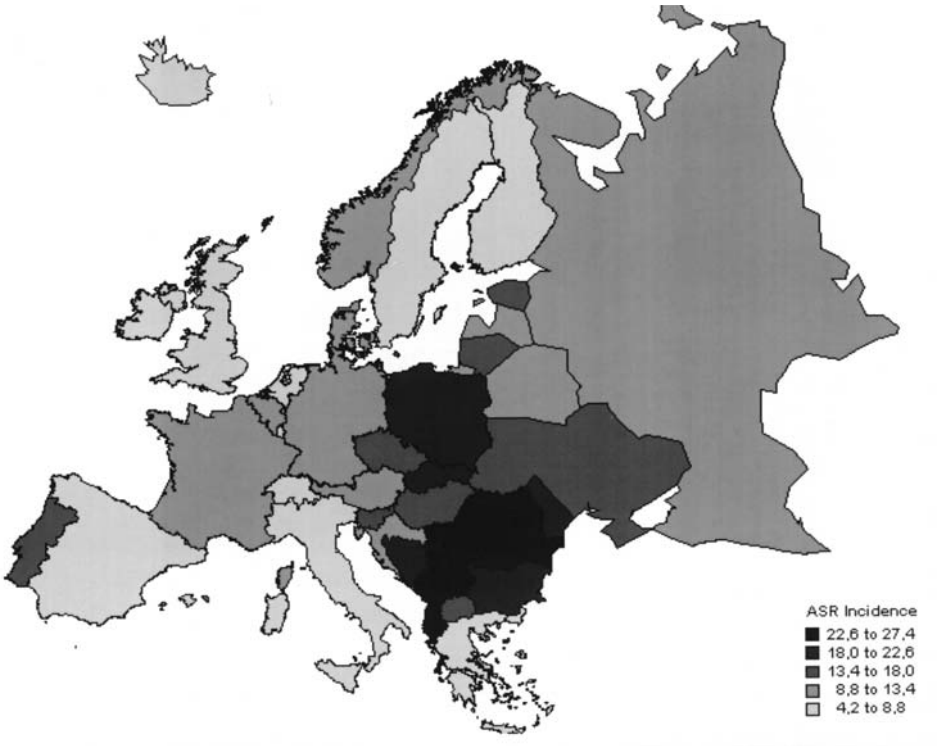

Figure 1. Age standardized rate (ASR) incidence in European countries.

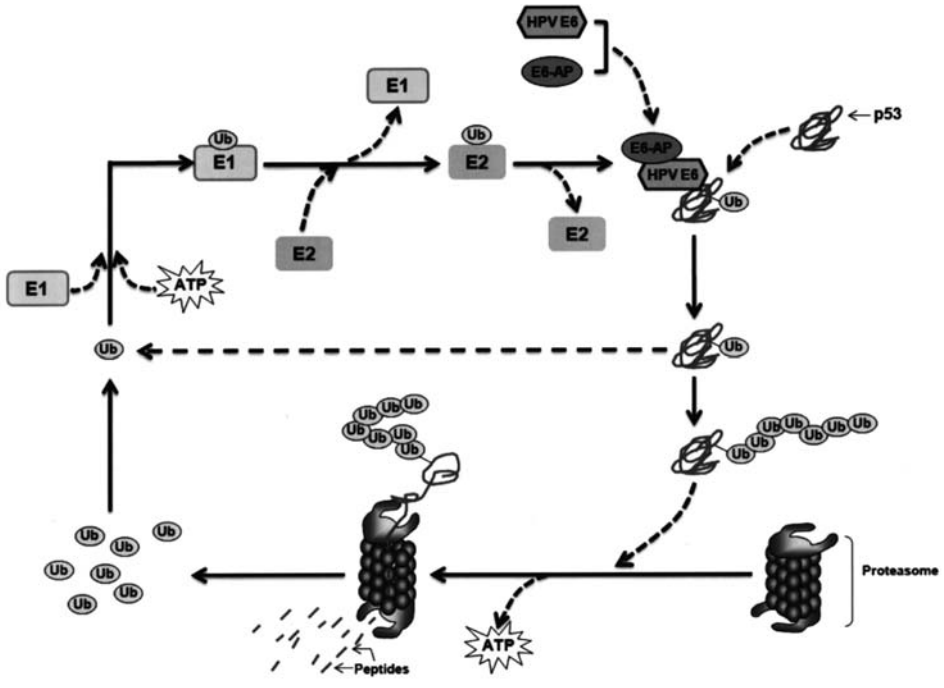

Figure 2. The ubiquitin proteasome-mediated degradation of p53. Ubiquitin (Ub) is a 76-amino acid protein highly conserved among eukaryotes and is involved in proteolysis and many other processes. Free ubiquitin is first activated by covalent attachment to the E1 enzyme in an ATP-dependent reaction and subsequently, ubiquitin is transferred to a ubiquitin-conjugating enzyme (E2). In the end, high-risk HPV-E6 binds to the cellular ubiquitin-protein ligase E6-associated protein (E6-AP) that then binds to p53. Poly-ubiquitinated p53 is recognized and degraded by the proteasome, and ubiquitin is regenerated.

the *TP53* gene, corresponding to p53 codon 72, causing an amino acid replacement from arginine (Arg) into proline (Pro) due to a transversion G→C (23). Although there is no obvious impact of proline at this position (24), this polymorphism confers two different structural and functional forms of p53 (25,26), and it has been largely investigated for its association with different neoplasias such as cervical cancer (27-29), bladder cancer (30,31), colorectal cancer (32), breast cancer (33), nasopharyngeal cancer (21), ovarian carcinoma (34) and lung adenocarcinoma (35). Storey *et al* suggested that the Arg p53 variant is seven times more susceptible to HPV-E6 targeting than the Pro p53 variant,

and thus women with the Arg/Arg genotype have an increased risk for cervical cancer development (36-39). However, since the results of Storey *et al*, several studies have tried to corroborate this association, but the data remain controversial (29,40). Several authors have shown that this *TP53* polymorphism is segregated differentially among different ethnic populations, the Arg allele being more common in Caucasian than in African or Asiatic populations (41-47). These findings require profound analysis to provide explanations for the differential susceptibility to cervical cancer development based on the genetic background of populations.

To the best knowledge of the authors, no previous study has reported the influence of geographical and ethnic location as an important factor in defining a genetic profile and the susceptibility for cervical cancer development. Therefore, we reviewed the literature in order to analyse the distribution of the *TP53* genotypes among European populations and its association with the development of cervical cancer, using a meta-analysis study design to summarize the overall risk.

Materials and methods

Study selection. All studies included in this review were selected from a PUBMED database search with the words 'tp53 polymorphism cervical cancer' and were published between 1998 (date of the first study) and 2005. In order to compile a list of studies regarding these data, we examined the reference list from all the identified studies. Nevertheless, studies which were conducted with cervical adenocarcinomas were excluded from the study due to the distinctive aetiology and behaviour of this type of carcinoma.

The decision to perform a meta-analysis regarding the role of *TP53* polymorphism in cervical cancer only within European populations was based on the evidence of differential segregation depending on ethnic group (45,46). A total of 27 case-control studies within European populations were selected and reviewed by the authors in order to evaluate the best approach for data analysis.

Study outcome. We reviewed the literature in order to analyse the role of the p53 codon 72 polymorphism in the development of pre-invasive and invasive cervical lesions, among European populations. If different types of lesions were considered in a study, the outcome was examined separately. Histological data from selected studies were adjusted to the Bethesda classification system: pre-invasive lesions of the cervix (CIN I, II and III) were designated as SIL, independently of being low grade (LGSIL) or high grade (HGSIL), and invasive lesions were designated as invasive cervical cancer (ICC).

Data extraction. To perform a more accurate analysis, despite the fact that some studies revealed the odds ratios (ORs) and respective 95% confidence intervals comparing the *Arg/Arg* genotype versus *Arg/Pro* or *Pro/Pro*, we calculated the adjusted ORs for all studies, taking into consideration the frequencies and Chi-square (χ^2) data provided. The *Arg/Pro* and *Pro/Pro* genotypes were grouped and ORs were calculated assuming the *Arg/Arg* genotype as the risk factor, since the first evidence suggested a 6-fold increased risk of ICC development (37). We also considered the heterogeneity of the studies by taking into account their different characteristics, such as the source of samples (blood versus cervical specimen) and genotyping method [allele specific-polymerase chain reaction (AS-PCR) versus other methods] in a meta-regression.

Statistical analysis. We used computer software Review Manager (RevMan) version 4.2 for Windows (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2003) to perform a meta-analysis and determine the overall ORs. The heterogeneity between studies was evaluated using χ^2 analysis in order to determine the odds ratio (OR) and its 95% confidence interval (CI) for the association between the p53 codon 72 polymorphism and the development of either pre-invasive and invasive cervical lesions.

Results were analysed regarding geographical distribution taking into account the longitude of the capital city of each country. For each country we performed a single meta-analysis of all published studies in order to reach an adjusted OR for the association of the p53 codon 72 polymorphism and the development of cervical lesions.

Results

The 27 studies published within European populations between 1998 and 2005, included in this review, analyse the role of the p53 codon 72 polymorphism in cervical cancer development among 12 different populations (Table I). It was clearly evident that the majority of studies were conducted in Italy with 7 studies, followed by the UK and Sweden with 4 studies each. Among the published studies, 3 considered exclusively pre-invasive cervical lesions, 10 considered invasive cervical lesions and 14 studies were conducted with both pre-invasive and invasive lesions. The number of cases and controls varied among the studies analysed, from 12 to 484 and 30 to 626, respectively. The total number of cases included in this meta-analysis was 3183 (1826 SIL and 1357 ICC) which were compared to a total of 3273 controls.

The majority of studies showed no statistical significant differences between cases and controls, suggesting no effect of the Arg/Arg genotype on the development of cervical lesions, although a few studies revealed an increased risk of development. Curiously, these findings were reported only in studies from 4 countries: Greece, Italy, the UK and Sweden; whereas studies from Greece and the UK showed the highest OR values (Table I).

We observed no significant differences when comparing the studies according to some heterogeneity factors, such as the origin of samples (blood or cervical specimen), or the genotyping method [allele specific-polymerase chain reaction (AS-PCR) or other method] (data not shown).

The data from selected studies were analysed according to the type of lesion, and were further studied according to the geographical distribution of the studies. The results from the meta-analysis of each outcome are presented in Figs. 3-6.

The meta-analysis revealed that the overall risk for ICC development was relatively low (OR, 1.27; 95% CI, 1.11-

Table I. Description of the characteristics of the studies regarding the p53 codon 72 polymorphism in cervical lesions.

Study	Country	Protocol	Source	Controls		Cases			P	OR	CI (95%)
				n	%A/A	n	%A/A	Type			
Storey *et al*, 1998 (37)	UK	AS-PCR	Tissue	41	36.6	30	76.7	ICC	**<0.001**	**5.70**	**1.98-16.41**
Rosenthal *et al*, 1998 (40)	UK	AS-PCR	Tissue	246	63.0	50	54.0	ICC	0.233	0.69	0.37-1.27
Helland *et al*, 1998 (67)	Norway	PCR-RFLP	Blood	225	54.2	77	57.1	ICC	0.656	1.13	0.67-1.90
						92	56.5	SIL	0.709	1.10	0.67-1.79
Heyes *et al*, 1998 (68)	Netherlands	AS-PCR	Tissue	158	57.0	25	68.0	ICC	0.326	1.56	0.64-3.84
						192	53.6	SIL	0.459	0.85	0.56-1.30
Josefsson *et al*, 1998 (69)[a]	Sweden	PCR-RFLP	Tissue	626	49.7	63	54.0	ICC	**0.516**	**1.19**	**0.69-2.06**
						484	52.9	SIL	**0.288**	**1.14**	**0.89-1.45**
Zehbe *et al*, 1999 (39)[a]	Sweden	PCR-SSCP	Tissue			30	73.3	ICC	**0.011**	**2.79**	**1.22-6.35**
						54	50.0	SIL	0.964	1.01	0.58-1.77
Zehbe *et al*, 1999 (39)	Italy	PCR-SSCP	Tissue	40	52.5	28	78.6	ICC	**0.028**	**3.32**	**1.11-9.92**
						24	50.0	SIL	0.846	0.90	0.33-2.49
Brady *et al*, 1999 (70)	UK	AS-PCR	Blood, tissue	74	58.1	85	69.4	ICC	0.138	1.64	0.85-3.14
Giannoudis *et al*, 1999 (71)	UK	AS-PCR	Tissue	30	56.7	43	62.8	ICC	0.598	1.29	0.50-3.34
						236	48.7	SIL	0.413	0.73	0.34-1.56
Klaes *et al*, 1999 (72)	Germany	PCR-RFLP	Tissue	151	55.6	87	52.9	ICC	0.680	0.89	0.53-1.52
Tachezy *et al*, 1999 (73)	Czech Republic	AS-PCR	Blood, tissue	172	53.5	71	52.1	ICC	0.845	0.95	0.54-1.65
Bertorelle *et al*, 1999 (74)	Italy	AS-PCR	Blood, tissue	130	54.6	105	51.4	SIL	0.681	0.88	0.53-1.47
Szarka *et al*, 2000 (75)	Hungary	AS-PCR	Blood	87	60.0	82	63.0	ICC	0.630	1.17	0.60-2.28
						86	53.0	SIL	0.400	0.77	0.40-1.48
Dybikowska *et al*, 2000 (76)	Poland	AS-PCR	Tissue	52	73.1	44	70.4	ICC	0.775	0.88	0.36-2.14
Tong *et al*, 2000 (77)	Austria	AS-PCR-Probe	Blood	133	62.4	105	54.3	ICC	0.206	0.72	0.43-1.20
Dokianakis *et al*, 2000 (49)	Greece	AS-PCR	Tissue	135	20.0	38	55.3	ICC	**<0.001**	**4.94**	**2.30-10.63**
						22	31.8	SIL	0.212	1.87	0.69-5.03
Agorastos *et al* 2000 (48)	Greece	AS-PCR	Tissue	30	20.0	12	66.7	ICC	**0.003**	**8.00**	**1.79-35.74**
						46	56.5	SIL	**0.012**	**5.20**	**1.79-15.13**

Table I. Continued.

Study	Country	Protocol	Source	Controls		Cases			P	OR	CI (95%)
				n	%A/A	n	%A/A	Type			
Tenti *et al*, 2000 (53)	Italy	AS-PCR	Tissue	140	61.4	64	53.1	ICC	0.264	0.71	0.39-1.29
van Dun *et al*, 2000 (78)	Netherlands	AS-PCR	Tissue	86	57.0	71	62.0	ICC	0.526	1.23	0.65-2.34
						89	42.7	SIL	0.059	0.56	0.31-1.02
Zehbe *et al*, 2001 (50)	Sweden	PCR-SSCP	Tissue	188	47.3	72	63.9	ICC	**0.017**	**1.97**	**1.12-3.44**
						98	38.8	SIL	0.447	0.70	0.43-1.16
Zehbe *et al*, 2001 (50)[b]	Italy	PCR-SSCP	Tissue	40	52.5	43	76.7	ICC	**0.020**	**2.99**	**1.17-7.65**
						37	51.4	SIL	0.120	0.96	0.39-2.34
Rezza *et al*, 2001 (79)	Italy	PCR-RFLP	Tissue	172	50.0	71	49.3	SIL	0.920	0.97	0.56-1.69
Gustafsson *et al*, 2001 (80)	Sweden	Pyrosequence	Tissue	36	50.0	20	60.0	ICC	**0.472**	**1.50**	**0.43-5.27**
						46	56.5	SIL	**0.556**	**1.30**	**0.49-3.43**
Humbey *et al*, 2002 (52)	France	PCR-DGGE	Tissue	50	52.0	68	45.6	SIL	0.491	0.77	0.37-1.61
Cenci *et al*, 2003 (51)	Italy	AS-PCR	Tissue	50	46.0	30	46.0	ICC	0.954	1.03	0.38-2.18
Comar *et al*, 2004 (81)	Italy	PCR-Probe	Tissue	76	60.5	23	52.2	ICC	0.476	0.71	0.28-1.82
Santos *et al*, 2005 (29)	Portugal	AS-PCR	Blood	145	63.4	164	67.1	ICC	0.504	1.17	0.73-1.88
						76	56.6	SIL	0.320	0.75	0.43-1.32

[a]These two studies used the same control group but different methodologies. [b]The results from the controls were the same as a previous study from Zehbe *et al*, 2001. ICC, invasive cervical cancer; SIL, squamous intraepithelial lesions; P, Pearson Chi-square; OR, odds ratio; CI, confidence interval.

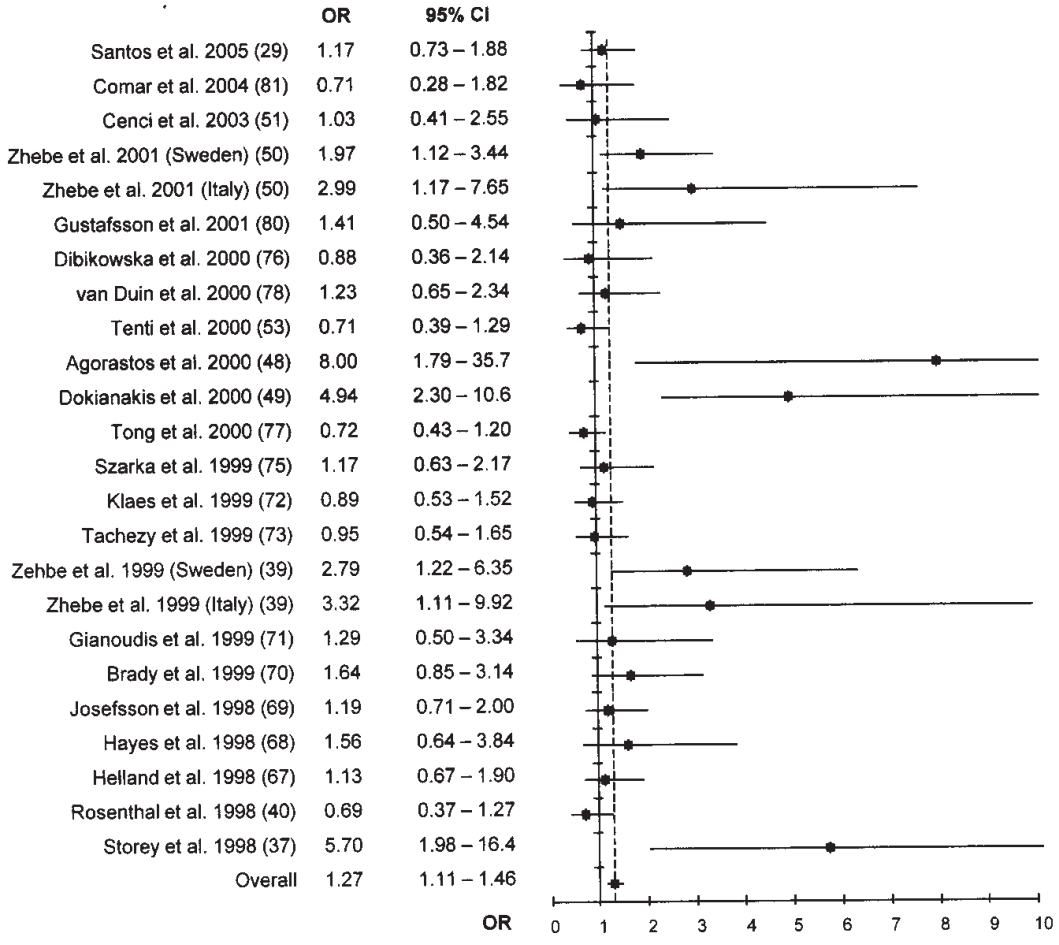

Figure 3. Odds ratios (OR) and 95% confidence intervals (CI) from studies regarding the p53 codon 72 genotype (*Arg*/*Arg* versus *Arg*/*Pro* or *Pro*/*Pro*) and its association with invasive cervical cancer development. The center dot represents the OR and the horizontal line indicates the 95% CI. The overall OR and its corresponding 95% CI are represented below the list of studies.

1.46) (Fig. 3), and moreover, in the case of SIL development the overall risk is approximately null (OR, 0.97; 95% CI, 0.85-1.10) (Fig. 5).

By merging studies by country origin and analysing data according to geographical distribution using longitude, it was observed that the majority of the studies did not show an increased risk for the development of ICC and, in the case of SIL, data pointed somehow to a protective role of the *TP53* Arg/Arg genotype. Furthermore, the individual meta-analysis for countries with more than one published study revealed that only Italy and the UK had statistically significant results (P=0.050 and P=0.007, respectively) for ICC development, whereas for SIL none of the countries had significant results.

These results emphasize the fact that since the results of Storey *et al*, few studies revealed a significant increased risk for Arg/Arg genotype carriers for the development of either SIL or ICC, but the implication of this polymorphism remains unexplained.

Discussion

In vitro studies suggest that HPV-E6 protein binds more efficiently to the Arg p53 variant at position 72, than to the Pro p53 variant, increasing its degradation through the ubiquitin proteasome pathway (15,17). Without functional p53, cell cycle deregulation occurs and cells start to proliferate without control leading to the development of neoplastic cells.

Storey *et al* made the first effort to show the role of the p53 codon 72 polymorphism in cancer development, emphasizing that the Arg/Arg genotype increased the risk of cervical cancer development by ~6-fold (37). Since then, and despite all the criticism about the reduced number of samples of this study, the Arg/Arg genotype has been suggested as a potential susceptibility marker for cervical cancer development. Several other studies were conducted in numerous countries worldwide, and despite some that supported the findings of Storey *et al* (39,48-50), the great majority did not corroborate them suggesting that the Arg/Arg genotype had no evidence for an increasing susceptibility for the development of cervical cancer (29,40,51-53). This controversy has already led to an increasing number of reviews about the role of this polymorphism. However, as far as the authors are concerned, no study has reported the influence of the geographical and ethnic location as an important factor in the definition of genetic profile and susceptibility for cervical cancer development (27,28,54).

Among the several reports that attempted to explain the role of the *TP53* polymorphism in cervical cancer many aspects were not considered that might have interfered in the analysis. Makni *et al* made the first effort to study the

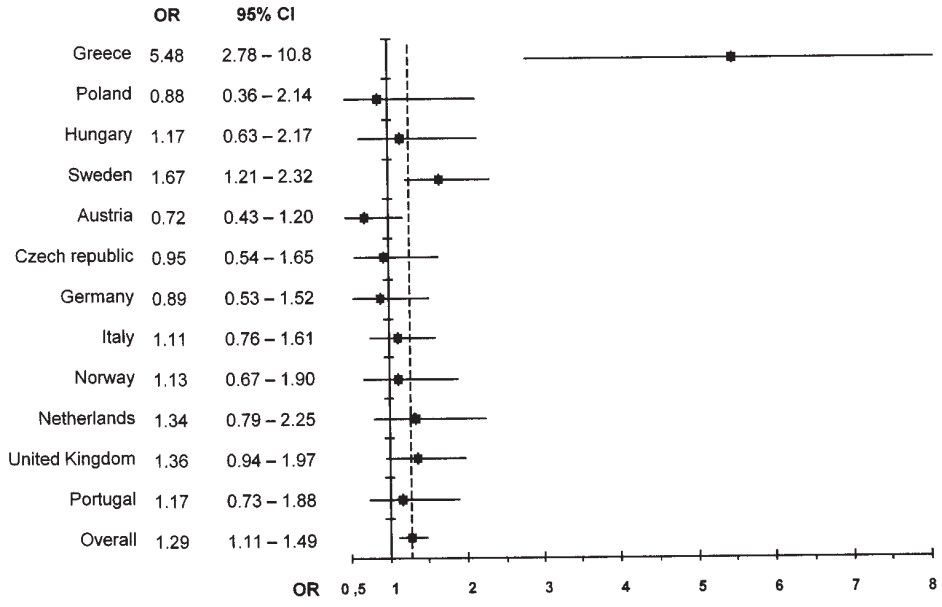

Figure 4. Odds ratios (OR) and 95% confidence intervals (CI) from the meta-analysis data for each country regarding the association of invasive cervical cancer and the p53 codon 72 genotype (*Arg*/*Arg* versus *Arg*/*Pro* or *Pro*/*Pro*). The center dot represents the OR and the horizontal line indicates the 95% CI. The overall OR and its corresponding 95% CI are represented below the list of studies.

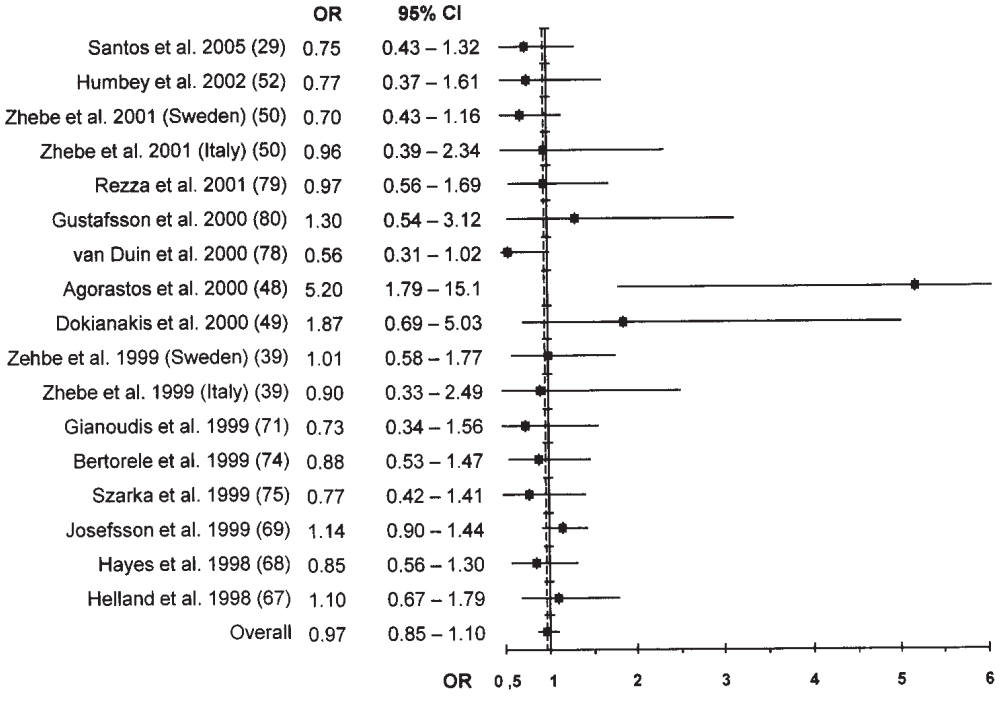

Figure 5. Odds ratios (OR) and 95% confidence intervals (CI) from studies regarding the p53 codon 72 genotype (*Arg*/*Arg* versus *Arg*/*Pro* or *Pro*/*Pro*) and its association with squamous intraepithelial lesion development. The center dot represents the OR and the horizontal line indicates the 95% CI. The overall OR and its corresponding 95% CI are represented below the list of studies.

accuracy of the results in different laboratories, suggesting that the protocol selected for the allelic discrimination and the source of the sample (blood or tissue) were extremely important in the analysis and could represent biased results (55). Therefore, we considered these characteristics among the studies in the European countries here resumed, and we observed that the greatest majority used allele-specific polymerase chain reaction (AS-PCR) as protocol, and tissues as samples. Although the use of blood samples would reduce the possible misclassification due to mutations and loss of heterozygosis (LOH) present in the local tumor as a consequence of neoplastic changes, the use of tissues is still accepted. Nevertheless, the use of another sample source or protocol did not have a direct implication on the accuracy of the results (54).

Another important factor that must be taken into consideration due to its possible interference with the analysis is the number of cases and controls. Most studies

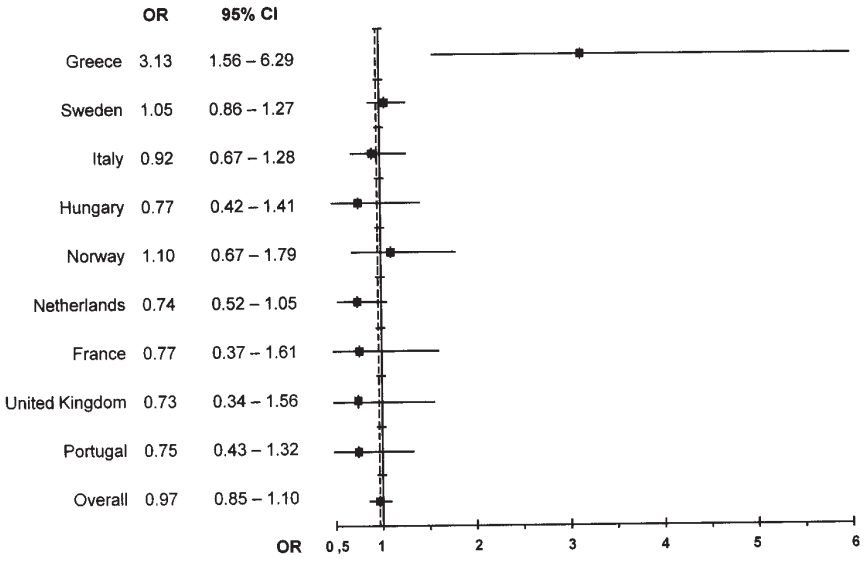

Figure 6. Odds ratios (OR) and 95% confidence intervals (CI) from the meta-analysis data for each country regarding the association of squamous intraepithelial lesions and the p53 codon 72 genotype (*Arg*/*Arg* versus *Arg*/*Pro* or *Pro*/*Pro*). The center dot represents the OR and the horizontal line indicates the 95% CI. The overall OR and its corresponding 95% CI are represented below the list of studies.

frequently use a reduced number of samples, thus many do not achieve statistically significant results. In this review, we observed that several studies used a reduced number of cases and controls; therefore without a large range of samples it was not possible, even with statistical analysis, to accurately reach overall conclusions for the population. To increase the accuracy of the studies, we suggested that the number of cases should be >100, so the sample could be representative, and that the controls should be at least equal or double. This condition was not observed in any of the studies analysed here, but this was somehow understandable due to difficulties in the selection and collection of samples from either cases or controls.

Table I shows the data collected from all published studies, and it is evident that a few studies revealed significantly higher frequencies of the Arg/Arg genotype in the cases analysed than in the controls, revealing an increased risk for invasive cervical cancer. Similar results were observed for the development of pre-invasive lesions of the cervix (SIL). Table I also reveals that, despite their high ORs, the studies from the UK, Italy, Greece and Sweden should be analysed more carefully.

From the five studies made in the UK, only one, and the first to be known, showed statistically significant association (37). Nevertheless, this study was conducted using a reduced number of samples and it revealed a large confidence interval. As a result, we believe that the study of Storey *et al* might not have been the most representative study for this population. Among the seven studies from Italy, only two suggested an increased risk of developing cervical cancer in individuals carrying the Arg/Arg genotype. Although the CI was satisfactory in both, we considered that this association was only achieved due to the small number of samples used and that probably it would not be statistically significant if the number of samples increased. Moreover, the two Greek studies which showed an increased risk had very large CIs, most likely due to the small number of cases and controls analysed, and so these results must be considered with extreme prudence. Also, to note, the two studies made in Sweden showed 2- to 3-fold increased risk of invasive cervical cancer development. One important finding from this analysis is that Greece and Sweden are countries with very low incidence rates of cervical cancer. Moreover, Greece and Sweden are countries located at the borders of Europe and might have different genetic backgrounds compared to the other European countries. These data combined with the information from the analysis, might confirm the *TP53* polymorphism as a specific genetic biomarker for these populations concerning the development of ICC. Other notable data evident from Table I show that Poland and the Czech Republic, which have the highest incidences of cervical cancer (Fig. 1), have no statistically significant association with the polymorphism. These data allow us to theorize that, while in countries with high incidence rates of ICC this polymorphism might not represent a susceptibility marker, in countries with low incidence rates it can represent a significant risk marker.

The data from the meta-analysis presented in Fig. 3 confirmed the first evidence that an increased risk for ICC development could be found only in studies from Greece, Italy, the UK and Sweden. Although the overall analysis provided a statistically significant association between the Arg/Arg genotype and ICC development (P<0.001) the overall risk was not significant (OR, 1.27; 95% CI, 1.11-1.46). By merging studies from each country (Fig. 4) we observed that, despite Italy and the UK having statistically significant results (P=0.050 and P=0.007), respectively for ICC development, only Sweden and Greece had results that deviated from the other countries. With this analysis we summarized all data regarding the association between the *TP53* Arg/Arg genotype and the development of ICC in European populations. Our data point to an overall risk for Europe of ~1.2-fold, which does not provide a strong association of this genotype as a susceptibility marker for ICC development as it was first suggested by previously published data.

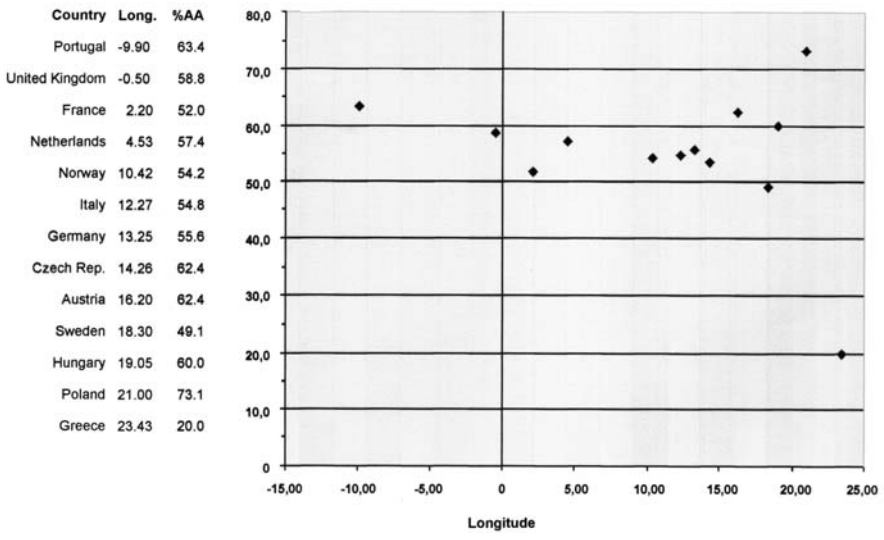

Figure 7. p53 codon 72 *Arg/Arg* genotype distribution among different populations analysed by considering the longitude of each country represented by its capital city.

The same analysis was performed for SIL development, and the data revealed that only studies from Greece, the UK and one from Sweden revealed an increased risk (Fig. 5). Despite the first evidence, individual meta-analysis for countries with more than one published study revealed that there were no countries with significant results for SIL development. Despite that the meta-analysis did not provide statistically significant results (P=0.170), it did suggest that the Arg/Arg genotype might not have an influence on the development of SIL (OR, 0.97; 95% CI, 0.85-1.10) (Fig. 6). Our analysis summarized all the data from European countries and indicated that the Arg/Arg genotype does not represent a risk marker for SIL development.

It is extremely important to mention that, although our meta-analysis revealed an overall risk for invasive cervical cancer development in Europe of ~1.27 fold, we believe that the studies from Greece, two studies from both Sweden and Italy, and the one from Storey *et al* have introduced deviating factors in the analysis. In fact, if we do not consider these studies in the analysis, due to their different frequencies, the overall risk would be 1.02 (95% CI, 0.89-1.10). As we stated before, if we take into consideration that these countries have low incidence rates of ICC, there might be evidence that this polymorphism represents a susceptibility marker in countries with low incidences, but not in all populations (43,56,57).

This fact supports the need of more meta-analysis reviews within this subject to allow a better explanation of the role of the p53 codon 72 polymorphism in cancer development. Summarizing the meta-analysis, this original study emphasizes the evidence of the most recently published studies worldwide revealing no association of the *TP53* polymorphism with the development of any modification on cervix epithelium (54,55,58-66).

Another notable finding from our review was the comparison of the Arg/Arg genotype frequency among the controls of the different studies (Fig. 7). By analysing the frequencies considering the longitude of each country represented by its capital, we observed that central European countries had similar frequencies of the Arg/Arg genotype (52-62%), while the countries at the edges showed some differences in the frequency. Additionally and despite the fact that Portugal and Sweden had slightly different frequencies compared to the others, 63.4 and 49.0% respectively, countries from the Eastern edge such as Poland and Greece showed the largest differences. While Poland showed a significantly higher frequency of the Arg/Arg genotype (73.1%), Greek studies revealed a frequency among the control population of only 20%, which is significantly different compared to the others. By analysing the data from Greece we observed that the studies used a reduced number of controls, and thus this might have biased the presented data.

Hence, several authors have studied the ethnic variations of the *TP53* polymorphism worldwide, showing that the Arg allele is more common in Caucasian than in African and Asiatic populations (41-47,58). Beckman *et al* conducted a noteworthy study regarding the potential natural selection of p53 during intrauterine development and suggest that this p53 codon 72 polymorphism might balance natural selection (41).

This is the first review that reports geographical location as an important marker in the population genetic background for the influence of the p53 codon 72 polymorphism. Despite the laboratory differences and methodologies, our data indicates that the Arg/Arg genotype does not represent a susceptibility risk marker for the development of any cervical lesion in most of the countries of Europe, although in countries with low incidence rates of ICC this polymorphism might represent a significant genetic marker (43,44,56,57). Furthermore, future investigations are required with appropriate attention to the design and methodological issues, mainly by considering larger study samples.

Acknowledgements

The authors are grateful to Professor Eduardo Franco, McGill University, Montreal, Canada, for his constructive criticisms on an earlier draft of this manuscript; Professor Mário Dinis-

Ribeiro from the Portuguese Institute of Oncology of Oporto, Portugal, for providing the necessary software tools for this meta-analysis; and to the Portuguese League Against Cancer (Núcleo Regional do Norte da Liga Portuguesa Contra o Cancro) for supporting our research group, and to the Caloust Gunbenkian Foundation for the financial support to the study (project 35-76491-S).

References

1. Sankaranarayanan R and Ferlay J: Worldwide burden of gynaecological cancer: the size of the problem. Best Pract Res Clin Obstet Gynaecol 20: 207-225, 2006.
2. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R and Shah KV: Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International Biological Study on Cervical Cancer (IBSCC) Study Group. J Natl Cancer Inst 87: 796-802, 1995.
3. Bosch FX and Munoz N: The viral etiology of cervical cancer. Virus Res 89: 183-190, 2002.
4. Bosch FX and de Sanjose S: Human papillomavirus in cervical cancer. Curr Oncol Rep 4: 175-183, 2002.
5. Castellsague X, Bosch FX and Munoz N: Environmental co-factors in HPV carcinogenesis. Virus Res 89: 191-199, 2002.
6. Franco EL, Rohan TE and Villa LL: Epidemiologic evidence and human papillomavirus infection as a necessary cause of cervical cancer. J Natl Cancer Inst 91: 506-511, 1999.
7. Matos A, Moutinho J, Pinto D and Medeiros R: The influence of smoking and other cofactors on the time to onset to cervical cancer in a southern European population. Eur J Cancer Prev 14: 485-491, 2005.
8. Moreno V, Munoz N, Bosch FX, de Sanjose S, Gonzalez LC, Tafur L, Gili M, Izarzugaza I, Navarro C and Vergara A: Risk factors for progression of cervical intraepithelial neoplasm grade III to invasive cervical cancer. Cancer Epidemiol Biomarkers Prev 4: 459-467, 1995.
9. Bosch FX, Munoz N, de Sanjose S, Navarro C, Moreo P, Ascunce N, Gonzalez LC, Tafur L, Gili M and Larranaga I: Human papillomavirus and cervical intraepithelial neoplasia grade III/carcinoma *in situ*: a case-control study in Spain and Colombia. Cancer Epidemiol Biomarkers Prev 2: 415-422, 1993.
10. Cannistra SA and Niloff JM: Cancer of the uterine cervix. N Engl J Med 334: 1030-1038, 1996.
11. Cooper B, Schneider S, Bohl J, Jiang Y, Beaudet A and Vande PS: Requirement of E6AP and the features of human papillomavirus E6 necessary to support degradation of p53. Virology 306: 87-99, 2003.
12. Nuber U, Schwarz SE and Scheffner M: The ubiquitin-protein ligase E6-associated protein (E6-AP) serves as its own substrate. Eur J Biochem 254: 643-649, 1998.
13. Vogelstein B and Kinzler KW: p53 function and dysfunction. Cell 70: 523-526, 1992.
14. Scheffner M, Werness BA, Huibregtse JM, Levine AJ and Howley PM: The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell 63: 1129-1136, 1990.
15. Scheffner M, Huibregtse JM, Vierstra RD and Howley PM: The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell 75: 495-505, 1993.
16. Scheffner M: Ubiquitin, E6-AP, and their role in p53 inactivation. Pharmacol Ther 78: 129-139, 1998.
17. Scheffner M and Whitaker NJ: Human papillomavirus-induced carcinogenesis and the ubiquitin-proteasome system. Semin Cancer Biol 13: 59-67, 2003.
18. Duarte I, Santos A, Sousa H, Catarino R, Pinto D, Matos A, Pereira D, Moutinho J, Canedo P, Machado JC and Medeiros R: G-308A TNF-alpha polymorphism is associated with an increased risk of invasive cervical cancer. Biochem Biophys Res Commun 334: 588-592, 2005.
19. Medeiros RM, Morais A, Vasconcelos A, Costa S, Pinto D, Oliveira J, Ferreira P and Lopes C: Outcome in prostate cancer: association with endothelial nitric oxide synthase Glu-Asp298 polymorphism at exon 7. Clin Cancer Res 8: 3433-3437, 2002.
20. Pinto D, Vasconcelos A, Costa S, Pereira D, Rodrigues H, Lopes C and Medeiros R: HER2 polymorphism and breast cancer risk in Portugal. Eur J Cancer Prev 13: 177-181, 2004.
21. Sousa H, Santos AM, Catarino R, Pinto D, Vasconcelos A, Lopes C, Breda E and Medeiros R: Linkage of TP53 codon 72 pro/pro genotype as predictive factor for nasopharyngeal carcinoma development. Eur J Cancer Prev 15: 362-366, 2006.
22. Zusman I, Vol A and Kozlenko M: Polymorphism of cellular physicochemical features and cancer: a review. In Vivo 14: 683-689, 2000.
23. Matlashewski GJ, Tuck S, Pim D, Lamb P, Schneider J and Crawford LV: Primary structure polymorphism at amino acid residue 72 of human p53. Mol Cell Biol 7: 961-963, 1987.
24. Sun Y, Keshava C, Sharp DS, Weston A and McCanlies EC: DNA sequence variants of *p53*: Cancer and aging. Am J Hum Genet 65: 1779-1782, 1999.
25. Dumont P, Leu JI, Della PA III, George DL and Murphy M: The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 33: 357-365, 2003.
26. Pim D and Banks L: p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. Int J Cancer 108: 196-199, 2004.
27. Jee SH, Won SY, Yun JE, Lee JE, Park JS and Ji SS: Polymorphism p53 codon-72 and invasive cervical cancer: a meta-analysis. Int J Gynaecol Obstet 85: 301-308, 2004.
28. Koushik A, Platt RW and Franco EL: p53 codon 72 polymorphism and cervical neoplasia: a meta-analysis review. Cancer Epidemiol Biomarkers Prev 13: 11-22, 2004.
29. Santos AM, Sousa H, Catarino R, Pinto D, Pereira D, Vasconcelos A, Matos A, Lopes C and Medeiros R: TP53 codon 72 polymorphism and risk for cervical cancer in Portugal. Cancer Genet Cytogenet 159: 143-147, 2005.
30. Chen WC, Tsai FJ, Wu JY, Wu HC, Lu HF and Li CW: Distributions of p53 codon 72 polymorphism in bladder cancer - proline form is prominent in invasive tumor. Urol Res 28: 293-296, 2000.
31. Soulitzis N, Sourvinos G, Dokianakis DN and Spandidos DA: p53 codon 72 polymorphism and its association with bladder cancer. Cancer Lett 179: 175-183, 2002.
32. Sjalander A, Birgander R, Athlin L, Stenling R, Rutegard J, Beckman L and Beckman G: P53 germ line haplotypes associated with increased risk for colorectal cancer. Carcinogenesis 16: 1461-1464, 1995.
33. Papadakis EN, Dokianakis DN and Spandidos DA: p53 codon 72 polymorphism as a risk factor in the development of breast cancer. Mol Cell Biol Res Commun 3: 389-392, 2000.
34. Santos AM, Sousa H, Pinto D, Portela C, Pereira D, Catarino R, Duarte I, Lopes C and Medeiros R: Linking TP53 codon 72 and P21 nt590 genotypes to the development of cervical and ovarian cancer. Eur J Cancer 42: 958-963, 2006.
35. Fan R, Wu MT, Miller D, Wain JC, Kelsey KT, Wiencke JK and Christiani DC: The p53 codon 72 polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 9: 1037-1042, 2000.
36. Hildesheim A, Schiffman M, Brinton LA, Fraumeni JF Jr, Herrero R, Bratti MC, Schwartz P, Mortel R, Barnes W, Greenberg M, McGowan L, Scott DR, Martin M, Herrera JE and Carrington M: p53 polymorphism and risk of cervical cancer. Nature 396: 531-532, 1998.
37. Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G and Banks L: Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature 393: 229-234, 1998.
38. Tommasino M, Accardi R, Caldeira S, Dong W, Malanchi I, Smet A and Zehbe I: The role of TP53 in cervical carcinogenesis. Hum Mutat 21: 307-312, 2003.
39. Zehbe I, Voglino G, Wilander E, Genta F and Tommasino M: Codon 72 polymorphism of p53 and its association with cervical cancer. Lancet 354: 218-219, 1999.
40. Rosenthal AN, Ryan A, Al-Jehani RM, Storey A, Harwood CA and Jacobs IJ: p53 codon 72 polymorphism and risk of cervical cancer in UK. Lancet 352: 871-872, 1998.
41. Beckman G, Birgander R, Sjalander A, Saha N, Holmberg PA, Kivela A and Beckman L: Is p53 polymorphism maintained by natural selection? Hum Hered 44: 266-270, 1994.
42. de la Calle-Martin O, Fabregat V, Romero M, Soler J, Vives J and Yague J: AccII polymorphism of the p53 gene. Nucleic Acids Res 18: 4963, 1990.

43. Inserra P, Abrahamsen M, Papenfuss M and Giuliano AR: Ethnic variation of the P53 codon 72 polymorphism, HPV persistence, and cervical cancer risk. Int J STD AIDS 14: 800-804, 2003.
44. rbel-Alon S, Menczer J, Feldman N, Glezerman M, Yeremin L and Friedman E: Codon 72 polymorphism of p53 in Israeli Jewish cervical cancer patients and healthy women. Int J Gynecol Cancer 12: 741-744, 2002.
45. Sjalander A, Birgander R, Kivela A and Beckman G: p53 polymorphisms and haplotypes in different ethnic groups. Hum Hered 45: 144-149, 1995.
46. Sjalander A, Birgander R, Saha N, Beckman L and Beckman G: p53 polymorphisms and haplotypes show distinct differences between major ethnic groups. Hum Hered 46: 41-48, 1994.
47. Weston A, Perrin LS, Forrester K, Hoover RN, Trump BF, Harris CC and Caporaso NE: Allelic frequency of a p53 polymorphism in human lung cancer. Cancer Epidemiol Biomarkers Prev 1: 481-483, 1992.
48. Agorastos T, Lambropoulos AF, Constantinidis TC, Kotsis A and Bontis JN: p53 codon 72 polymorphism and risk of intraepithelial and invasive cervical neoplasia in Greek women. Eur J Cancer Prev 9: 113-118, 2002.
49. Dokianakis DN and Spandidos DA: P53 codon 72 polymorphism as a risk factor in the development of HPV-associated cervical cancer. Mol Cell Biol Res Commun 3: 111-114, 2000.
50. Zehbe I, Voglino G, Wilander E, Delius H, Marongiu A, Edler L, Klimek F, Andersson S and Tommasino M: p53 codon 72 polymorphism and various human papillomavirus 16 E6 genotypes are risk factors for cervical cancer development. Cancer Res 61: 608-611, 2001.
51. Cenci M, French D, Pisani T, Alderisio M, Lombardi AM, Marchese R, Colelli F and Vecchione A: p53 polymorphism at codon 72 is not a risk factor for cervical carcinogenesis in central Italy. Anticancer Res 23: 1385-1387, 2003.
52. Humbey O, Aubin F, Cairey-Remonnay S, Riethmuller D, Pretet JL, Fest T, Seilles E and Mougin C: TP53 polymorphism at exon 4 in Caucasian women from eastern France: lack of correlation with HPV status and grade of cervical precancerous lesions. Eur J Obstet Gynecol Reprod Biol 103: 60-64, 2002.
53. Tenti P, Vesentini N, Rondo SM, Zappatore R, Migliora P, Carnevali L and Ranzani GN: p53 codon 72 polymorphism does not affect the risk of cervical cancer in patients from northern Italy. Cancer Epidemiol Biomarkers Prev 9: 435-438, 2000.
54. Koushik A, Ghosh A, Duarte-Franco E, Forest P, Voyer H, Matlashewski G, Coutlee F and Franco EL: The p53 codon 72 polymorphism and risk of high-grade cervical intraepithelial neoplasia. Cancer Detect Prev 29: 307-316, 2005.
55. Makni H, Franco EL, Kaiano J, Villa LL, Labrecque S, Dudley R, Storey A and Matlashewski G: P53 polymorphism in codon 72 and risk of human papillomavirus-induced cervical cancer: effect of inter-laboratory variation. Int J Cancer 87: 528-533, 2000.
56. Saranath D, Khan Z, Tandle AT, Dedhia P, Sharma B, Contractor R, Shrivastava S and Dinshaw K: HPV16/18 prevalence in cervical lesions/cancers and p53 genotypes in cervical cancer patients from India. Gynecol Oncol 86: 157-162, 2002.
57. Menczer J: The low incidence of cervical cancer in Jewish women: has the puzzle finally been solved? Isr Med Assoc J 5: 120-123, 2003.
58. Abba MC, Villaverde LM, Gomez MA, Dulout FN, Laguens MR and Golijow CD: The p53 codon 72 genotypes in HPV infection and cervical disease. Eur J Obstet Gynecol Reprod Biol 109: 63-66, 2003.
59. Baek WK, Cho JW, Suh SI, Suh MH, Shin DH, Cho CH, Lee TS and Cha SD: p53 codon 72 polymorphism and risk of cervical carcinoma in Korean women. J Korean Med Sci 15: 65-67, 2000.
60. Brenna SM, Silva ID, Zeferino LC, Pereira JS, Martinez EZ and Syrjanen KJ: Prognostic value of P53 codon 72 polymorphism in invasive cervical cancer in Brazil. Gynecol Oncol 93: 374-380, 2004.
61. Katiyar S, Thelma BK, Murthy NS, Hedau S, Jain N, Gopalkrishna V, Husain SA and Das BC: Polymorphism of the p53 codon 72 Arg/Pro and the risk of HPV type 16/18-associated cervical and oral cancer in India. Mol Cell Biochem 252: 117-124, 2003.
62. Kim JW, Roh JW, Park NH, Song YS, Kang SB and Lee HP: Polymorphism of TP53 codon 72 and the risk of cervical cancer among Korean women. Am J Obstet Gynecol 184: 55-58, 2001.
63. Kucera E, Tong D, Reinthaller A, Leodolter S, Zeillinger R and Sliutz G: p53 polymorphism at codon 72 - Does it constitute a risk for squamous intraepithelial lesions and invasive cancer of the cervix in Central Europeans? Wien Klin Wochenschr 112: 817-820, 2000.
64. Pegoraro R, Moodley J, Naiker S, Lanning P and Rom L: The p53 codon 72 polymorphism in black South African women and the risk of cervical cancer. Br J Obstet Gynaecol 107: 1164-1165, 2000.
65. Pillai MR, Sreevidya S, Pollock BH, Jayaprakash PG and Herman B: Polymorphism at codon 72 of p53, human papillomavirus, and cervical cancer in South India. J Cancer Res Clin Oncol 128: 627-631, 2002.
66. Wang NM, Tsai CH, Yeh KT, Chen SJ and Chang JG: p53 codon 72Arg polymorphism is not a risk factor for carcinogenesis in the Chinese. Int J Mol Med 4: 249-252, 1999.
67. Helland A, Langerod A, Johnsen H, Olsen AO, Skovlund E and Borresen-Dale AL: p53 polymorphism and risk of cervical cancer. Nature 396: 530-531, 1998.
68. Hayes VM, Hofstra RM, Buys CH, Hollema H and van der Zee AG: Homozygous arginine-72 in wild type p53 and risk of cervical cancer. Lancet 352: 1756, 1998.
69. Josefsson AM, Magnusson PK, Ylitalo N, Quarforth-Tubbin P, Ponten J, Adami HO and Gyllensten UB: p53 polymorphism and risk of cervical cancer. Nature 396: 531, 1998.
70. Brady CS, Duggan-Keen MF, Davidson JA, Varley JM and Stern PL: Human papillomavirus type 16 E6 variants in cervical carcinoma: relationship to host genetic factors and clinical parameters. J Gen Virol 80: 3233-3240, 1999.
71. Giannoudis A, Graham DA, Southern SA and Herrington CS: p53 codon 72 ARG/PRO polymorphism is not related to HPV type or lesion grade in low- and high-grade squamous intraepithelial lesions and invasive squamous carcinoma of the cervix. Int J Cancer 83: 66-69, 1999.
72. Klaes R, Ridder R, Schaefer U, Benner A and von Knebel DM: No evidence of p53 allele-specific predisposition in human papillomavirus-associated cervical cancer. J Mol Med 77: 299-302, 1999.
73. Tachezy R, Mikyskova I, Salakova M and Van RM: Correlation between human papillomavirus-associated cervical cancer and p53 codon 72 arginine/proline polymorphism. Hum Genet 105: 564-566, 1999.
74. Bertorelle R, Chieco-Bianchi L and Del MA: Papillomavirus and p53 codon 72 polymorphism. Int J Cancer 82: 616-617, 1999.
75. Szarka K, Veress G, Juhasz A, Konya J, Sapy T, Soos G, Hernadi Z and Gergely L: Integration status of virus DNA and p53 codon 72 polymorphism in human papillomavirus type 16 positive cervical cancers. Anticancer Res 20: 2161-2167, 2000.
76. Dybikowska A, Dettlaff A, Konopa K and Podhajska A: p53 codon 72 polymorphism in cervical cancer patients and healthy women from Poland. Acta Biochim Pol 47: 1179-1182, 2000.
77. Tong D, Kucera E, Stimpfl M, Kolbl H, Leodolter S and Zeillinger R: Detection of p53 polymorphism at codon 72 by PCR and allele-specific oligonucleotide hybridization on microtiter plates. Clin Chem 46: 124-126, 2000.
78. van Dun M, Snijders PJ, Vossen MT, Klaassen E, Voorhorst F, Verheijen RH, Helmerhorst TJ, Meijer CJ and Walboomers JM: Analysis of human papillomavirus type 16 E6 variants in relation to p53 codon 72 polymorphism genotypes in cervical carcinogenesis. J Gen Virol 81: 317-325, 2000.
79. Rezza G, Giuliani M, Garbuglia AR, Serraino D, Cappiello G, Migliore G, Branca M, Benedetto A and Ippolito G: Lack of association between p53 codon-72 polymorphism and squamous intraepithelial lesions in women with, or at risk for, human immunodeficiency virus and/or human papillomavirus infections. Cancer Epidemiol Biomarkers Prev 10: 565-566, 2001.
80. Gustafsson AC, Guo Z, Hu X, Ahmadian A, Brodin B, Nilsson A, Ponten J, Ponten F and Lundeberg J: HPV-related cancer susceptibility and p53 codon 72 polymorphism. Acta Derm Venereol 81: 125-129, 2001.
81. Comar M, Molin GD, Guaschino S and Campello C: p53 at codon 72 polymorphism, human papillomavirus infection and cervical lesions: a cross-sectional study from northeastern Italy. Eur J Obstet Gynecol Reprod Biol 114: 210-214, 2004.