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TP53 and *EGFR* mutations in combination with lifestyle risk factors in tumours of the upper aerodigestive tract from South America

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Cancers of the upper aerodigestive tract [(UADT): oral cavity, pharynx, larynx and oesophagus] have high incidence rates in some parts of South America. Alterations in the *TP53* gene are common in these cancers. In our study, we have estimated the prevalence and patterns of *TP53* mutations (exons 4–10) in 236 UADT tumours from South America in relation to lifestyle risk factors, such as tobacco smoking and alcohol drinking. Moreover, we have conducted a pilot study of *EGFR* mutations (exons 18–21) in 45 tumours from the same population. *TP53* mutation prevalence was high: 59% of tumours were found to carry mutant *TP53*. We found an association between *TP53* mutations and tobacco smoking and alcohol drinking. The mutation rate increased from 38% in never-smokers to 66% in current smokers (*P*-value for trend = 0.09). G:C>T:A transversions were found only in smokers (15%). Alcohol drinkers carried more G:C>A:T transitions (*P* = 0.08). Non-exposed individuals were more probable to carry G:C>A:T transitions at CpG sites (*P* = 0.01 for never-smokers and *P* < 0.001 for never-drinkers). *EGFR* mutations were found in 4% of cases. Inactivation of *TP53* by mutations is a crucial molecular event in the UADT carcinogenesis and it is closely related to exposure to lifestyle risk factors. *EGFR* mutations do not appear to be a common event in UADT carcinogenesis in this population.

Introduction

Cancers of the upper aerodigestive tract (UADT) comprise cancers of the oral cavity, oropharynx, hypopharynx and larynx, frequently referred to as head and neck cancers, as well as cancers of the oesophagus. It is estimated that >1 million new UADT cancer cases were diagnosed in the year 2002 and that these types of cancers were responsible for >680 000 deaths. There is a striking geographical variation in the incidence of UADT cancers worldwide, with some parts of South America (Brazil, Uruguay and Argentina) being high-risk areas (1). The major risk factors for UADT cancers in Europe and America are alcohol drinking and tobacco smoking, with evidence of a synergistic interaction (2,3) (IARC Monographs: Alcohol drinking, in preparation). However, in developing countries, other factors, like betel and tobacco chewing, human papillomavirus infections or drinking of hot beverages, also play an important role (4–7).

Abbreviations: SCC, squamous-cell carcinoma; UADT, upper aerodigestive tract.

The molecular mechanisms underlying the development of UADT tumours are not fully understood. The gene that is most frequently targeted for inactivation by mutations in UADT cancers is the *TP53* tumour suppressor gene. It encodes a protein that plays multiple roles in growth suppression, apoptosis and DNA repair in response to various types of stress. The IARC *TP53* mutation database, which compiles *TP53* mutations reported in the literature, records 3557 *TP53* mutations found in 8802 UADT cancer samples analysed (40%) (8). However, only four studies so far have investigated the prevalence and patterns of *TP53* mutations in South America (9–12). Another gene that is a common molecular target in human cancers is *EGFR*. It encodes the epidermal growth factor receptor and is mutated in a wide range of epithelial cancers, in particular adenocarcinoma of the lung in never-smokers (13). Its role in UADT cancers has not been extensively studied. Some studies report *EGFR* overexpression but few mutations have been found (14–16) and the *EGFR* mutation prevalence in UADT cancers in South America has never been estimated.

In our study, we assessed the prevalence and patterns of *TP53* mutations in tumours of the UADT from six centres in South America in combination with lifestyle risk factors. We have also conducted a pilot study of *EGFR* mutations on a subset of 45 tumours from the same study population.

Materials and methods

The study and subjects

An international multi-centre case–control study of upper aerodigestive cancers was initiated in 1998 in seven centres in South America (São Paulo, Goiania, Rio de Janeiro, Pelotas and Porto Alegre in Brazil, Buenos Aires in Argentina and La Havana in Cuba). Cases were patients with UADT cancers, newly diagnosed in one of the participating hospitals or referred to one of these hospitals for primary therapy, with no prior treatment, either local or systemic.

A total of 2479 incidental cancer cases and 1825 hospital controls were initially recruited, with a response rate of 95% for the cases and 86% for the controls. Informed consent was obtained from all study subjects and ethical approvals were obtained from relevant ethical committees. Each individual answered a detailed lifestyle questionnaire, including basic demographic characteristics and a detailed history of tobacco, alcohol and maté use, which was administered face-to-face in the hospital by a trained interviewer. Blood and buccal cells were collected from all cases and controls, and fresh tumour material was collected from cases whenever possible. Tumour tissue was not collected in the Cuban centre.

The cases were grouped into three main tumour site categories on the basis of the ICD-O classification of the site of origin:

(i) oral cavity and oropharynx, including floor of the mouth, tongue, other parts of oral cavity, oral cavity not otherwise specified, oropharynx and overlapping tumours with the origin in the oral cavity (overlapping oral cavity—oropharynx—hypopharynx not otherwise specified) (C00.3–C00.8, C01.9, C02.0–4, C02.8–9, C03.0–1, C03.9, C04.0–1, C04.8–9, C05.0–2, C05.8–9, C06.0–2, C06.8–9, C09.0–1, C09.8–9, C10.0–4, C10.8–9, C14.0, C14.2 and C14.8);
(ii) hypopharynx and larynx (C12.9, C13 and C32) and
(iii) oesophagus (C15).

Tumours overlapping more than one of the above categories were kept as a separate category.

DNA extraction and mutation analysis

DNA was extracted from 748 tumours (frozen tissue) using a standard QIAGEN DNA Tissue Kit protocol. A subset of 242 tumours stratified to oversample rare subgroups (e.g. never-smokers and never-alcohol drinkers) was selected for genetic analysis. This series of 242 tumours was screened for mutations in exons 5–9 of the TP53 gene by denaturing high-performance liquid chromatography and sequencing as described elsewhere (17). Results were obtained for 236 tumours. One hundred and eleven of the 113 tumours that were found wild-type in exons 5–9 were also analysed for mutations in exons 4 and 10 by direct sequencing. Additionally, 45 tumours were screened for mutations in exons 18–21 of the EGFR gene by denaturing high-performance liquid chromatography/sequencing (exon 19) or direct sequencing (other exons). This pilot series

was selected to include about the same number of cases for each tumour site (no overlapping locations included) and the maximum number of never-smokers.

Serology

The anti-p53 antibodies were detected in plasma samples using glutathione *S*-transferase fusions of full-length proteins as described elsewhere (18). The cut-offs for positive values were determined by the mean plus 5 SDs of the seroreactions of 106 female participants from a cross-sectional study among Korean students (19) and set to 1479 median fluorescence intensity units for p53.

Statistical analysis

Ever-smokers were defined as having smoked on average one cigarette, one cigar or one pipefill a day for at least 1 year. Individuals who quit smoking more than a year before the interview were considered to be former smokers. Ever-alcohol/maté drinkers were defined as having ever consumed alcoholic drinks/maté. Individuals who quit drinking more than a year before the interview were considered to be former drinkers.

The associations between *TP53* mutations and lifestyle characteristics of the patients were assessed by estimating odds ratios and 95% confidence intervals by unconditional multivariate logistic regression using Stata Intercooled, version 8.0, with adjustments for sex, age (continuous), centre and education, as well as alcohol or tobacco consumption. Depending on the number of observations, *P*-values were calculated by a chi-square test (two-sided) or by a two-tailed Fisher's exact test, with the exclusion of the missing values. Only confirmed squamous-cell carcinomas (SCCs) were included in the analysis of lifestyle risk factors.

Results

Patients and tumours

Detailed characteristics of the study subjects and tumours are presented in Table I. The largest number of cases was from Pelotas. Goiania provided only two cases and there were no cases from Cuba. Oesophageal cases were available only from Pelotas (45 cases) and Rio de Janeiro (1 case). Sixty-seven per cent of all cases were males. Thirteen per cent of patients were never-smokers, 19% had never drunk alcohol (Table 1) and 9% were both never-smokers and never-alcohol drinkers

Table I. Patients and tumours

	All sites (n = 242)[a]		Oral cavity + oropharynx (n = 119)		Hypopharynx + larynx (n = 73)		Oesophagus (n = 46)	
	n	%	*n*	%	*n*	%	*n*	%
Centre								
Argentina, Buenos Aires	42	17.4	24	20.2	15	20.5	0	0.0
Brazil, Porto Alegre	13	5.4	7	5.9	6	8.2	0	0.0
Brazil, Rio de Janeiro	55	22.7	31	26.1	23	31.5	1	2.2
Brazil, São Paulo	57	23.6	42	35.3	15	20.5	0	0.0
Brazil, Pelotas	73	30.2	15	12.6	12	16.4	45	97.8
Brazil, Goiania	2	0.8	0	0.0	2	2.7	0	0.0
Age (years)								
<40	8	3.3	5	4.2	2	2.7	1	2.2
40–49	39	16.1	21	17.6	14	19.2	4	8.7
50–59	86	35.5	45	37.8	25	34.2	13	28.3
60–69	67	27.7	34	28.6	18	24.7	14	30.4
70–79	32	13.2	13	10.9	12	16.4	7	15.2
>79	10	4.1	1	0.8	2	2.7	7	15.2
Sex								
Male	163	67.4	78	65.5	49	67.1	32	69.6
Female	79	32.6	41	34.5	24	32.9	14	30.4
Smoking								
Never-smokers	32	13.2	12	10.1	7	9.6	12	26.1
Former smokers (quit >1 year ago)	66	27.3	40	33.6	14	19.2	12	26.1
Current smokers	144	59.5	67	56.3	52	71.2	22	47.8
Alcohol drinking								
Never-drinkers	45	18.6	14	11.8	18	24.7	13	28.3
Former drinkers (quit >1 year ago)	62	25.6	33	27.7	16	21.9	13	28.3
Current drinkers	135	55.8	72	60.5	39	53.4	20	43.5
Tumour histology								
SCC	215	88.8	107	89.9	70	95.9	34	73.9
Not SCC	19	7.9	11	9.2	1	1.4	7	15.2
Missing values	8	3.3	1	0.8	2	2.7	5	10.9
Tumour stage								
T-stage								
TIS[b]	4	1.7	3	2.5	1	1.4	0	0.0
T1	18	7.4	5	4.2	5	6.8	8	17.4
T2	53	21.9	30	25.2	20	27.4	3	6.5
T3	54	22.3	24	20.2	16	21.9	14	30.4
T4	71	29.3	42	35.3	19	26.0%	6	13.0
Missing values	42	17.4	15	12.6	12	16.4	15	32.6
N-stage								
N0	103	42.6	50	42.0	37	50.7	15	32.6
N1	40	16.5	17	14.3	9	12.3	13	28.3
N2	47	19.4	29	24.4	16	21.9	0	0.0
N3	12	5.0	8	6.7	4	5.5	0	0.0
Missing values	40	16.5	15	12.6	7	9.6	18	39.1
M-stage								
M0	170	70.2	89	74.8	55	75.3	24	52.2
M1	9	3.7	1	0.8	2	2.7	5	10.9
Missing values	63	26.0	29	24.4	16	21.9	17	37.0

[a]Four cases were overlapping more than one UADT site.
[b]TIS, carcinoma in situ.

(data not shown). Eighty-nine per cent of the tumours were SCC. The series included tumours from all stages of tumour growth (no particular T-stage group prevailed). Forty-three per cent of them had no extension to lymph nodes and 70% did not metastasize (Table I).

TP53 mutations

A total of 159 *TP53* mutations in exons 4–10 were found in 143 of 236 analysable tumours (61% mutant tumours). Fifteen samples carried more than one *TP53* mutation (14 samples—two distinct mutations and 1 sample—three distinct mutations). Additionally, eight tumours (two of them carrying mutant *TP53*) contained a known A/G polymorphism at codon 213 (exon 6) and eight others (three with mutant *TP53*) a G/C polymorphism at base 13964 of intron 6. Twelve of the mutations and two of the polymorphisms were detected in tumours that were not confirmed SCC (supplementary material is available at *Carcinogenesis* Online).

The mutations were about evenly distributed throughout exons 4–10 of the *TP53* gene, especially in exons 5–8. Eighteen base changes (11%) were detected in introns, 14 of which were within the splice site regions. The codons that were most frequently targeted by point mutations were codons 179 and 248 (5 and 7% of all exonic point mutations, respectively).

The prevalence of mutations by tumour site ranged from 56% in cancers of the hypopharynx and larynx and 57% in cancers of the oral cavity and oropharynx, up to 80% in cancers of the oesophagus. When only confirmed SCCs were considered, the prevalence was 59% in oral cavity and oropharynx, 58% in hypopharynx and larynx and 88% in oesophagus (P-value = 0.03; Table II). The *TP53* mutation status was neither associated with the TNM stage of the tumour (P-value = 0.40 for the T-stage, $P = 0.19$ for the N-stage and $P = 0.71$ for the M-stage) nor with the presence of the p53 antibodies ($P = 1.00$) (Table II). There were differences in mutation prevalence by country—in Brazil, 70% of the SCCs were mutated, whereas in Argentina (Buenos Aires), the prevalence was only 26% (P-value < 0.001). This difference was maintained after excluding from the analysis the oesophageal cases, which were available only in Brazil (66% mutant head and neck cases in Brazil versus 26% in Buenos Aires, P-value < 0.001). In a stratification by cancer site, a significant difference in mutation prevalence by country was observed for cancers of the hypopharynx and larynx (69% mutant cases in Brazil versus 14% in Buenos Aires, P-value < 0.001) but less for oral cavity and oropharynx (63% mutant cases in Brazil versus 39% in Buenos Aires, P-value = 0.06).

Mutation types were grouped into six categories—A:T all (A:T>G:C, A:T>C:G and A:T>T:A), G:C>A:T, G:C>A:T at CpG sites, G:C>C:G, G:C>T:A and complex mutations (deletions, insertions, inversions, tandem and other complex mutations). Fifty-one per cent of all *TP53* alterations (81/159) were missense mutations and 24% (38/159) were complex mutations, including insertions, deletions, inversions and other complex mutations (supplementary material is available at *Carcinogenesis* Online). Eighty per cent of them resulted in proteins that were reported not to retain any wild-type transactivation functions as based on the yeast assay (8). No particular pattern appeared in an analysis by tumour site, with the exception of G:C>T:A transversions. The lowest proportions of these mutations were detected in the proximal part of the UADT (9% in oral cavity and oropharynx). These proportions increased to 22% in the larynx and to 14% in the oesophagus; however, this was based on low numbers.

TP53 mutations in relation to lifestyle risk factors

The prevalence, distribution and types of *TP53* mutations were analysed in relation to lifestyle risk factors in SCC patients (209 cases and 145 mutations). Smokers were more probable to carry *TP53* mutations than never-smokers and the prevalence of mutations increased from 38% in never-smokers through 64% in former smokers (adjusted mutation odds ratio: 3.98, 95% confidence interval: 1.05–15.12) to 66% in current smokers (odds ratio: 4.01, confidence interval: 1.15–14.02; P-value for linear trend 0.09). Alcohol and maté drinking were not associated with increased prevalence of *TP53* mutations (Table III).

Although the numbers of non-exposed individuals were small, the codon distribution of *TP53* mutations differed with different lifestyle

Table II. TP53 status and clinical and biological characteristics of the UADT SCC tumours

	TP53 status			
	Wt ($n = 78$; 37%)		Mut ($n = 131$; 63%)	
Tumour site				
Oral cavity + oropharynx	42	41%	60	59%
Hypopharynx + larynx	29	42%	40	58%
Oesophagus	4	12%	30	88%
Overlapping sites other than oral	3	75%	1	25%
Fisher's exact test P-value[a]	$P = 0.002$			
Tumour stage				
T-stage				
TIS	2	67%	1	33%
T1	8	53%	7	47%
T2	20	43%	27	57%
T3	15	31%	34	69%
T4	25	40%	37	60%
Missing values	8	24%	25	76%
Fisher's exact test P-value[a]	$P = 0.397$			
N-stage				
N0	37	42%	51	58%
N1	9	25%	27	75%
N2	19	43%	25	57%
N3	6	55%	5	45%
Missing values	7	23%	23	77%
Fisher's exact test P-value[a]	$P = 0.188$			
M-stage				
M0	58	38%	95	62%
M1	2	29%	5	71%
Missing values	18	37%	31	63%
Fisher's exact test P-value[a]	$P = 0.712$			
Anti-p53 antibodies				
No	62	38%	103	62%
Yes	11	37%	19	63%
Missing values	5	36%	9	64%
Chi-square test P-value[a]	$P = 1.000$			

Mut, mutant; TIS, carcinoma in situ; Wt, wild-type.
[a]P-values were calculated from a two-sided Fisher's exact test or chi-square test, with the exclusion of the missing values.

Table III. *TP53* mutation status and lifestyle characteristics of the SCC patients

	TP53 status				OR (95% CI)[a]
	Wt		Mut		
	n	%	*n*	%	
Tobacco smoking					
Never-smoker ($n = 21$)	13	62	8	38	1.00 (ref)
Ex-smoker >1year ($n = 58$)	21	36	37	64	3.98 (1.05–15.12)
Current smoker ($n = 130$)	44	34	86	66	4.01 (1.15–14.02)
P for linear trend					$P = 0.091$
Alcohol drinking					
Never-drinkers ($n = 31$)	12	39	19	61	1.00 (ref)
Ever-drinkers ($n = 178$)	66	37	112	63	1.34 (0.47–3.82)
Maté drinking					
Never-drinkers ($n = 116$)	44	38	72	62	1.00 (ref)
Ever-drinkers ($n = 93$)	34	37	59	63	1.63 (0.59–4.55)

Mut, mutant; Wt, wild-type; OR, odds ratio; CI, confidence interval.
[a]Odds ratios were adjusted for age, sex, centre, education and alcohol and/or tobacco consumption as relevant.

exposures. In never-smokers, 38% of all exonic point mutations in SCC cases (and 45% if also non-SCC cases were considered) were detected at two codons of exon 8: 271 (13%) and 273 (25%), whereas no mutations at codon 273 and 1% mutations at codon 271 were found in current smokers. An inverse trend was observed for mutations at codon 248; the prevalence increased from no mutation in never-smokers through 5% in former smokers up to 9% in current smokers. In never-alcohol drinkers with SCC, the most frequently targeted codon was codon 282. The point mutation prevalence at this codon decreased from 13% in never-drinkers, through 4% in former drinkers, to 2% in current drinkers.

Also mutation patterns differed depending on exposure to different lifestyle risk factors. G:C>T:A transversions were found only in tobacco smokers and they represented 15% of all mutations found in this group (*P*-value for G:C>T:A transversions in smokers versus never-smokers $P = 0.40$). G:C>A:T transitions were more prevalent in alcohol drinkers than in never-drinkers (23% as compared with 5%, $P = 0.08$). Non-exposed individuals had a higher prevalence of endogenous G:C>A:T mutations at CpG sites. Over a half of all mutations found in never-smokers (56% as compared with 11% in smokers, $P = 0.01$) and 43% in never-drinkers as compared with 9% in drinkers ($P < 0.001$) were mutations targeting these sites (Figure 1). Maté drinking was not associated with any particular *TP53* mutation type pattern (data not shown).

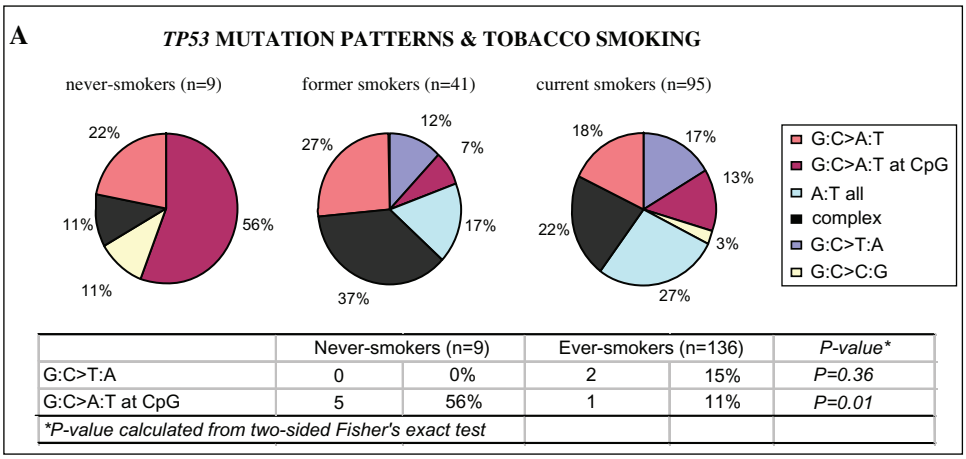
Serology data were available for 195 of the 209 SCC patients for whom the *TP53* mutation status was assessed. Only 15% of them tested positive for antibodies against p53 with no associations with the *TP53* status.

EGFR mutations

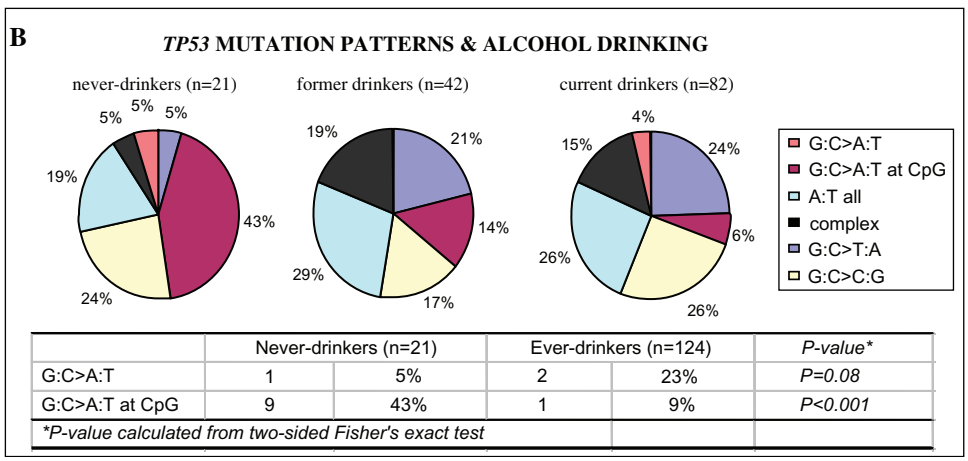
A pilot series of 45 tumours was additionally screened for mutations in exons 18–21 of the *EGFR* gene by denaturing high-performance liquid chromatography/sequencing or direct sequencing. Thirty-five of them were confirmed SCC cases. Seven DNA alterations were detected in 45 tumours, however, three of them were in introns in regions not affecting the splicing patterns and of the remaining four mutations, two were silent. Overall, only two tumours (4%) carried *EGFR* mutations that had an impact on protein structure: one of them (a current smoker) had a missense base substitution at codon 848 (exon 21) and the other (never-smoker) had an in-frame 15 bp deletion starting at codon 745 (exon 19) (supplementary material is available at *Carcinogenesis* Online). Both mutant tumours were SCC. Besides, two known polymorphisms were detected in 24 of 45 samples (53%): a G/A polymorphism at the third base of codon 787 (exon 20) was detected in 23 tumours and a C/G polymorphism at the third base of codon 836 (exon 21) in one tumour.

Discussion

In this study, we have analysed genetic alterations in relation to aetiological factors in a large series of cancers of the UADT in patients from South America, a part of the world where these cancers are frequent. We report an overall *TP53* mutation prevalence of 63% among the 209 SCC in our study population, which is higher than that reported in the IARC *TP53* database for all UADT tumours worldwide (40%) (8) and close to that reported by Nagai *et al.* (11) for head and neck cancers in Brazil. However, if we restrict our



	Never-smokers (n=9)		Ever-smokers (n=136)		*P-value**
G:C>T:A	0	0%	2	15%	*P=0.36*
G:C>A:T at CpG	5	56%	1	11%	*P=0.01*
**P-value calculated from two-sided Fisher's exact test*					



	Never-drinkers (n=21)		Ever-drinkers (n=124)		*P-value**
G:C>A:T	1	5%	2	23%	*P=0.08*
G:C>A:T at CpG	9	43%	1	9%	*P<0.001*
**P-value calculated from two-sided Fisher's exact test*					

Fig. 1. *TP53* mutation patterns in relation to lifestyle risk factors in SCC cases. (**A**) TP53 mutations and tobacco smoking; (**B**) *TP53* mutations and alcohol drinking.

estimate to Brazil, the prevalence reaches 70%. Our estimates for particular UADT sites are also higher than in previous studies (9,12), ranging from 58% for hypopharynx and larynx to as much as 88% for oesophagus. So far, such a high prevalence of *TP53* mutations have been reported only in specific areas of high incidence for SCC, in particular SCC of the oesophagus (e.g. Normandy, France: 80% and Lixian Province, China: >65%) (8). Furthermore, we observed a significant increase in the prevalence of mutations in smokers as compared with never-smokers. Altogether, these results are consistent with the notion that *TP53* mutation prevalence in SCC of the UADT is a good marker of the mutation load due to environmental mutagens.

Our analysis shows the importance of p53 mutations in tobacco-related UADT carcinogenesis. The evidence for this comes from both the increased likelihood of mutations in smokers and from the presence in smokers of a higher proportion of G:C to T:A transversions, a type of mutation that has been described as resulting from mutagenesis by bulky carcinogens from tobacco smoke, such as polycyclic aromatic hydrocarbons. In lung cancers of heavy smokers, this type of mutation accounts for >30% of all mutations but is infrequent (~4%) in never-smokers (20). Conversely, among smokers, we observed a significant decrease in the proportion of G:C to A:T transversions at CpG sites, a type of mutation commonly associated with endogenous mutagenic processes such as spontaneous deamination of methylated cytosine into thymine. This phenomenon has also been observed in lung cancers of never-smokers (20). An additional argument in favour of a direct mutagenic effect of tobacco is the tendency for the proportion of G:C to T:A transversions to increase from proximal (oral cavity and oropharynx) to distal parts of the respiratory tract (hypopharynx and larynx) and to the bronchial tree.

We also found differences in mutation patterns in relation with the consumption of alcoholic beverages. In this case, there was no difference in the prevalence of mutations between the exposed and non-exposed patients, but there was a change in the mutations pattern, with a shift in the proportion of G:C to A:T transitions. In never-drinkers, these transitions represented 48% of all mutations and a vast majority of them (90%) occurred at CpG sites. In current drinkers, these mutations accounted for 31%, 80% of them being at non-CpG sites. This shift in the position and sequence context of G:C to A:T transitions may correspond to a shift in mutagenic processes, from essentially endogenous, non-carcinogen-induced processes to exogenous and carcinogen-induced mechanisms. Many carcinogens may induce G:C to A:T transitions, including several types of nitrosamines such as those found in different types of alcoholic beverages, although at low levels. In previous studies on oesophageal cancer, it has been proposed that a high proportion (>40%) of G:C>A:T mutations may be suggestive of mutagenesis by acetaldehyde, a metabolite of ethanol. Indeed, this type of mutation is particularly frequent in oesophageal SCC from areas where alcohol is thought to play a major aetiological role, such as north-west France (21). Moreover, there is *in vitro* evidence that acetaldehyde can induce A:T base pair mutations in various experimental systems (22).

In contrast to tobacco and alcohol, maté drinking did not appear to leave any specific fingerprint in *TP53* mutation prevalence or patterns. This observation, however, does not demonstrate that maté in itself does not contribute to SCC of the UADT as a source of mutagens.

We did not find any associations between the *TP53* status and clinico-pathological features of the tumours or patient seropositivity for p53. There have been no studies so far investigating the correlation between the *TP53* status in the tumour and the presence of anti-p53 antibodies in UADT cancers in South America. The only such study was conducted for oral cancer in South Africa and no correlation was found (23).

Our study is the largest and most comprehensive study on *TP53* mutations in UADT cancers in South America. Only four studies so far have estimated the prevalence of *TP53* mutations in South America, three of which studied Brazilian populations (9,11,12). In a study of 90 SCC of the head and neck from São Paulo, Nagai *et al.* (11) have found *TP53* mutations in 53% of cases using single strand conformation polymorphism and sequencing. Chaves *et al.* (9) reported *TP53* mutations in 40% of oral cancer patients alone, in a study of 76 SCC cases collected in Porto Alegre using the same detection method. In the only study devoted to oesophageal cancer, Putz *et al.* (12) detected *TP53* mutations in 36% of 135 SCCs from Rio Grande do Sul using immunohistochemistry as a prescreening method and single strand conformation polymorphism and sequencing for mutation identification.

Our study also provides the first ever estimate of the prevalence of *EGFR* mutations in UADT cancers in South America. Only two mutations were found in a pilot series of 45 tumours. This low prevalence is consistent with the results of *EGFR* studies conducted in North America and Asia, which report *EGFR* overexpression but state that *EGFR* mutations are infrequent (14–16). However, it should be kept in mind that in all lung cancers taken together, the prevalence of *EGFR* mutations is equally low (up to 5%). These mutations are essentially restricted to a specific histological and exposure category: lung adenocarcinomas in never-smokers (24). In our study, one of the two mutations was found in a current smoker. However, our results are too limited to determine whether activating *EGFR* mutations may be restricted to a particular category of UADT SCC patients in South America. Still, this observation raises the possibility that a small proportion of patients may benefit from therapy using tyrosine kinase inhibitors. Given the poor therapeutic outcome of many SCC of the UADT, the possibility to improve survival by the use of a targeted therapy deserves further investigations.

Overall, we conclude that inactivation of the *TP53* tumour suppressor gene by mutations is an important molecular event in the tumorigenesis of the UADT in South America. The prevalence and patterns of *TP53* mutations in UADT tumours in these populations are closely related to exposures to environmental carcinogens, especially tobacco smoke. An important next step will be to investigate whether the presence of mutations in *TP53* can be used as prognostic marker. Further studies are needed to estimate the prevalence of *EGFR* mutations in particular exposure categories and potentially identify groups of patients that could benefit from targeted therapy.

Supplementary material

Supplementary material can be found at http://carcin.oxfordjournals.org/

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