

# A meta-analysis of cancer risk associated with the *TP53* intron 3 duplication polymorphism (rs17878362): geographic and tumor-specific effects

C Sagne<sup>1,2,3</sup>, V Marcel<sup>1,2,4</sup>, A Amadou<sup>3</sup>, P Hainaut<sup>3,5</sup>, M Olivier<sup>3</sup> and J Hall<sup>\*1,2</sup>

We have performed a meta-analysis of cancer risk associated with the rs17878362 polymorphism of the *TP53* suppressor gene (*PIN3*, (polymorphism in intron 3), 16 bp sequence insertion/duplication in intron 3), using a compilation of a total of 25 published studies with 10 786 cases and 11 760 controls. Homozygote carriers of the duplicated allele (A2A2) had a significantly increased cancer risk compared with A1A1 carriers (aggregated odds ratio (OR) = 1.45, 95% confidence interval (CI) = 1.22–1.74). However, there was no significant effect for the A1A2 heterozygotes (A1A2 versus A1A1 aggregated OR = 1.08, 95% CI = 0.99–1.18). No significant heterogeneity or publication bias was detected in the data set analysed. When comparing populations groups, increased cancer risk was associated with A2A2 carriage in Indian, Mediterranean and Northern Europe populations but not in the Caucasian population of the United States. Analysis by cancer site showed an increased risk for A2A2 carriers for breast and colorectal, but not for lung cancers. These results support that the A2A2 genotype of rs17878362 is associated with increased cancer risk, with population and tumour-specific effects.

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The *TP53* gene (OMIM 191170), encoding the p53 protein, is frequently inactivated in sporadic human tumours, disabling a wide range of anti-proliferative responses regulating cell cycle progression, apoptosis, autophagy, differentiation, senescence, DNA repair and oxidative metabolism.<sup>1–4</sup> The activity of p53 is regulated by multiple transcriptional, post-transcriptional, translational and post-translational mechanisms in response to a wide range of physical and biological stresses, endowing this protein with a pivotal role in preventing DNA replication and cell division in conditions that threaten genetic integrity.<sup>1,5–7</sup> Among these mechanisms, the expression of p53 as multiple protein isoforms with different N- and/or C-terminal domains has recently emerged as a form of regulation that may participate in the diversity of the repertoire of biological effects mediated by p53 (reviewed in Marcel *et al*).<sup>8</sup>

Close to 100 genetic polymorphisms have been identified in *TP53* (listed at <http://p53.iarc.fr>),<sup>9</sup> many of which show geographic and population frequency variations. However, their effects on cancer risk appear to be inconsistent across studies.<sup>10,11</sup> The most studied polymorphism is a single-nucleotide polymorphism (SNP) in exon 4 encoding an arginine (R) or a proline (P) at codon 72 (rs1042522, G>C, R>P at codon 72, PEX4 (polymorphism in exon 4)).<sup>12</sup>

There is *in vitro* evidence that the rs1042522 R72 and P72 p53 protein variants differ by their biological activities.<sup>13,14</sup> However, results from systematic studies and meta-analyses have failed to identify a consistent association with cancer risk.<sup>15–19</sup>

The most common intronic variation in *TP53* is a 16-base pair (bp)<sup>11</sup> insertion/duplication in intron 3 (rs17878362, consisting of one copy (A1 allele) or two copies (A2 allele) of the sequence ACCTGGAGGGCTGGGG, *PIN3* (polymorphism in intron 3 (rs17878362))).<sup>20</sup> Several case–control studies have reported an increased risk of various cancer types associated with the rs17878362 A2 allele in Caucasians, with the most consistent association reported for breast,<sup>21,22</sup> and colorectal cancers.<sup>23,24</sup> A recent meta-analysis identified a small but significant increase in overall cancer risk of 14% (95% confidence interval (CI) = 1.02–1.27) in homozygote carriers of the A2 allele.<sup>25</sup> However, this conclusion was questioned because of apparent discrepancies between data selected for meta-analysis and the original publications.<sup>26</sup> At the mechanistic level, there is some evidence that rs17878362 may have an impact on the levels<sup>23</sup> and alternative splicing of the *TP53* mRNA, and thus on the ratios of p53 protein isoforms.<sup>8</sup> However, the precise mechanisms underlying an increased cancer risk associated with the rs17878362 A2 allele are not clearly understood.

<sup>1</sup>INSERM U612, Bât 110-112, Centre Universitaire, Orsay, France; <sup>2</sup>Institut Curie, Centre de Recherche, Bât 110-112, Centre Universitaire, Orsay, France and <sup>3</sup>Molecular Carcinogenesis Group, International Agency for Research on Cancer, 150 cours Albert Thomas, Lyon, France

\*Corresponding author: Dr J Hall, Institut Curie, Centre de Recherche, INSERM U612, Bât 110-112, Centre Universitaire, Orsay 91405, France.

Tel: +33 (0)1 69 86 30 61; Fax: +33 (0)1 69 07 53 27; E-mail: [janet.hall@curie.fr](mailto:janet.hall@curie.fr)

<sup>4</sup>Present address: Cancer Research Centre of Lyon, UMR INSERM 1052 CNRS 5286, Centre Léon Bérard, FNCLCC, Nuclear Domains and Pathologies, 28 rue Laennec, 69373 Lyon, France.

<sup>5</sup>Present address: International Prevention Research Institute, 95 cours Lafayette, 69006 Lyon, France.

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**Abbreviations:** Bp, base pair; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; PEX4, polymorphism in exon 4 (rs1042522); *PIN3*, polymorphism in intron 3 (rs17878362); *PIN6*, polymorphism in intron 6 (rs1625895); SNP, single-nucleotide polymorphism

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To assess whether the rs17878362 polymorphism may represent a potentially important and relevant genetic marker contributing to cancer susceptibility, we have performed an independent, two-stage meta-analysis on a total of 10 786 cancer cases and 11 377 controls from 25 published case–control studies. First, we have analysed the overall cancer risk associated with the A2 allele and second we have performed sub-group analyses to examine this association in different populations and for specific cancer types. Data for the rs1042522 and rs1625895 (rs1625895, intron 6, G>A, PIN6 (polymorphism in intron 6)) variant alleles in relation to cancer risk was also compiled and analysed from the same publication set to assess their potential confounding effect.

## Results

**Characteristics of selected publications.** A total of 25 publications out of the 299 identified met the necessary inclusion criteria for the meta-analysis that required the reporting of odds ratio (OR) data and information on the frequency of each allele, which has been verified to be in Hardy–Weinberg equilibrium in each control population (Table 1 and Supplementary Table 1). Two studies<sup>24,27</sup> used the same control populations and they were included only once to avoid over-representation. Overall, nine individual studies reported a significant increase in cancer risk associated with the rs17878362 A2 allele compared with the A1 allele, 16 showed no statistical association between either allele and cancer susceptibility and no study reported an association between the A2 allele and decreased cancer risk (Table 1).

**The A2A2 genotype of rs17878362 polymorphism increases cancer risk.** On the basis of the results of the heterogeneity testing, a random model was used for the meta-analysis to assess the overall cancer risk in A2 allele carriers (A1A2 or A2A2) (Table 2).<sup>28</sup> The rs17878362 minor allele frequency (MAF) was inferior to 0.17 in control subjects in the different sub-groups and allele ratios were compatible with Hardy–Weinberg equilibrium (data not presented). No significant association with cancer risk was found in the heterozygous A1A2 carriers compared with the homozygous A1A1 carriers (A1A2 *versus* A1A1 aggregated OR = 1.08, 95% CI = 0.99–1.18), however, a significantly increased risk was found for the A2A2 carriers (A2A2 *versus* A1A1 aggregated OR = 1.45, 95% CI = 1.22–1.74). Leave-one-out analyses showed that the aggregated OR for the A1A2 *versus* A1A1 genotypes varied between 1.06 and 1.10 (95% CI between 0.97 and 1.20) and for the A2A2 *versus* A1A1 genotypes between 1.37 and 1.55 (95% CI between 1.15 and 1.91) (Supplementary Table 2). The Egger's bias coefficient was determined to assess a possible bias introduced by any single study. The ORs for Egger's bias coefficient were 0.07, (95% CI = 1.32–1.46) for the A1A2 genotype, and 0.79 (95% CI = 0.62–2.19) for the A2A2 genotype, suggesting no significant publication bias.

To assess the possibility that the overall result might be biased by initial publications reporting a large effect, a

cumulative inclusion over time analysis was conducted. For the A1A2 genotype, the first set of studies (four reports published before 2006) had the highest ORs for the association between the A1A2 genotype and cancer risk (Supplementary Table 3). Lower values were reported in the following 2 years, after which the overall result remained stable (aggregated OR 1.08 for 2010 and 2011). For the A2A2 allele, the time trend for the aggregated OR showed little variation, with ORs between 1.37 and 1.45 being reported since 2007, in support of the robustness of this association.

**rs17878362-related cancer risk is dependent on ethnicity and geographical origin.** To investigate whether rs17878362 related cancer susceptibility varies between populations and geographical regions, the data from the 25 studies were divided into four geographical sub-groups (India, Northern Europe, North America and the Mediterranean area) each containing at least 1000 cases and 1000 controls from a minimum of five independent case–control studies (Table 2, see Table 1 and Supplementary Table 1 for population details). Differences in genotype distribution were noted with that of the Indian controls being statistically different from the three other control sub-groups (India *versus* Mediterranean countries:  $\chi^2$  *P*-value 0.01, India *versus* Northern Europe or United States:  $\chi^2$  *P*-values <0.01). The genotype distribution found in the United States' controls (reported as a Caucasian population in the original publications) was also different from that of the Northern Europe controls ( $\chi^2$  *P*-value 0.01). No difference in genotype distribution was observed between controls from the Mediterranean and from Northern Europe or United States (Mediterranean countries *versus* Northern Europe:  $\chi^2$  *P*-value 0.49, Mediterranean countries *versus* United States:  $\chi^2$  *P*-value 0.14).

In this geographical sub-group analysis, the homozygous A2A2 genotype was associated with an increased cancer risk in Indian (A2A2 *versus* A1A1 aggregated OR = 1.63, 95% CI = 1.10–2.42) and Northern Europe populations (A2A2 *versus* A1A1 aggregated OR = 1.70, 95% CI = 1.26–2.31) compared with the homozygous A1A1 genotype. For the Mediterranean population, both the A1A2 and A2A2 genotypes were associated with increased cancer susceptibility in an A2 allelic dose-dependent manner (A1A2 *versus* A1A1 aggregated OR = 1.25, 95% CI = 1.03–1.51; A2A2 *versus* A1A1 aggregated OR = 2.54, 95% CI = 1.53–4.24, *P*-trend <0.01). In contrast, in the United States' sub-group (3,963 cases and 3,731 controls), no increased cancer susceptibility was associated with carriage of the rs17878362 A2 allele (A1A2 *versus* A1A1 aggregated OR = 1.09, 95% CI = 0.87–1.38; A2A2 *versus* A1A1 aggregated OR = 1.02, 95% CI = 0.73–1.43).

**rs17878362-related cancer risk is dependent on cancer type.** The risk of developing cancer was assessed for three cancer types: lung, colon and breast, with over 1600 cases and controls included in the analysis (Table 3). For colorectal cancer, homozygous A2A2 carriage was associated with increased susceptibility compared with homozygous A1A1 carriage (A2A2 *versus* A1A1 aggregated

**Table 1** Characteristics of the 25 case–control studies selected for TP53 rs17878362 (PIN3) polymorphism meta-analysis

Study numbers and study	Cancer type	Cases	Controls	Population	Minor allele frequency in controls (MAF)			Hardy–Weinberg equilibrium P-value for controls			
					rs17878362 (A2)	rs1042522 (P72)	rs1625895 (A)	rs17878362 (A2)	rs1042522 (P72)	rs1625895 (A)	
1	Jha <i>et al.</i> <sup>40 a</sup>	Glial tissue	84	76	India	0.18	0.55	NA	0.23	0.01 <sup>b</sup>	NA
2	Umar <i>et al.</i> <sup>41 a</sup>	Oesophagus	255	255	India	0.19	NA	NA	0.33	NA	NA
3	Alawadi <i>et al.</i> <sup>42 a</sup>	Breast	229	133	NC	0.31	0.44	NA	0.58	0.01 <sup>b</sup>	NA
4	Mittal <i>et al.</i> <sup>43 a</sup>	Prostate	177	265	India	0.15	0.24	0.21	0.12	0.28	0.11
5	Malik <i>et al.</i> <sup>27 c</sup>	Oesophagus	135	<sup>d</sup> 195	India	0.21	NA	NA	0.08	NA	NA
6	Malik <i>et al.</i> <sup>27 c</sup>	Gastric	108	<sup>d</sup> 195	India	0.21	NA	NA	0.08	NA	NA
7	Naccarati <i>et al.</i> <sup>44 a</sup>	Pancreas	240	743	Northern Europe	0.16	0.29	NA	0.10	0.40	NA
8	Polakova <i>et al.</i> <sup>45 a</sup>	Colon	612	613	Northern Europe	0.14	0.27	NA	0.15	0.52	NA
9	Ashton <i>et al.</i> <sup>30 a</sup>	Endometrial	190	291	NC	0.14	0.24	0.11	0.81	0.97	0.12
10	de Feo <i>et al.</i> <sup>46 a</sup>	Gastric	114	295	Mediterranean	0.16	0.25	0.20	0.35	0.13	0.15
11	Hrstka <i>et al.</i> <sup>47 a</sup>	Breast	117	108	Northern Europe	0.14	0.45	0.13	0.46	0.00 <sup>b</sup>	0.78
12	Gaudet <i>et al.</i> <sup>48 a</sup>	Breast	578	390	United States	0.16	0.74	0.85	0.85	0.08	0.93
13	Costa <i>et al.</i> <sup>21 c</sup>	Breast	191	216	Mediterranean	0.17	0.17	NA	0.29	0.29	NA
14	Ye <i>et al.</i> <sup>49 a</sup>	Bladder	636	618	United States	0.15	0.22	0.15	0.29	0.00 <sup>b</sup>	0.13
15	de Vecchi <i>et al.</i> <sup>50 a</sup>	Breast	350	352	Mediterranean	0.15	0.23	NA	0.62	0.23	NA
16	Chen <i>et al.</i> <sup>51 a</sup>	Head and neck	821	818	United States	0.14	0.27	0.12	0.75	0.07	0.67
17	Tan <i>et al.</i> <sup>52 a</sup>	Colon	467	563	Northern Europe	0.17	0.22	NA	0.23	0.98	NA
18	Wang <i>et al.</i> <sup>53 a</sup>	Lung	1412	1363	United States	0.13	0.26	0.12	0.45	0.54	0.14
19	Hung <i>et al.</i> <sup>54 c</sup>	Lung	2126	2140	Northern Europe	0.13	0.27	NA	0.50	0.74	NA
20	Perfumo <i>et al.</i> <sup>24 a</sup>	Colon	60	188 <sup>e</sup>	Mediterranean	0.15	0.20	NA	0.21	0.81	NA
21	Perfumo <i>et al.</i> <sup>24 c</sup>	Colon	124	188 <sup>e</sup>	Mediterranean	0.15	0.20	NA	0.21	0.81	NA
22	Mitra <i>et al.</i> <sup>55 a</sup>	Oral cancer	307	342	India	0.19	0.48	NA	0.56	0.20	NA
23	Gemignani <i>et al.</i> <sup>23 c</sup>	Colon	374	322	Mediterranean	0.12	0.21	NA	0.60	0.09	NA
24	Wang-Gohrke <i>et al.</i> <sup>22 c</sup>	Breast	563	549	Northern Europe	0.16	0.26	0.15	0.92	0.49	0.60
25	Wu <i>et al.</i> <sup>56 c</sup>	Lung	516	542	United States	0.10	0.20	0.12	0.05	0.01 <sup>b</sup>	0.18

Abbreviations: NA, not available; NC, not classified

<sup>a</sup>no significant increase in cancer risk associated with rs17878362 (TP53 PIN3)

<sup>b</sup>P-value < 0.05 indicates a Hardy–Weinberg disequilibrium: study exclusion

<sup>c</sup>Significant increase in cancer risk associated with rs17878362 (TP53 PIN3)

<sup>d</sup>Same control population

<sup>e</sup>Same control population

OR = 1.67, 95% CI = 1.02–2.74) (Table 3). A slight but significant increased breast cancer risk was observed in the heterozygous A1A2 carriers compared with the A1A1 carriers (A1A2 *versus* A1A1 aggregated OR = 1.18, 95% CI = 1.02–1.37). However, no altered breast cancer risk was seen in the A2A2 carriers (A2A2 *versus* A1A1 aggregated OR = 1.41, 95% CI = 0.97–2.06), although a significant trend towards increased cancer risk was noted as the number of A2 alleles carried was increased (*P*-trend < 0.01). No increased risk of lung cancer was observed for any genotype despite the inclusion of 4101 cases and 4052 controls in the analysis (A2A2 *versus* A1A1 aggregated OR = 1.46, 95% CI = 0.71–3.00).

**Association of rs1042522 and rs1625895 genotypes with cancer susceptibility.** Among the 25 selected publications, several have analysed cancer risk associated with the rs1042522 and rs1625895 variant alleles (Table 1). For rs1625895, the 10 studies reporting rs1625895-related ORs showed rs1625895 allele ratios compatible with Hardy–Weinberg equilibrium, allowing the pooling of 5011 cancer cases and 5100 controls. For the rs1042522 polymorphism, 8517 cases and 9311 controls were pooled from 17 studies (Supplementary Table 4), while 5 other studies were excluded as the allele ratios for rs1042522 in controls were not compatible with Hardy–Weinberg equilibrium (Table 1). When compared with the respective common homozygous carriers, a small but significant association with cancer

risk was observed for heterozygous carriers of the variant allele (rs1042522 R72/P72 *versus* R72/R72 aggregated OR = 1.16, 95% CI = 1.05–1.18; rs1625895 GA *versus* GG aggregated OR = 1.19, 95% CI = 1.02–1.40) (Supplementary Table 4). However, no increased risk was observed in association with the homozygous carriage of the variant alleles at either position.

## Discussion

A large number of studies have addressed the association of common *TP53* polymorphisms with cancer risk (reviewed in Whibley *et al.*<sup>10</sup>). Overall, the reported effects are of small amplitude and many studies have reported contradictory results that may result from many causes: small numbers of cases and controls and thus limited statistical power, the selection of specific tumour types, differences between populations and the lack of reliability in SNP genotyping, in particular in earlier studies. Of the *TP53* intronic polymorphisms rs17878362 is the most studied. In this meta-analysis, based on 10786 cases and 11377 controls we detected an aggregated OR of 1.45 (95% CI = 1.22–1.74) for increased cancer risk in homozygous carriers of the rare rs17878362 A2 genotype as compared with homozygous carriers of the common A1 genotype. However, no risk was observed when A2A1 carriers were compared with the A1A1 carriers, suggesting that the increased risk associated with rs17878362 follows a recessive model. This result is in

**Table 2** Meta-analysis results for the selected case-control studies focused on the TP53 rs17878362 polymorphism

Genotypes	Cases, n (%)	Controls, n (%)	Heterogeneity, P-value	OR	(95% CI)	P-trend <sup>a</sup>
<i>Overall (25 studies, MAF = 0.15)</i>						
Total	10 786 (100.0)	11 377 (100.0)		1.00	—	<0.01
A1A1	7 639 (70.8)	8 254 (72.5)	0.03 <sup>b</sup>	1.08	(0.99–1.18)	
A1A2	2 823 (26.2)	2 871 (25.2)	0.06 <sup>b</sup>	1.45	(1.22–1.74)	
A2A2	324 (3.0)	252 (2.3)				
<i>Geographical origin of studies India (study numbers: 1, 2, 4, 5, 6, 22; MAF = 0.19)</i>						
Total	1 066 (100.0)	1 133 (100.0)		1.00	—	0.19
A1A1	699 (65.6)	750 (66.2)	0.54 <sup>c</sup>	0.94	(0.79–1.13)	
A1A2	304 (28.5)	345 (30.5)	0.07 <sup>c</sup>	1.63	(1.10–2.42)	
A2A2	63 (5.9)	38 (3.3)				
<i>Mediterranean countries (study numbers: 10, 13, 15, 20, 21, 23; MAF = 0.15)</i>						
Total	1 213 (100.0)	1 373 (100.0)		1.00	—	<0.01
A1A1	806 (66.4)	994 (72.4)	0.475 <sup>c</sup>	1.25	(1.03–1.51)	
A1A2	357 (29.4)	348 (25.4)	0.701 <sup>c</sup>	2.54	(1.53–4.24)	
A2A2	50 (4.2)	31 (2.2)				
<i>Northern Europe (study numbers: 7, 8, 11, 17, 19, 24; MAF = 0.15)</i>						
Total	4 125 (100.0)	4 716 (100.0)		1.00	—	0.03
A1A1	2 944 (71.4)	3 428 (72.7)	0.247 <sup>c</sup>	1.05	(0.95–1.17)	
A1A2	1 063 (25.8)	1 205 (25.5)	0.795 <sup>c</sup>	1.70	(1.26–2.31)	
A2A2	118 (2.8)	83 (1.8)				
<i>United States (study numbers: 12, 14, 16, 18, 25; MAF = 0.14)</i>						
Total	3 963 (100.0)	3 731 (100.0)		1.00	—	0.65
A1A1	2 947 (74.3)	2 801 (75.0)	0.003 <sup>b</sup>	1.09	(0.87–1.38)	
A1A2	938 (23.7)	849 (22.8)	0.344 <sup>c</sup>	1.02	(0.73–1.43)	
A2A2	78 (2.0)	81 (2.2)				

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio

<sup>a</sup>Fisher's exact test<sup>b</sup>Heterogeneity P-value ≤ 0.05: performed random model for meta-analysis<sup>c</sup>Heterogeneity P-value > 0.05: performed fixed model for meta-analysis**Table 3** Meta-analysis results for the TP53 rs17878362 polymorphism by cancer type

Genotypes	Cases, n (%)	Controls, n (%)	Heterogeneity P-value	OR	(95% CI)	P-trend <sup>a</sup>
<i>Breast (Study numbers: 3, 11, 12, 13, 15, 25; MAF = 0.17)</i>						
Total	2 028 (100.0)	1 748 (100.0)		1.00	—	<0.01
A1A1	1 307 (64.5)	1 212 (69.3)	0.57 <sup>b</sup>	1.18	(1.02–1.37)	
A1A2	642 (31.7)	483 (27.6)	0.08 <sup>b</sup>	1.41	(0.97–2.06)	
A2A2	79 (3.9)	53 (3.0)				
<i>Colon (study numbers: 8, 17, 20, 21, 23; MAF = 0.15)</i>						
Total	1 637 (100.0)	1 686 (100.0)		1.00	—	0.08
A1A1	1 143 (69.8)	1 214 (72.0)	0.04 <sup>*</sup>	1.15	(0.87–1.50)	
A1A2	453 (27.7)	444 (26.3)	0.33 <sup>b</sup>	1.67	(1.02–2.74)	
A2A2	41 (2.5)	28 (1.7)				
<i>Lung (study numbers: 18, 19, 25; MAF = 0.13)</i>						
Total	4 054 (100.0)	4 045 (100)		1.00	—	<0.01
A1A1	2 977 (73.4)	3 076 (76.0)	0.02 <sup>*</sup>	1.22	(0.96–1.54)	
A1A2	979 (24.2)	898 (22.2)	0.03 <sup>*</sup>	1.46	(0.71–3.00)	
A2A2	98 (2.4)	71 (1.8)				

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio

<sup>\*</sup>Heterogeneity P-value ≤ 0.05: performed random model for meta-analysis<sup>a</sup>Fisher's exact test<sup>b</sup>Heterogeneity P-value > 0.05: performed fixed model for meta-analysis

agreement with the recent meta-analysis of Hu and collaborators, despite the fact that the two studies differed in the selection and analysis of data to be included as we used original ORs reported in each publication, which was not the case in the study of Hu *et al.*<sup>25,26</sup> When sub-grouping data

according to tumour site, different associations were seen for breast, colon and lung cancer, which were the only three tumour sites for which over 1600 cases and controls was available with the data drawn from at least three different reports. These differences suggest that the contribution of

rs17878362 to susceptibility might be different from one tumour type to the other. In the case of breast cancer, the increased risk was associated only with the heterozygosity status. Tumour type heterogeneity, in term of pathology and molecular profiles including the frequency of *TP53* mutations, may explain these results although this clearly needs further evaluation.<sup>29,30</sup> The lack of significant effect in lung cancer might reflect the overwhelming effect of tobacco smoke as a causative risk factor, masking the much smaller contribution of genetic susceptibility factors such as rs17878362.

Few studies have investigated the impact of rs17878362 on cancer susceptibility with respect to the geographical origin of the cohorts. Here, the observed difference across countries could be due to a different distribution in rs17878362 polymorphism between different ethnic groups. Indeed, Sjalander *et al.*<sup>31</sup> reported a difference in rs17878362 distribution across latitudes, between Swedish, Asian and Mongolian populations, which is independent of rs1042522 distribution. However, in the present meta-analysis, although some differences in the rs17878362 A2 allele frequency were seen between the different geographical regions, no heterogeneity was observed in the overall data set independently of any geographical consideration. Thus, the difference in rs17878362 A2 allele-related cancer susceptibility in the different countries suggests that additional factors, such as environmental factors, lifestyle and other genetic modifiers, may modulate cancer susceptibility associated with this allele.

Several studies have shown that the rs17878362 polymorphism is in linkage disequilibrium with other common TP53 SNPs, including rs1042522.<sup>31,32</sup> In a previous study, we have haplotyped rs17878362 and rs1042522 in a group of mostly Caucasian subjects from Brazil and reported that 71% of the tested population carried the haplotype combining rs17878362 A1 and rs1042522 R72, whereas the haplotype rs17878362 A2/rs1042522 R72 was detected in only 1.5% of the population.<sup>33</sup> In contrast, the A1/P72 and A2/P72 haplotypes were almost equally represented (15 and 12.5% of the population, respectively). This observation suggests that the rs17878362 A2 allele most frequently occurs on a haplotype that also contains rs1042522 P72,<sup>33</sup> raising the possibility that the susceptibility associated with rs17878362 might be driven, or confounded, by other common TP53 SNPs. To evaluate this possibility, we have used the data compiled from the same set of publications to assess cancer risk associated with rs1042522 and rs1625895 variants in the same data set. The aggregated ORs for the overall analysis showed that the heterozygote carriers of either variant allele had an increased cancer risk, consistent with several previous meta-analyses.<sup>14,25,34</sup> However, the effects observed for rs1042522 and rs1625895 were clearly smaller than for rs17878362 and were observed only in heterozygote carriers of rs1042522 or rs1625895, whereas the effect of rs17878362 appears to follow a recessive model. This would suggest that if rs1042522 and rs1625895 contribute to susceptibility, this effect could occur independently of their association with rs17878362. These results should be interpreted with caution, as no corrections for multiple testing have been performed. Indeed, it is not possible to calculate the number of tests carried in the original

papers in order to correct for multiple comparisons. Moreover, it has to be recognized that this analysis was not designed to specifically assess the cancer risk of these two alleles. The linkage disequilibrium between rs17878362 (tagged by rs2909430, which is in linkage disequilibrium with rs17878362,  $r^2 > 0.9$ ), rs1042522 and rs1625895 also shows ethnic differences as is reflected in the haplotype frequencies calculated based on published data<sup>9</sup> for three different HapMap populations (Supplementary Table 5). The most frequently found haplotype in the Caucasian and Asian HapMap populations was found to be rs17878362 A1/rs1042522 R72/rs1625895 G (78.13% of the Caucasian and 53.70% of the Asian population), while this only represented 31.67% of the haplotypes seen in the African population. The rs17878362 A1/rs1042522 P72/rs1625895 G haplotype was more frequent in the Asian (43.83%) and African (38.33%) populations than the Caucasian population (11.46%), while the rs17878362 A2/rs1042522 P72/rs1625895 A haplotype was seen in only 1.85% of Asian population compared with 9.37 and 26.11% of the Caucasian and African populations, respectively. Clearly further studies analysing TP53 haplotypes are needed to clarify the specific contribution of each of these common SNPs to cancer susceptibility.

The mechanistic basis of this altered risk associated with the carriage of the rs17878362 A2 allele is still poorly understood. Some evidence links rs17878362 status to differential expression of different p53 isoforms. In lymphoblastoid cell lines established from breast cancer patients the A1A1 genotype was associated with higher constitutive levels of *TP53* mRNA than for the A1A2 and A2A2 alleles.<sup>28</sup> Recently, we have shown that *TP53* intron 3 is involved in the splicing regulation of the *TP53* intron 2, influencing the generation of the fully spliced p53 (FSp53) and the intron-2-retaining p53 (p53I2) mRNA transcripts.<sup>7</sup> These transcripts generate the canonical p53 protein and the N-truncated  $\Delta 40$ p53 isoform, respectively, the latter being a regulator of p53 activity.<sup>8</sup> Using *in silico* algorithms, biophysical measurements and *in vitro* assays we have shown that the RNA sequences present in *TP53* intron 3 pre-RNA can form G-quadruplex structures, whose stability alters the balance of FSp53/p53I2 mRNA species through the modulation of intron 2 splicing.<sup>7</sup> On the basis of the same *in silico* algorithms, it appears that the rs17878362 duplication may alter the topology of the G4 structures in intron 3 that may impact on the FSp53/p53I2 balance. As the  $\Delta 40$ p53 isoform encoded by the p53I2 mRNA can inhibit p53 transcriptional activity and growth suppressive activity *in vitro* and appears to represent the main form of p53 expressed in mouse embryonic stem cells.<sup>2,35–37</sup> It is possible that the presence of the rs17878362 A2 variant allele could impact on p53 regulatory activity through the modulation of TP53 mRNA transcript patterns, subsequent isoform expression and maintenance of stem cell-like phenotype. Recent evidence suggesting that mRNA encoding  $\Delta 40$ p53 and  $\Delta 133$ p53 isoforms are over-expressed in some forms of ovarian carcinoma is in support of the hypothesis that changes in expression of these isoforms may contribute to carcinogenesis.<sup>38</sup> The mechanism by which the rs17878362 polymorphism modulates cancer risk needs to be fully addressed in appropriate functional genetics studies.

## Materials and methods

**Literature search and selection criteria.** Publications relative to the association between the rs17878362 polymorphism and cancer risk examined in case-control studies were identified using two databases: Pubmed Central (NCBI, NIH) (<http://www.ncbi.nlm.nih.gov/pubmed>) and Web of Science (Thomson Reuters) (<http://apps.webofknowledge.com>). The publication search was carried out from June 1993, when rs17878362 was first described<sup>20</sup> to December 2011. Several individual search terms, as well as combinations, were used: 'TP53', 'p53', 'intron3', 'rs17878362', 'polymorphism', 'intron', 'PIN3' and '16bp-Def', as in several publications the major A1 allele is referred to as a deletion of the 16 bp sequence. The publications were reviewed to identify those that met the following inclusion criteria: (i) that the publication reported a formal case-control study analysing cancer susceptibility associated with rs17878362, (ii) results were given as an OR and (iii) the publication was in English.

**Statistical analysis.** The methodological approach described by Thakkinstantian and collaborators was used to carry out our analyses on the association of the rs17878362 polymorphism with cancer risk variant allele with cancer risk and also those on rs1042522 and rs1625895 when data were available in the same panel of selected studies.<sup>28</sup> First, data from both controls and cases were extracted from the selected studies for the TP53 polymorphisms of interest, including the number of subjects, ORs<sup>11</sup> and the corresponding 95% CIs (Table 1 and Supplementary Table 1). Second, the Hardy-Weinberg equilibrium was tested by  $\chi^2$  goodness of fit in each study. Third, heterogeneity was determined using the *Q*-test and was considered as present when *Q*-test *P*-value was < 0.05. According to the *Q*-test *P*-value, the association between a polymorphism and cancer risk was investigating using either the fixed- or the random-effects models, according to the method of DerSimonian and Laird.<sup>39</sup> Using the same methodology, sub-group analyses were performed by geographic location of the population and cancer type. Sensitivity analyses were conducted to assess the impact of any single study (leave-one-out analysis, cumulative inclusion over time analysis). Publication bias was tested using the Egger test. Statistical analyses were performed using the commercial STATA software (version 11.1, StataCorp LP, College Station, Texas, USA).

## Conflict of Interest

The authors declare no conflict of interest.

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