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

C Sagne1,2,3, V Marcel1,2,4, A Amadou3, P Hainaut3,5, M Olivier3 and J Hall*1,2

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.1–4 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.1,5–7 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.).

Close to 100 genetic polymorphisms have been identified in TP53 (listed at http://p53.iarc.fr),9 many of which show geographic and population frequency variations. However, their effects on cancer risk appear to be inconsistent across studies.10,11 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)).12 There is in vitro evidence that the rs1042522 R72 and P72 p53 protein variants differ by their biological activities.13,14 However, results from systematic studies and meta-analyses have failed to identify a consistent association with cancer risk.15–19 The most common intronic variation in TP53 is a 16-base pair (bp)11 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))).20 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.21,22 and colorectal cancers.23,24 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.25 However, this conclusion was questioned because of apparent discrepancies between data selected for meta-analysis and the original publications.26 At the mechanistic level, there is some evidence that rs17878362 may have an impact on the levels23 and alternative splicing of the TP53 mRNA, and thus on the ratios of p53 protein isoforms.6 However, the precise mechanisms underlying an increased cancer risk associated with the rs17878362 A2 allele are not clearly understood.

1INSERM U612, Bât 110-112, Centre Universitaire, Orsay, France; 2Institut Curie, Centre de Recherche, Bât 110-112, Centre Universitaire, Orsay, France and 3Molecular 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

2Present address: International Prevention Research Institute, 95 cours Lafayette, 69006 Lyon, France.
3Present 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.

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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 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). 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 carriage (A2A2 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
versus carriers (A1A2 heterozygous A1A2 carriers compared with the A1A1 significant increased breast cancer risk was observed in the OR

eSame control population

Significant increase in cancer risk associated with rs17878362 (TP53 PIN3)

Abbreviations: NA, not available; NC, not classified

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 (A2) | rs1042522 R72 (P72) | rs1625895 A (A) | rs17878362 A2 (A2) | rs1042522 R72 (P72) | rs1625895 A (A) |
| 1 Jha et al.40 a         | Gliatissue  | 84    | 76       | India      | 0.18                                      | 0.55                                        | NA                        | 0.23                                      | 0.01b          | NA |
| 2 Umair et al.41 a       | Esophagus   | 255   | 255      | India      | 0.19                                      | NA                                         | NA                        | 0.33                                      | NA            | NA |
| 3 Alawadi et al.42 a     | Breast      | 229   | 133      | NC         | 0.31                                      | 0.44                                        | 0.58                      | 0.015                                     | NA           | NA |
| 4 Mittal et al.43 a      | Prostate    | 177   | 265      | India      | 0.15                                      | 0.24                                        | 0.21                      | 0.12                                      | 0.28          | 0.11 |
| 5 Malik et al.44 a       | Oesophagus  | 135   | 195      | India      | 0.21                                      | NA                                         | NA                        | 0.08                                      | NA            | NA |
| 6 Malik et al.45 a       | Gastric     | 198   | 195      | India      | 0.21                                      | NA                                         | NA                        | 0.08                                      | NA            | NA |
| 7 Naccarali et al.46 a   | Pancreas    | 240   | 743      | Northern Europe | 0.16                                  | 0.29                                        | NA                        | 0.10                                      | NA            | NA |
| 8 Polakova et al.47 a    | Colon       | 612   | 613      | Northern Europe | 0.14                                      | 0.27                                        | NA                        | 0.15                                      | 0.52          | NA |
| 9 Ashion et al.30 a      | Endometrial | 190   | 291      | NC         | 0.14                                      | 0.24                                        | 0.11                      | 0.81                                      | 0.97          | 0.12 |
| 10 de Foco et al.48 a    | Gastric     | 114   | 295      | Mediterranean | 0.16                                  | 0.25                                        | 0.20                      | 0.35                                      | 0.13          | 0.15 |
| 11 Hrstka et al.49 a     | Breast      | 117   | 108      | Northern Europe | 0.14                                      | 0.45                                        | 0.13                      | 0.46                                      | 0.00e         | 0.78 |
| 12 Gaudet et al.50 a     | Breast      | 578   | 390      | United States | 0.16                                      | 0.74                                        | 0.85                      | 0.85                                      | 0.08          | 0.93 |
| 13 Costa et al.51 c      | Breast      | 191   | 216      | Mediterranean | 0.17                                      | 0.17                                        | 0.11                      | 0.29                                      | 0.29          | NA |
| 14 Ye et al.52 a         | Bladder     | 636   | 618      | United States | 0.15                                      | 0.22                                        | 0.15                      | 0.29                                      | 0.00n         | 0.13 |
| 15 de Vecchi et al.53 a  | Breast      | 350   | 352      | Mediterranean | 0.15                                      | 0.23                                        | 0.23                      | 0.62                                      | 0.23          | NA |
| 16 Chen et al.54 a       | Head and neck | 821   | 818      | United States | 0.14                                      | 0.27                                        | 0.12                      | 0.75                                      | 0.07          | 0.67 |
| 17 Tan et al.55 a        | Colon       | 467   | 563      | Northern Europe | 0.17                                      | 0.22                                        | 0.22                      | 0.23                                      | 0.98          | NA |
| 18 Wang et al.56 a       | Colon       | 1412  | 1363     | United States | 0.13                                      | 0.26                                        | 0.12                      | 0.45                                      | 0.54          | 0.14 |
| 19 Hung et al.57 a       | Lung        | 2126  | 2140     | Northern Europe | 0.13                                      | 0.27                                        | 0.27                      | 0.50                                      | 0.74          | NA |
| 20 Perfumo et al.58 a    | Colon       | 60    | 188     | Mediterranean | 0.15                                      | 0.20                                        | 0.20                      | 0.21                                      | 0.81          | NA |
| 21 Perfumo et al.59 a    | Colon       | 124   | 188     | Mediterranean | 0.15                                      | 0.20                                        | 0.20                      | 0.21                                      | 0.81          | NA |
| 22 Mitra et al.60 a      | Oral cancer | 307   | 342     | India      | 0.19                                      | 0.48                                        | 0.48                      | 0.56                                      | 0.20          | NA |
| 23 Gemignani et al.61 c  | Colon       | 374   | 322     | Mediterranean | 0.12                                      | 0.21                                        | 0.21                      | 0.60                                      | 0.09          | NA |
| 24 Wang-Gohre et al.62 c | Breast      | 563   | 549     | Northern Europe | 0.16                                      | 0.26                                        | 0.15                      | 0.92                                      | 0.49          | 0.60 |
| 25 Wu et al.63 a         | Lung        | 516   | 542     | United States | 0.10                                      | 0.20                                        | 0.12                      | 0.05                                      | 0.01b         | NA |

Abbreviations: NA, not available; NC, not classified

*No significant increase in cancer risk associated with rs17878362 (TP53 PIN3)

**P-value < 0.05 indicates a Hardy–Weinberg disequilibrium: study exclusion

Significant increase in cancer risk associated with rs17878362 (TP53 PIN3)

Same control population

Same control population

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.10). 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 intrinsic 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
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.\textsuperscript{25,26} 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

| Genotypes | Cases, n (%) | Controls, n (%) | Heterogeneity, $P$-value | OR   | (95% CI)   | $P$-trend\textsuperscript{a} |
|-----------|--------------|-----------------|--------------------------|------|------------|--------------------------|
| Overall (25 studies, MAF = 0.15) | | | | | | |
| Total     | 10 786 (100.0) | 11 377 (100.0) | | 1.00 | — | <0.01 |
| A1A1       | 7 639 (70.8) | 8 254 (72.5) | | 0.03\textsuperscript{b} | 1.08 | (0.99–1.18) |
| A1A2       | 2 823 (26.2) | 2 871 (25.2) | | 0.06\textsuperscript{b} | 1.45 | (1.22–1.74) |
| A2A2       | 324 (3.0) | 252 (2.3) | | | | |

Geographical origin of studies India (study numbers: 1, 2, 4, 5, 6, 22; MAF = 0.19) | | | | | | |
| Total     | 1 066 (100.0) | 1 133 (100.0) | | 1.00 | — | 0.19 |
| A1A1       | 699 (65.6) | 750 (66.2) | | | | |
| A1A2       | 304 (28.5) | 345 (30.5) | | 0.54\textsuperscript{c} | 0.94 | (0.79–1.13) |
| A2A2       | 63 (5.9) | 38 (3.3) | | 0.07\textsuperscript{c} | 1.63 | (1.10–2.42) |

Mediterranean countries (study numbers: 10, 13, 15, 20, 21, 23; MAF = 0.15) | | | | | | |
| Total     | 1 213 (100.0) | 1 373 (100.0) | | 1.00 | — | <0.01 |
| A1A1       | 806 (66.4) | 994 (72.4) | | | | |
| A1A2       | 357 (29.4) | 348 (25.4) | | 0.475\textsuperscript{c} | 1.25 | (1.03–1.51) |
| A2A2       | 50 (4.2) | 31 (2.2) | | 0.701\textsuperscript{c} | 2.54 | (1.53–4.24) |

Northern Europe (study numbers: 7, 8, 11, 17, 19, 24; MAF = 0.15) | | | | | | |
| Total     | 4 125 (100.0) | 4 716 (100.0) | | 1.00 | — | 0.03 |
| A1A1       | 2 944 (71.4) | 3 428 (72.7) | | | | |
| A1A2       | 1 063 (25.8) | 1 205 (25.5) | | 0.247\textsuperscript{c} | 1.05 | (0.95–1.17) |
| A2A2       | 50 (4.2) | 83 (1.8) | | 0.795\textsuperscript{c} | 1.70 | (1.26–2.31) |

United States (study numbers: 12, 14, 16, 18, 25; MAF = 0.14) | | | | | | |
| Total     | 3 963 (100.0) | 3 731 (100.0) | | 1.00 | — | 0.65 |
| A1A1       | 2 947 (74.3) | 2 801 (75.0) | | | | |
| A1A2       | 938 (23.7) | 849 (22.8) | | 0.003\textsuperscript{b} | 1.09 | (0.87–1.38) |
| A2A2       | 78 (2.0) | 81 (2.2) | | 0.344\textsuperscript{b} | 1.02 | (0.73–1.43) |

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio
\textsuperscript{a}Fisher’s exact test
\textsuperscript{b}Heterogeneity $P$-value $\leq$ 0.05: performed random model for meta-analysis
\textsuperscript{c}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\textsuperscript{a} |
|-----------|--------------|-----------------|--------------------------|------|------------|--------------------------|
| Breast (Study numbers: 3, 11, 12, 13, 15, 25; MAF = 0.17) | | | | | | |
| Total     | 2 028 (100.0) | 1 748 (100.0) | | 1.00 | — | <0.01 |
| A1A1       | 1 307 (64.5) | 1 212 (69.3) | | 0.57\textsuperscript{b} | 1.18 | (1.02–1.37) |
| A1A2       | 642 (31.7) | 483 (27.6) | | 0.08\textsuperscript{b} | 1.41 | (0.97–2.06) |
| A2A2       | 79 (3.9) | 53 (3.0) | | | | |

Colon (study numbers: 8, 17, 20, 21, 23; MAF = 0.15) | | | | | | |
| Total     | 1 637 (100.0) | 1 668 (100.0) | | 1.00 | — | 0.08 |
| A1A1       | 1 143 (69.8) | 1 214 (72.0) | | | | |
| A1A2       | 453 (27.7) | 444 (26.3) | | 0.04\textsuperscript{b} | 1.15 | (0.87–1.50) |
| A2A2       | 41 (2.5) | 28 (1.7) | | 0.33\textsuperscript{b} | 1.67 | (1.02–2.74) |

Lung (study numbers: 18, 19, 25; MAF = 0.13) | | | | | | |
| Total     | 4 054 (100.0) | 4 045 (100) | | 1.00 | — | <0.01 |
| A1A1       | 2 977 (73.4) | 3 076 (76.0) | | | | |
| A1A2       | 979 (24.2) | 898 (22.2) | | 0.02\textsuperscript{b} | 1.22 | (0.96–1.54) |
| A2A2       | 98 (2.4) | 71 (1.8) | | 0.03\textsuperscript{b} | 1.46 | (0.71–3.00) |

Abbreviations: CI, confidence interval; MAF, minor allele frequency; OR, odds ratio
\textsuperscript{a}Heterogeneity $P$-value $\leq 0.05$: performed random model for meta-analysis
\textsuperscript{b}Fisher’s exact test
\textsuperscript{c}Heterogeneity $P$-value $> 0.05$: performed fixed model for meta-analysis
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.29,30 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.31 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 rs1042522.31,32 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.33 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 P72,33 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 assessed 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.14,25,34 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, r2 > 0.9), rs1042522 and rs1625895 also shows ethnic differences as is reflected in the haplotype frequencies calculated based on published data9 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 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.28 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.7 These transcripts generate the canonical p53 protein and the N-truncated Δ40p53 isoform, respectively, the latter being a regulator of p53 activity.8 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.7 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 Δ40p53 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,2,35–37 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 Δ40p53 and Δ133p53 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.38 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 described20 to December 2011. Several individual search terms, as well as combinations, were used: ‘TP53’, ‘p53’, ‘introm3’, ‘rs17878362’, ‘polymorphism’, ‘intron’, ‘PIN3’ and ‘16bp-Del’, 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 Thakkinstan 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 tian and collaborators was used to carry out our analyses on the association of the Statistical analysis. (i) that the publication reported a formal case–control study analysing (ii) results were given as an OR (iii) the publication was in English.

Conflict of Interest

The authors declare no conflict of interest.

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