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TP53 polymorphisms and lung cancer risk: a systematic review and meta-analysis

A.Matakidou[1,2,3], T.Eisen[2] and R.S.Houlston[1]

[1]Section of Cancer Genetics, Institute of Cancer Research, Sutton SM2 5NG, UK and [2]Cancer Research Unit, Section of Medicine, Royal Marsden Hospital, Downs Road, Sutton, UK

To examine the risk of lung cancer associated with the codon 72, intron 6 and intron 3 *TP53* polymorphisms a meta-analysis of published case–control studies was undertaken. The principle outcome measure was the odds ratio (OR) for the risk of lung cancer using homozygosity of the 'wild-type allele' as the reference group. Data from 13 studies detailing the relationship between lung cancer and the codon 72 polymorphism of *TP53* and three studies examining the intron 3 and 6 polymorphisms of *TP53* were analysed. The ORs of lung cancer associated with the Pro-Pro and Pro-carrier genotypes of codon 72 were 1.18 [95% confidence interval (CI) 0.99–1.41] and 1.02 (95% CI 0.86–1.20), respectively. The ORs of lung cancer associated with homozygous and variant allele carrier genotypes of the intron 6 (*Msp*I RFLP) polymorphism were 1.13 (95% CI 0.55–2.27) and 1.30 (95% CI 0.75–2.26) and of the intron 3 (16 bp duplication) polymorphism were 1.50 (95% CI 0.76–2.97) and 1.11 (95% CI 0.53–2.35), respectively. Although polymorphic variations in *TP53* represent attractive candidate susceptibility alleles for lung cancer the results from this analysis provide little support for this hypothesis. Additional well-designed studies based on sample sizes commensurate with the detection of small genotypic risks may allow a more definitive conclusion.

Introduction

Lung cancer accounts for ~19% of all cancers and ~29% of all cancer deaths (Flehinger *et al*., 1993). It is the commonest cause of cancer death in men and is second only to breast cancer in women (Flehinger *et al*., 1993; Strauss, 1997). Tobacco smoking is undoubtedly the most important aetiological risk factor in the development of this cancer, the risk being at least 10 times higher in long-term smokers compared with non-smokers (Peto, 2001). Hence, it is frequently cited as an example of a malignancy solely attributed to environmental factors (exposure to carcinogens). There is, however, a growing appreciation that the development of most cancers results from a complex interaction between both environmental and genetic factors. Epidemiological studies have shown that relatives of lung cancer cases are at a 2-fold increased risk of developing the disease (Houlston and Peto, 1996), and although part of this is likely to be attributable to familial non-genetic factors, there is some support for an inherited predisposition. It is possible that part of an inherited susceptibility to lung cancer may be determined by inter-individual variation in genes encoding DNA repair proteins, cell cycle control proteins and metabolic enzymes responsible for the bioactivation and detoxification of carcinogens.

TP53 occupies a central role in mediating cellular responses to genotoxic insults through its effects on gene transcription, DNA synthesis and repair, genomic plasticity and programmed cell death (Vogelstein and Kinzler, 1992).

Germline mutation in *TP53* as a determinant of cancer risk has been the subject of considerable research over the last 10 years, especially in the context of Li–Fraumeni syndrome (reviewed in Evans and Lozano, 1997). Somatic mutations in *TP53* can be detected in over half of all human cancers (Hollstein *et al*., 1991), including lung cancer (Takahashi *et al*., 1989). Loss of TP53 function is an early event of lung tumorigenesis and *TP53* mutations have been observed in preneoplastic lesions, such as bronchial epithelial dysplasia (Bennett *et al*., 1993). Furthermore, mutations have been detected in non-tumorous peripheral lung tissue from lung cancer patients (Hussain *et al*., 2001).

TP53 is polymorphic, and 13 different variants have been described to date (Table I). It has recently been shown that certain polymorphic variants of *TP53* differentially affect the properties of the mutant p53 proteins (Thomas *et al*., 1999), raising the possibility that *TP53* polymorphic variation may directly influence individual susceptibility to lung cancer. The codon 72 polymorphism in exon 4, which is carried by 20–40% of the population, leads to an arginine to proline substitution. Weston *et al*. (1992a) first reported an association between this polymorphism and lung cancer. Since the publication of this report, over 14 studies have appeared in the literature either supporting or refuting an association. Similarly, studies of the 16 bp duplication in intron 3 and the *Msp*I restriction fragment length polymorphism (RFLP) in intron 6 have yielded conflicting results. To date, one study evaluating the intron 2 polymorphism and lung cancer risk has been reported. Most of the studies published are based on the analysis of small numbers of cases and controls. To clarify the effect of variation within *TP53* defined by these four polymorphisms on the risk of lung cancer, we have undertaken a systematic review and meta-analysis.

Materials and methods

Identification of studies

A search of the literature was made using the electronic database PUBMED (www.ncbi.nml.nih.gov/pubmed) for the years 1992–2002 to identify articles which have determined germline *TP53* status defined by codon 72, intron 6, intron 3 and intron 2 polymorphisms in lung cancer cases and controls. Additional articles were ascertained through references cited in these publications. Articles included for analyses were primary references and of case–control design. Care was taken to include only primary data or data which superseded earlier work. Characteristics of the studies were extracted from published articles and summarized in a consistent manner to aid comparison.

[3]To whom correspondence should be addressed at Section of Cancer Genetics, Institute of Cancer Research, Sutton SM2 5NG, UK. Tel: +44 208 643 8901; Fax: +44 208 643 0257; Email: athenam@icr.ac.uk

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Table I. *TP53* polymorphisms

Site	Consequence	Reference
Exonic		
Codon 21: C→T transition	Silent	Ahuja *et al.* (1990)
Codon 36: G→A transition	Silent	Felix *et al.* (1999)
Codon 47: C→T transition	Pro→Ser substitution	Felley-Bosco *et al.* (1993)
Codon 72: G→C transversion	Arg→Pro substitution	Matlashewski *et al.* (1987)
Codon 213: A→G transition	Silent	Carbone *et al.* (1991)
Intronic		
Intron 1: VNTR	Unknown effect	Hahn *et al.* (1993)
Intron 1: *Hae*III RFLP	Unknown effect	Ito *et al.* (1994)
Intron 2: G→C transversion	Unknown effect	Pleasants and Hansen (1994)
Intron 3: 16 bp duplication	Unknown effect	Lazar *et al.* (1993)
Intron 6: G→A transition (*Msp*I RFLP)	Unknown effect	Chumakov and Jenkins (1991)
Intron 6: G→C transversion	Unknown effect	Hillebrandt *et al.* (1997)
Intron 7: *Apa*I RFLP	Unknown effect	Prosser and Condie (1991)
Intron 10: A→T transversion	Unknown effect	Buller *et al.* (1995)

VNTR, variable number of tandem repeats; RFLP, restriction fragment length polymorphism.

Statistical analysis

The odds ratio (OR) of lung cancer associated with the variant allele carrier and homozygous genotype for each *TP53* polymorphism were based on homozygosity of the 'wild-type allele' as the reference group. Where adjusted ORs were provided, these were used in the analysis. Otherwise unadjusted ORs were computed from the data presented (sufficient data to allow calculation of adjusted ORs was not available).

Pooled estimates of the OR were obtained by calculating a weighted average of the logarithm of ORs (Breslow and Day, 1987). A P value of 0.05 was considered statistically significant. Studies were analysed jointly using both a fixed effects and a random effects model (if significant heterogeneity between studies was present a random effects model was utilized) (DerSimonian and Day, 1986). A random effects model assumes that the studies in question are a random sample of a hypothetical population of studies taking into account variability within and between studies. Specific analyses considering confounding factors were not possible because the raw data were not available.

The presence of publication bias was examined by plotting ORs in order according to the variance of the logOR estimate. Estimates from small studies that have less precision in estimating the underlying OR will scatter widely at the base of the graph, with a narrowing among larger studies. In the absence of publication bias the plot resembles a symmetrical inverted funnel (Egger *et al.*, 1997). Conversely, if there is bias, the funnel plot will be asymmetrical. Statistical manipulations were undertaken using the program STATA version 7.0 (Stata Corp., Texas, TX) utilizing the META (Sharpe and Sterne, 1998a,b) module.

In order to test for evidence of population stratification, the distribution of genotypes in controls was tested for a departure from Hardy–Weinberg equilibrium (HWE) by means of the χ^2 test.

The power of each study was computed as the probability of detecting an association between *TP53* carrier status for each polymorphism and lung cancer at the 0.05 level of significance, assuming a genotypic risk of 1.5 and 2.0. Estimates of power were performed using the method published by Fleiss *et al.* (1980), implemented in the statistical program POWER (Version 1.30; Epicenter Software; http://icarus2.hsc.usc.edu/epicenter).

Results

Codon 72 polymorphism

Table II details the studies examining the possible association between *TP53* codon 72 polymorphism and lung cancer risk that were suitable for analysis. Reports were excluded if the same data were available in more than one study (Murata *et al.*, 1996; Fan *et al.*, 2000; Biros *et al.*, 2001b; Miller et al., 2002), in which case the most recent publication or the publication with the largest study population were included, or if no information on genotype OR was presented (Weston *et al.*, 1992b). The study reported by Murata *et al.* in 1996 has been superseded by the study published by the same authors in 1998, hence the latter (Murata *et al.*, 1998) was used to estimate the overall risk. The 1996 study did, however, provide information on *TP53* polymorphic status by histology, which was used in the sub-analysis. The study by Miller *et al.* (2002) provided additional data to the study of Liu *et al.* (2001) and was used to estimate risk by ethnic group.

Data on smoking exposure in cases and controls has not been universally collected. Hospital disease controls were used exclusively in three studies (Table II). ORs adjusted for age, gender and ethnicity were provided in some but not all studies (Weston *et al.*, 1994; Pierce *et al.*, 2000; Liu *et al.*, 2001; Wu *et al.*, 2002). Some studies have analysed the relationship between *TP53* status and cancer risk in a stratified manner or by logistic regression, taking into account other covariates, such as histology or other polymorphisms.

Table II shows the power of each study to demonstrate an association between the *TP53* carrier status and lung cancer risk. Power >80% was attained by 10 of the 16 studies (63%) if the genotypic risk is $\geqslant$2.0 (data not shown). However, if the genotypic risk is 1.5, only four of 16 (25%) of the studies have 80% power.

The distribution of genotypes among controls are in HWE in most studies. However, the control groups in Weston *et al.* (1994), Wu *et al.* (2002), Miller *et al.* (2002), Papadakis *et al.* (2002) and Jin *et al.* (1995) show departure from HWE, which suggests possible population stratification.

Figures 1 and 2 show plots of ORs and associated 95% confidence intervals (CI) for the risk of developing lung cancer in Pro-Pro homozygotes and Pro-carriers for the 13 studies. There was evidence of heterogeneity between the studies in the Pro-carrier genotype analysis ($P = 0.005$). The pooled ORs for the Pro-Pro homozygotes and Pro-carriers are 1.18 (95% CI 0.99–1.41) and 1.02 (95% CI 0.86–1.20), respectively. There was no obvious evidence of publication bias based on the constructed funnel plots of the ORs.

These analyses are based on pooling data from studies of all ethnic groups. Restricting the analyses to the eight studies of Caucasians (Weston *et al.*, 1994; Birgander *et al.*, 1995; To-Figueras *et al.*, 1996; Pierce *et al.*, 2000; Biros *et al.*, 2001a; Miller et al., 2002; Papadakis *et al.*, 2002; Wu *et al.*, 2002), the pooled ORs associated with the Pro-Pro and the Pro-carrier genotypes are 1.12 (95% CI 0.74–1.71) and 0.98 (95% CI 0.75–1.29), respectively. There was some evidence of study heterogeneity in both analyses ($P < 0.05$). The respective ORs

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Table II. Summary of studies of lung cancer and *TP53* codon 72 polymorphism

Investigator	Place of study	Cases[a]			Controls[a]			Exposure/ other covariates	Power[b] (RR > 1.5; $\alpha = 0.05$)
			Pro/Pro (%)	Pro/Arg (%)		Pro/Pro (%)	Pro/Arg (%)		
Weston *et al.* (1992a)	Maryland, USA	78 prevalent cases from 2 centres recruited between 1985 and 1989; age 64; sex ratio NS; mean pack years 57.4; Caucasian (n = 42), African-Americans (n = 36)	20.5 (all cases)	48.7 (all cases)	47 controls with COPD and 25 controls with malignancies other than lung or urinary bladder; age 62/61; sex ratio NS; mean pack years 57.1; Caucasian (n = 38), African-Americans (n = 34)	17.0 44.0	51.0 32.0	Race, histology	14%
Kawajiri *et al.* (1993)	Saitama, Japan	328 incident cases from one centre; age NS; sex ratio NS; Japanese	16.2	38.7	347 unrelated controls randomly selected from individuals surveyed in a prospective cohort study of a Japanese general population; age NS; 50% male; Japanese	10.9	47.6	None specified	69%
Weston *et al.* (1994)	Maryland, USA	31 prevalent cases from one centre; age 61.2; 90% male; mean pack years 60.9;			48 controls with COPD and non-pulmonary cancers; age 63.1; 94% male; mean pack years 51.1;			Race, L-*myc* polymorphism	9%
		Caucasians (n = 18),	11.1	38.9	Caucasian (n = 36),	16.7	22.2		
		African-Americans (n = 13)	30.8	38.5	African-Americans (n = 12)	25.0	50.0		
Jin *et al.* (1995)	Texas, USA	109 incident cases; age 62.5; 79% male; mean pack years 49.8;			114 controls matched to cases by age, gender, ethnicity, residence (convenience sample); age 61.3; 71% male; mean pack years 14.6;			Race, histology, age at diagnosis, pack years	20%
		Mexican-Americans (n = 42),	4.8	52.4	Mexican-Americans (n = 40),	2.5	47.5		
		African-Americans (n = 67)	28.4	47.8	African-Americans (n = 74)	20.3	64.9		
Birgander *et al.* (1995)	Sweden	142 incident cases from central Sweden; age NS; sex ratio NS; median pack years 34; Caucasian	7.7	40.8	206 healthy controls from northern Sweden, 76 healthy controls from central Sweden,	9.6	37.6	Histology, other p53 polymorphisms	50%
					95 control patients with COPD; age NS; sex ratio NS; Caucasian	4.2	31.6		
To-Figueras *et al.* (1996)	Spain	139 incident cases from Catalonia; age 58 (10); 94% male; mean pack years 53 (23); Caucasian	7.2	37.4	147 controls selected from a DNA bank of healthy and unrelated volunteers from Catalonia; age 48 (22); 93% male; pack years NS; Caucasian	5.4	32.0	Histology, GSTM1 polymorphism	35%
Murata *et al.* (1996)	Chiba, Japan	191 incident cases from 1 centre; 33% <60 years; 74% male; 73% smokers; Japanese	11.5	46.6	152 controls with non-cancerous pulmonary diseases chosen from out-patients,	15.1	50.0	Histology, smoking	48%
					115 colorectal cancer patients; age NS; sex ratio NS; pack years NS; Japanese	12.2	47.8		
Murata *et al.* (1998)	Chiba, Japan	224 non-small cell lung cancer patients admitted to 2 centres between 1991–1995; 49% <65 years; sex ratio NS; 26% non-smokers; Japanese	12.9	46.9	148 controls with non-cancerous pulmonary diseases (enrolled in previous study) and 155 healthy individuals; 75% <65 years; sex ratio NS; 43% non-smokers; Japanese	13.9	42.6	Age, smoking	57%
Wang *et al.* (1999)	Taiwan	194 surgically resected lung cancer patients from one centre (DNA obtained from normal lung tissue); age 64; 73% male; 36% non-smokers; Taiwanese	26.8	38.1	152 non cancer and unrelated randomly selected individuals from a physical check-up centre (age matched to cases); age 62; 53% male; smoking NS; Taiwanese	19.7	49.3	Sex, histology, age (>60, <60)	34%

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Table II. *Continued*

Investigator	Place of study	Cases[a]			Controls[a]			Exposure/ other covariates	Power[b] (RR > 1.5; $\alpha = 0.05$)
			Pro/Pro (%)	Pro/Arg (%)		Pro/Pro (%)	Pro/Arg (%)		
Fan *et al.* (2000)	Boston, USA	482 incident lung cancer cases from 1 center (1992–1996); age 65.5 (10.2); 55% male; mean pack years 57.8 (39.3); 96% Caucasian	13.7	42.3	510 unrelated family or friends of patients with either lung cancer or other cardiothoracic problems; age 61.6 (10.2); 45% male; mean pack years 22.6 (27.3); 98% Caucasian	11.9	41.6	Histology	87%
Pierce *et al.* (2000)	Hawaii, USA	334 prevalent cases (1992–1997) in all main medical centres of Oahu, Hawaii; age 26–79; sex ratio NS; pack years NS;			446 controls randomly selected from a list of Oahu residents (interviewed as part of a health survey), matched to each case by sex, age, ethnicity;			Race, histology, H-*ras* polymorphism	76%
		Japanese ($n = 111$),	17.1	45.9	Japanese ($n = 170$),	13.5	38.2		
		Caucasian ($n = 138$),	5.8	33.3	Caucasian ($n = 173$),	5.9	43.9		
		Hawaiian ($n = 85$)	29.4	48.2	Hawaiian ($n = 103$)	23.3	49.5		
Biros *et al.* (2001a)	Slovakia	168 incident, histologically confirmed lung cancer cases from 1 centre; age: 61.7; 80% male; 83% smokers of at least 20 years; Caucasian	4.8	41.1	148 healthy controls; age NS; 59% male; pack years NS; Caucasian	13.5	39.9	Histology	38%
Liu *et al.* (2001)	Boston, USA	1168 incident, histologically confirmed lung cancer cases from one centre (since 1992); age 65; 54% male; mean pack years: 60; ? Caucasian	8.5	39.8	1256 unrelated family or friends of patients with either cancer or cardiothoracic problems; age 58; 46% male; mean pack years 32; ? Caucasian	6.9	35.1	Histology	100%
Wu *et al.* (2002)	Texas, USA	635 incident, histologically confirmed, previously untreated patients from 1 centre; age 61.4 (9.7); 53% male; mean pack years 52 (33.7);			635 healthy controls recruited from 20 area clinics of a health maintenance organisation; age 60.6 (9.8); 56% male; mean pack years 47.3 (32.1);			Race	94%
		Caucasian ($n = 517$),	7.7 (Caucasian only)	34.3	Caucasian ($n = 544$),	5.9	28.9		
		Mexican-American ($n = 54$),			Mexican-American ($n = 40$),	7.5	25.6		
		African-American ($n = 64$)			African-American ($n = 51$)	21.6	49.0		
Miller *et al.* (2002)	Boston, USA	767 incident, histologically confirmed lung cancer cases from 1 center (since 1992); age 67; 55% male; mean pack years 52; Caucasian	13.2	39.0	927 unrelated family or friends of patients with either lung cancer or other cardiothoracic problems; age 61; 45% male; mean pack years 27; Caucasian	9.9	36.6	GSTM1 and GSTP1 polymorphisms	98%
Papadakis *et al.* (2002)	Greece	54 incident, advanced lung cancer patients from 1 centre; age 66.4 (8.3); 85% male; mean pack years 64.6 (31.4); Caucasian	0.0	50.0	99 unrelated healthy individuals from same geographical area; age 65.8 (9.7); 85% male; mean pack years 63.2 (29.3); Caucasian	11.1	64.7	Histology	17%

[a]Ages and pack years of cases and controls: mean (SD) or range given where possible. ?, probable; NS, not specified.
[b]RR (relative risk) calculated as the probability of detecting an association between *p53* codon 72 Pro allele carriers and lung cancer risk.

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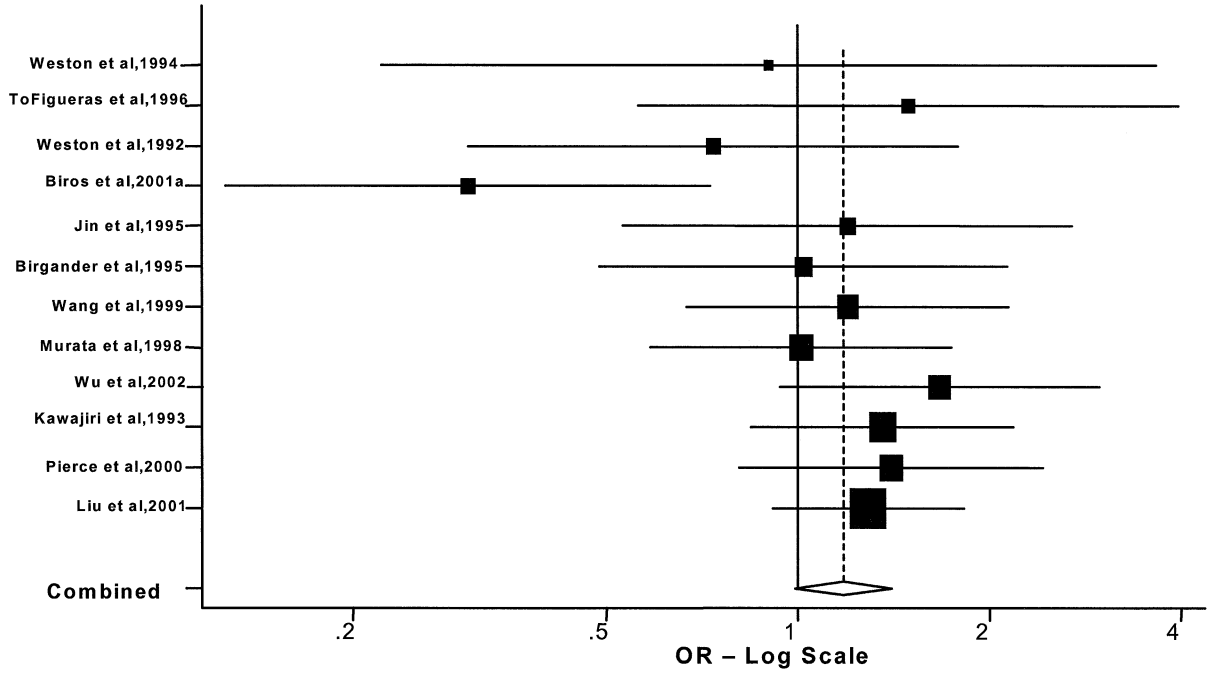

Fig. 1. Funnel plot of OR of lung cancer risk associated with the Pro/Pro genotype of *TP53*. Studies are plotted in order of decreasing variance of the logOR. Horizontal lines represent 95% confidence intervals. Each box represents the OR point estimate and its area is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with confidence interval given by its width. The unbroken vertical line is at the null value (RR = 1.0).

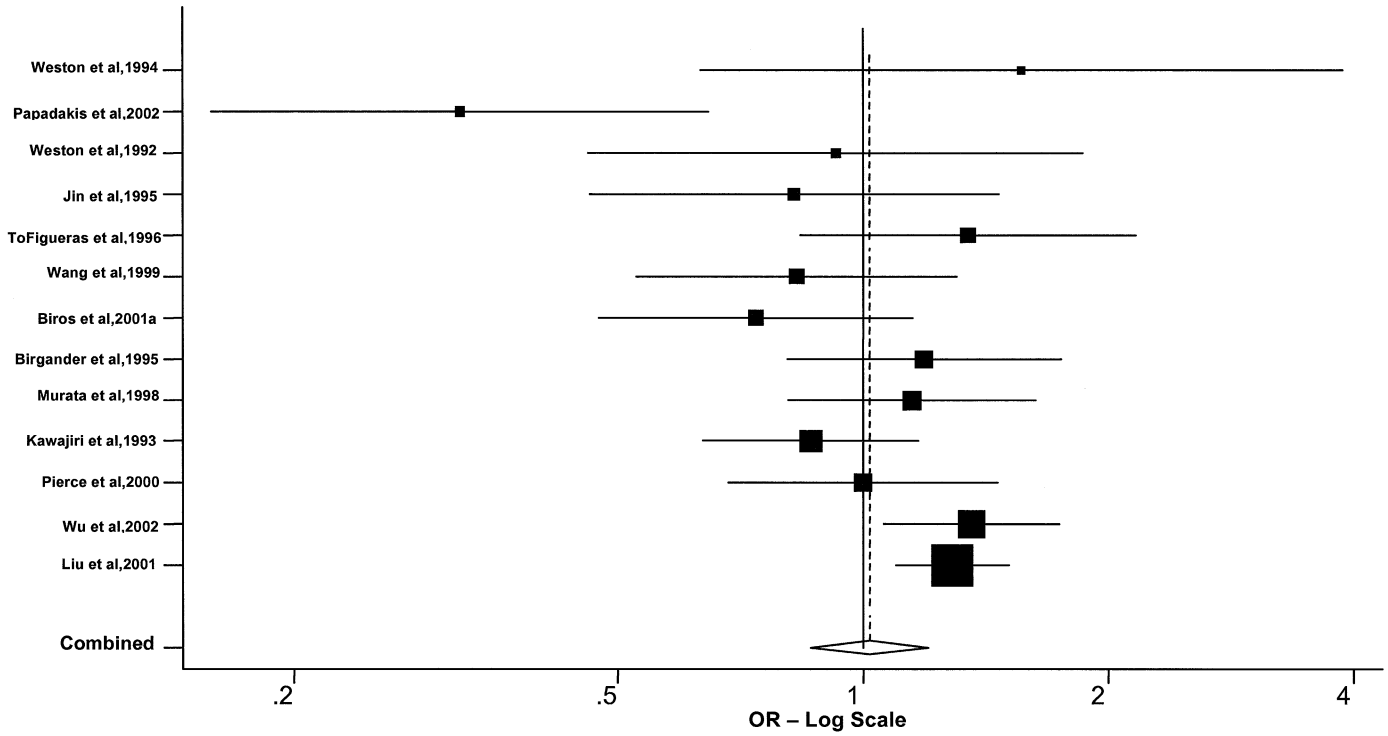

Fig. 2. Funnel plot of OR of lung cancer risk associated with the Pro allele carrier genotype of *TP53*. Refer to Figure 1 for details of the plot.

calculated for the Asian population (Kawajiri *et al*., 1993; Murata *et al*., 1998; Wang *et al*., 1999; Pierce *et al*., 2000) are 1.23 (95% CI 0.93–1.64) and 1.02 (95% CI 0.85–1.23). Two studies provided data on the African-American population (Weston *et al*., 1994; Jin *et al*., 1995), and the pooled OR(s) are 0.89 (95% CI 0.35–2.27) and 0.59 (95% CI 0.27–1.27) for the homozygous and Pro allele carrier genotypes. There was no evidence of heterogeneity between the studies. Jin *et al*. (1995) studied Mexican-Americans and reported the respective ORs to be 2.20 (95% CI 0.22–22.3) and 1.33 (95% CI 0.56–3.18). One

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Table III. Summary of studies of lung cancer and intron 6/intron 3 *TP53* polymorphisms

Investigator	Place of study	Cases[a]	Homozygous for variant allele (%)		Heterozygous for variant allele (%)		Controls[a]	Homozygous for variant allele (%)		Heterozygous for variant allele (%)		Exposure/other covariates	Power[b] (RR > 1.5, α = 0.05)	
			Intron 6	Intron 3	Intron 6	Intron 3		Intron 6	Intron 3	Intron 6	Intron 3		Intron 6	Intron 3
Birgander *et al.* (1995)	Sweden	142 incident cases from central Sweden; age NS; sex ratio NS; median pack years 34; Caucasian	0.7	2.8	19.0	17.6	206 healthy controls from northern Sweden, 76 healthy controls from central Sweden;	3.2	2.8	22.3	23.4	Histology, other p53 polymorphism	43%	44%
							95 control patients with COPD; age NS; sex ratio NS; Caucasian	0.0	0.0	23.2	24.2			
Biros *et al.* (2001b)	Slovakia	109 incident, histologically confirmed lung cancer cases from one centre; age 61.6; 77% male; pack years NS; Caucasian	0.9		33.0		113 healthy controls; age matched to cases; 56% male; pack years NS; Caucasian	2.7		21.2		Sex, histology, codon 72 polymorphism	22%	
Wu *et al.* (2002)	Texas, USA	635 incident, histologically confirmed, previously untreated patients from one centre; age 61.4 (9.7); 53% male; mean pack years: 52 (33.7);	2.7 (Caucasian only)	2.7	30.0 (Caucasian only)	24.2	635 healthy controls recruited from 20 area clinics; age 60.6 (9.8); 56% male; mean pack years 47.3 (32.1);					Race	76%	76%
		Caucasian (n = 517),					Caucasian (n = 544)	2.0	1.8	19.8	17.0			
		Mexican-American (n = 54),					Mexican-American (n = 40)	0.0	0.0	20.0	12.5			
		African-American (n = 64)					African-American (n = 51)	5.9	3.9	35.3	27.5			

[a]Ages and pack years of cases and controls: mean (SD) or range given where possible. NS, not specified.
[b]RR (relative risk) calculated as the probability of detecting an association between *TP53* intron 6 or 3 variant allele carriers and lung cancer risk.

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study of native Hawaiians (Pierce *et al*., 2000) did not ascertain a significant association between the *TP53* codon 72 polymorphism and lung cancer risk; OR 1.30 (95% CI 0.52–3.27) for Pro-Pro homozygotes and 1.30 (95% CI 0.66–2.53) for the Pro-carriers.

It is conceivable that this polymorphism may be associated with a specific histological form of lung cancer. Of the 16 studies that examined a relationship between genotype and lung cancer risk 10 contain information on histology in a form suitable for a pooled analysis (Table II). The pooled ORs for the homozygous Pro-Pro and the Pro-carrier genotypes are 1.14 (95% CI 0.90–1.45, no heterogeneity) and 0.87 (95% CI 0.67–1.13, evidence of heterogeneity, $P = 0.005$) for adenocarcinoma and 0.88 (95% CI 0.65–1.18, no heterogeneity) and 0.95 (95% CI 0.81–1.11, no heterogeneity), respectively, for squamous cell carcinoma. Insufficient data precluded this analysis for other histological types.

Intron 6 (MspI RFLP) polymorphism

Three studies have examined the association of the intron 6 polymorphism with lung cancer risk (Table III). All the controls were in HWE. Pooling data, absence of the *Msp*I restriction site is associated with an OR of 1.13 (95% CI 0.55–2.27, no heterogeneity) for the homozygous genotype and 1.30 (95% CI 0.75–2.26, evidence of heterogeneity, $P = 0.01$) for the carrier genotype.

Intron 3 (16 bp duplication) polymorphism

Two studies have examined the association between the intron 3 polymorphism and lung cancer risk (Table III). Birgander *et al*. (1995) did not detect a significant association between this polymorphism and lung cancer risk; OR 1.24 (95% CI 0.37–4.20) for variant allele homozygotes and 0.74 (95% CI 0.46–1.18) for carriers. Wu *et al*. (2002) reported an increased risk of lung cancer associated with homozygous (adjusted OR 2.37, 95% CI 0.76–7.39) and variant allele carrier genotypes (1.77, 95% CI 1.24–2.52) in Caucasians and Mexican-Americans (data not presented). For African-Americans there was no significant association reported (data not presented). The pooled ORs for the variant allele homozygous and carrier genotypes in the Caucasian population were 1.50 (95% CI 0.76–2.97) and 1.11 (95% CI 0.53–2.35), respectively.

Intron 2 polymorphism

Only one study of the association of intron 2 polymorphism and lung cancer risk has been published to date. Ge *et al*. (1996) studied 61 lung cancer patients, 27 healthy individuals, and 30 bronchiectasis patients of Chinese origin. The frequency of the A2 (G at position 38) homozygous genotype was 22% in the healthy population, 30% in bronchiectasis patients, 29.4% in non-small cell lung cancer patients and 29.6% in patients with small cell lung cancer. The ORs associated with the A2 homozygous and A2 allele carrier genotypes were 2.35 (95% CI 1.04–5.35) and 1.97 (95% CI 1.01–3.82), respectively. The distribution of genotypes in the control group shows a deviation from HWE.

Discussion

The hypothesis that variation in the function of genes responsible for DNA repair mechanisms and cell cycle control in the presence of carcinogen-mediated cell damage is an attractive mechanism for explaining any inter-individual variation in lung cancer susceptibility. As the *TP53* tumour suppressor gene is an important mediator against genotoxic insults it therefore represents a suitable candidate for a lung cancer susceptibility locus.

Although no attempt was made in this meta-analysis to quality score reports (Sacks *et al*., 1987; Dickersin and Berlin, 1992) for inclusion, it is clear that the design of some studies is not optimal. Population stratification is an area of concern, and can lead to spurious evidence for or against an association between the marker and disease. The frequency of *TP53* polymorphisms varies between ethnic groups and therefore a failure to match cases and controls represents the most serious source of bias. Testing the distribution of genotypes in controls for a deviation from HWE provides a simple method of assessing this, although deviation can also indicate genotyping errors. To address possible population stratification in future studies requires the identification of sub-populations defined in terms of factors which can influence disease and marker allele frequencies.

Smoking is the major risk factor for lung cancer and tobacco carcinogens have been shown to exert a direct mutagenic action on DNA of cancer-related genes and *TP53* in particular (Rodin and Rodin, 2000). The majority of the studies used in this analysis reported information on smoking habits of cases and controls. Unfortunately, most of the studies did not provide ORs adjusted for smoking history or the raw data to enable direct calculation of the relative risks, and hence a sub-analysis of the effect of *TP53* polymorphisms on lung cancer risk in relation to tobacco exposure was not possible.

A number of the studies were based on a comparison of cases and non-lung cancer disease controls. The use of a healthy population is preferable since it is conceivable that the *TP53* gene might confer susceptibility to both other cancer and non-cancer diseases. This is, however, unlikely to represent a major source of confounding.

Prime candidate genes for involvement in lung cancer development are those thought to be involved in the aetiology of the disease and variation of which affects protein structure or expression. The codon 72 polymorphism of *TP53* is a single base pair substitution resulting in the replacement of an arginine by a proline in the amino acid sequence of the encoded protein. There is some evidence suggesting that the two variants confer different properties to the TP53 protein. Thomas *et al*. (1999) reported that the Pro variant of *TP53* is less efficient in suppressing cell transformation and slower in inducing apoptosis than the Arg variant. Paradoxically, cells harbouring the Arg variant appear to be more susceptible to the degradation induced by human papillomavirus E6 protein (Storey *et al*., 1998). Marin *et al*. (2000) have suggested that the codon 72 polymorphism influences the ability of TP53 mutants to form stable complexes with p73 (a homologue of p53), correlating with a loss of p73 DNA-binding capability, and ability to induce apoptosis. They observed that the Arg allele was preferentially mutated and retained in squamous cell tumours arising in Arg-Pro germline heterozygotes and concluded that the codon 72 polymorphic residue within *TP53* affects mutant protein behaviour. If the intronic *TP53* polymorphisms confer an increase in lung cancer risk the effect has to be mediated through linkage disequilibrium with a functional variant or changes in gene expression.

Lohmueller *et al*. (2003) recently performed a meta-analysis of a number of genetic association studies (cancer and non-cancer) and reported that up to 25% of published associations are probably real associations but that the inconsistency of findings is accounted for by underpowered studies.

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Most of the studies reviewed in our meta-analysis of *TP53* polymorphisms and lung cancer risk had insufficient power to detect an association between the *TP53* polymorphisms and lung cancer risk, if the true increase in risk is less than 2.

The studies used in this meta-analysis provide data on over 3000 cases and controls. Based on this analysis there is limited evidence to support the hypothesis that polymorphic variation in *TP53* defined by the codon 72, intron 2, intron 3 and intron 6 polymorphisms represent risk factors for lung cancer. We cannot, however, preclude each being associated with a small increase in risk (~1.2). Moreover, it does not preclude other *TP53* variants acting as susceptibility alleles for lung cancer. Further studies of *TP53* polymorphisms should be based on sample sizes commensurate with the detection of small genotypic risks.

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