

International Lung Cancer Consortium: Pooled Analysis of Sequence Variants in DNA Repair and Cell Cycle Pathways

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Abstract

Background: The International Lung Cancer Consortium was established in 2004. To clarify the role of DNA repair genes in lung cancer susceptibility, we conducted a pooled analysis of genetic variants in DNA repair pathways, whose associations have been investigated by at least 3 individual studies.

Methods: Data from 14 studies were pooled for 18 sequence variants in 12 DNA repair genes, including *APEX1*, *OGG1*, *XRCC1*, *XRCC2*, *XRCC3*, *ERCC1*, *XPD*, *XPF*, *XPG*, *XPA*, *MGMT*, and *TP53*. The total number of subjects included in the analysis for each variant ranged from 2,073 to 13,955 subjects.

Results: Four of the variants were found to be weakly associated with lung cancer risk with borderline significance: these were *XRCC3* T241M [heterozygote odds ratio (OR), 0.89; 95% confidence interval (95% CI), 0.79-0.99 and homozygote OR, 0.84; 95% CI, 0.71-1.00]

based on 3,467 cases and 5,021 controls from 8 studies, *XPD* K751Q (heterozygote OR, 0.99; 95% CI, 0.89-1.10 and homozygote OR, 1.19; 95% CI, 1.02-1.39) based on 6,463 cases and 6,603 controls from 9 studies, and *TP53* R72P (heterozygote OR, 1.14; 95% CI, 1.00-1.29 and homozygote OR, 1.20; 95% CI, 1.02-1.42) based on 3,610 cases and 5,293 controls from 6 studies. *OGG1* S326C homozygote was suggested to be associated with lung cancer risk in Caucasians (homozygote OR, 1.34; 95% CI, 1.01-1.79) based on 2,569 cases and 4,178 controls from 4 studies but not in Asians. The other 14 variants did not exhibit main effects on lung cancer risk.

Discussion: In addition to data pooling, future priorities of International Lung Cancer Consortium include coordinated genotyping and multistage validation for ongoing genome-wide association studies. (Cancer Epidemiol Biomarkers Prev 2008;17(11):3081-9)

Introduction

Lung cancer continues to be the most common cancer overall and the leading cause of cancer death worldwide. In 2006, there were an estimated 1,352,000 new cases and 1,179,000 deaths (1). Disease survival continues to be

poor with a 5-year mortality of ~90%. The only current option for disease control is through avoidance of exposure to lung carcinogens. However, much research remains to be done among women, never smokers, and

the young (2, 3). This emphasizes the importance of further understanding its etiology, including carcinogenesis in subgroup of interests and among histopathologic subtypes.

With the aim of sharing comparable data from ongoing lung cancer case-control and cohort studies, as well as increasing power for focused analysis of special subgroups, we established the International Lung Cancer Consortium (ILCCO) in 2004. The overall objectives are to achieve greater power, especially for subgroup analyses, reduce duplication of research effort, replicate novel findings, and maximize the cost efficiency through large collaborative efforts.

The consortium is operated under the guidelines and the policies addressing issues of data sharing, intellectual properties, authorship, and organization. These guidelines and policies followed the general principles adopted by InterLymph (4) and were amended and approved by ILCCO members.⁴² Working groups were formed to oversee research areas that were considered priorities for the consortium. The current working groups include (a) Genetic Susceptibility, (b) Family History, (c) Risks among Nonsmokers, (d) Rare Histologic Types, (e) Occupational Factors, (f) Medical Conditions, and (g) Statistical Analysis (a working group that provides consultation to other groups on a project-specific basis). The coordinators and current research projects of each working group are posted on the ILCCO Web portal.

As the first proof-of-principle study, we conducted pooled analysis on sequence variants in the pathways of DNA repair, a critical defense mechanism against human carcinogenesis, and cell cycle control. The DNA repair system maintains the integrity of the human genome by reducing the mutation frequency of cancer-related genes, minimizing replication errors, removing DNA damage, and minimizing deleterious rearrangements arising via aberrant recombination (5). Cells with damaged DNA must either pause in the cell cycle to allow for repair or succumb to elimination by apoptosis, and the activation of cell cycle checkpoints is a critical component of the cellular response to DNA damage (6). Defects in DNA repair and cell cycle control pathway are likely to play a crucial role in tobacco-related lung cancer *a priori*. It has been hypothesized that a combination of low-penetrance genetic variants may account for a proportion of the genetic component for lung cancer susceptibility, and the candidate gene approach has guided research in this field in the past decade. However, most of the associations in such studies have not been replicated. The likely reasons include lack of statistical power leading to false-negative and false-positive results due to multiple comparisons, the latter being exacerbated due to publication bias. To evaluate whether genetic variants in DNA repair and cell cycle control pathways might influence the predisposition to lung cancer, we studied 18 variants in 12 DNA repair enzymes in a total of 14 lung cancer studies in ILCCO.

Materials and Methods

Study Population. Investigators who had conducted epidemiologic studies of lung cancer were invited to participate in ILCCO and requested to complete a consortium questionnaire. The eligibility criteria of studies to be included in ILCCO were that they had a study protocol for subject recruitment and a structured questionnaire for lifestyle information. Lung cancer researchers with expertise in molecular biology, pathology, and other relevant fields were also invited to join the consortium. The consortium was established with funding from the National Cancer Institute (NCI) and the IARC.

Worldwide, 39 lung cancer studies have participated in ILCCO to date including 19 population-based case-control studies, 14 hospital-based case-control studies, 2 case-control studies with mixed types of controls, and 3 cohort studies, which comes up to a total of more than 46,000 case-control pairs. Fifteen studies were conducted in North America, 13 studies were conducted in Europe, and 11 studies were conducted in Asia and Oceania. The basic characteristics of these 39 studies are summarized at the Table 2.

Pooled Analysis of DNA Repair and Cell Cycle Control Pathways. Fourteen studies that participated in ILCCO had genetic data on DNA repair and cell cycle control pathways and contributed data to this pooled analysis as indicated in Table 1. Six studies were conducted in European countries, four in the United States, and four in Asia or the Pacific islands. Seven studies recruited hospital-based controls, whereas the other seven recruited population-based controls with one nested in a cohort. The control groups in most of the studies were frequency matched with cases on age and sex, whereas some also matched on ethnicity or residence area, and three studies did not apply any matching factors. Written informed consents were obtained from all study subjects, and the investigations were approved by the ethical review board at each study center.

Statistical Methods. The individual-level data from each participating study were sent to the IARC for data pooling. Data elements submitted from the studies included demographic variables (e.g., age, sex, ethnicity, and country of residence), tobacco exposures, family history of cancer, and histology classification of the cases. The data submitted from all 14 studies were checked for inadmissible values, aberrant distributions, inconsistencies, and missing values. Queries were sent to the investigators to resolve all discrepancies and possible errors. Subjects with unknown age or sex were excluded from the analysis. Thus, data from 14 studies with a total of 8,454 lung cancer cases and 9,344 controls were pooled for the present project.

The frequency distribution of demographic variables and putative risk factors of lung cancer, including age, sex, ethnicity, and smoking, was examined for cases and controls. The ethnicity of the subjects were categorized according to the NIH definition as non-Hispanic Whites, Blacks or African Americans, Hispanic or Latinos,

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⁴² <http://ilcco.iarc.fr>

Table 1. Summary of studies participating in ILCCO

	Study	Principal investigator	Control source	Study period	Study location	Cases, <i>n</i>	Controls/ cohort, <i>n</i>
North America	Family Health Study-NS	A.G. Schwartz	Population	1984-1987	Detroit, MI	305	308
	Family Health Study-Young	A.G. Schwartz	Population	1990-2003	Detroit, MI	702	871
	Mayo-P	P. Yang	Population	1997-2006	United States	400	2,000
	University of California at San Francisco	J. Wiencke	Population	1998-2002	San Francisco, CA	1,011	900
	University of California at Los Angeles (29)*	Z.F. Zhang	Population	1999-2004	Los Angeles, CA	600	600
	Women's Epidemiology of Lung Disease	A.G. Schwartz	Population	2001-2005	Detroit, MI	576	575
	New England Lung Cancer Study	E. Duell	Population	2005-2008	New Hampshire, USA	285	250
	NCI-Maryland (30)*	C. Harris	Mixed	1998-2004	Baltimore, MD	800	800
	Samuel Lunenfeld Research Institute	J. McLaughlin	Mixed	1997-2002	Toronto, Ontario, Canada	516	660
	M. D. Anderson (24)*	M. Spitz	Hospital	1992-2004	Houston, TX	3,000	3,000
	Harvard (31)*	D. Christiani	Hospital	1992-2008	Boston, MA	3,300	3,300
	Mayo-H	P. Yang	Hospital	1997-2006	United States	4,000	2,000
	Moffitt	P. Lazarus	Hospital	1999-2003	Florida, USA	500	910
	Cancer Prevention Study-II	M. Thun	Cohort	1982-	United States	150	184,000
	CARET	G. Goodman	Cohort	1985-	United States	1,275	18,314
	Europe	Norway (14)*	A. Haugen	Population	1986-	Norway	2,000
GenAir (10) (European Prospective Investigation into Cancer and Nutrition)*		P. Vineis	Population	1993-1998	10 European countries	271	2,977
Danish Cancer Society		H. Skuladottir	Population	1998-2001	Denmark	281	281
Liverpool Lung Project		J. Field	Population	1998-2016	Liverpool, UK	1,500	3,000
Lung Cancer of Young (32)*		H.E. Wichmann	Population	2000-2004	Germany	800	2,000
deCODE		T. Rafnar	Population	2000-2012	Iceland	800	3,000
INSERM-P		I. Stucker	Population	2002-2005	France	3,000	3,000
NCI-Italy		M.T. Landi	Population	2002-2005	Lombardy, Italy	2,101	2,120
INSERM-CEPH (11)*		S. Benhamou	Hospital	1987-1992	Paris, France	151	171
Lung Cancer Study		I. Stucker	Hospital	1990-1992	Paris, France	310	301
German Cancer Research Center (33)*		A. Risch	Hospital	1996-	Heidelberg, Germany	1,200	650
IARC (12)*		P. Boffetta	Hospital	1998-2002	Central/eastern Europe	2,861	3,118
Asia and Oceania	Institute of Cancer Research	R. Houlston	Hospital		Sutton, UK	6,000	10,000
	NCI-China 1	Q. Lan	Population	1985-1990	Xuan Wei, People's Republic of China	500	500
	Hawaii-P (13)*	L. Le Marchand	Population	1992-1997	Hawaii, USA	582	582
	Kyushu (34)*	C. Kiyohara	Population	1994-1996	Japan	192	130
	NCI-China 2 (35)*	Q. Lan	Population	1995-1996	Xuan Wei, People's Republic of China	122	122
	Singapore 1 (36)*	A. Seow	Hospital	1996-1998	Singapore	303	765
	Aichi	K. Tajima/K. Mastuo	Hospital	2000-2003	Aichi, Japan	400	800
	Seoul	Y.C. Hong	Hospital	2001-2008	Seoul, Korea	1,150	1,150
	Eastern China	H. Shen	Hospital	2002-2007	Eastern China	2,000	2,000
	Singapore 2	A. Seow	Hospital	2005-2007	Singapore	400	800
Total	Hawaii	L. Le Marchand	Cross-sectional	2000-2004	Hawaii, USA	600	
	NCI-China cohort	Q. Lan	Cohort	1975-1995	People's Republic of China	1,600	42,663
					46,544	300,618	

*Indicates the 14 studies contributing to the DNA repair pooled analysis with references provided.

Asians, Native Hawaiians or other Pacific islanders, American Indians, or others. Former smokers were defined as smokers who quit smoking at least 2 years before interview or diagnosis when the exact duration of time since quitting was available or based on self-reports when the duration of quitting was not available. Cumulative tobacco consumption was calculated as the product of smoking duration and intensity and expressed as pack-years.

Data from 14 studies were pooled for 18 sequence variants in 12 DNA repair genes, including *APEX1* D148E; *OGG1* S326C; *XRCC1* R194W, R280H, and R399Q; *XRCC2* R188H; *XRCC3* T241M; *ERCC1* T354C and C8092A; *XPD* D312N and K751Q; *XPF* R415Q; *XPG* H11054D; *XPA* G23A; *MGMT* L84F, I143V, and K178R; and *TP53* R72P. We assessed the deviations from Hardy-Weinberg equilibrium in the control population for individual studies. Studies in which the allele frequency among control group departed from Hardy-Weinberg equilibrium with $P < 0.01$ were excluded from the analysis, including the Norway study from the analysis of *APEX1* D148E, *OGG1* S326C, and *XPD* K751Q (Hardy-Weinberg equilibrium $P < 0.0001$ for all markers mentioned above) and the Harvard study from the analysis of *OGG1* S326C (Hardy-Weinberg equilibrium $P = 0.006$).

Genotypes were categorized into three groups (major allele homozygous, heterozygous, and homozygous variant). We estimated the genotype-specific odds ratios (OR), OR per allele, and their associated confidence intervals (95% CI) of lung cancer in each study using unconditional logistic regression modeling, adjusting for age, sex, cumulative tobacco smoking (expressed as pack-years), and country (when the study was conducted in multiple countries). When information on cumulative tobacco smoking was missing, it was imputed using the median of the study-specific control population. When there were at least three studies available, the summary estimates were obtained using a two-stage random-effect model, which allows for unexplained sources of heterogeneity among studies (7). Studies in which the OR could not be estimated (because one or more cells in the 4-fold table had no subjects) were excluded from the pooled analysis. The number of studies may appear to be different in each stratum depending on the amount of data provided in each stratum. A test of heterogeneity based on Q statistics was done for each summary estimate.

When there was evidence of heterogeneity across the study-specific ORs, we evaluated the source of heterogeneity by meta-regression and by stratified analysis on ethnicity and type of controls (8). If the heterogeneity was not due to any study characteristic, we conducted influence analysis and evaluated the source of heterogeneity from any single study by a Galbraith plot and comparing the Q values. The study contributing the most heterogeneity was excluded from the summary estimate.

We conducted stratified analyses by histology of lung cancer to investigate the potential modification in the effect of each polymorphism by histologic subtype. We also evaluated the modulating effects of tobacco smoking and family history of cancer by stratifying and comparing the strata-specific estimates. All statistical analysis above was conducted with STATA software version 9.

For genes that have multiple single nucleotide polymorphisms that are in linkage disequilibrium ($D' > 0.7$) such as *XRCC1* (R194W, R280H, and R399Q), *ERCC1* (T354C and C8092A), *XPD* (D312N and K751Q), and *MGMT* (I143V and K178R), we have also conducted haplotype analysis based on the pooled data set. Haplotype dosages for variants in linkage disequilibrium were estimated based on E-M algorithm by the tag single nucleotide polymorphism program to indicate an individual's probability of being heterozygote or homozygote and for a log-additive model (9). The haplotype dosage was then analyzed as a continuous variable in multivariate logistic regression adjusting for age, gender, geographic area, and study.

Results

The demographic distribution of the pooled data set for DNA repair genes is shown in Table 2. Individual studies contributed between 1.4% and 26.6% of cases and similar range of controls. More than 60% of the cases and controls were males, and the majority of the subjects were ages >60 years. More than 85% of the cases and controls were non-Hispanic Whites, and ~6% to 7% of the cases and controls were Asians. As expected, the prevalence of smoking was higher among cases than controls. Squamous cell carcinoma and adenocarcinoma were the two predominant histologic subtypes of lung cancer in this analysis.

Table 3 shows the summary ORs for each variant. The total number of subjects included in the analysis for each variant ranged from 2,073 to 13,955. Overall, 2 of the 18 variants were suggested to be associated with lung cancer risk: *XRCC3* T241M (heterozygote OR, 0.89; 95% CI, 0.79-0.99 and homozygote OR, 0.84; 95% CI, 0.71-1.00; P value per allele = 0.01) based on 3,467 cases and 5,021 controls from 8 studies and *TP53* R72P (heterozygote OR, 1.14; 95% CI, 1.00-1.29 and homozygote OR, 1.20; 95% CI, 1.02-1.42; P value per allele = 0.01) based on 3,610 cases and 5,293 controls from 6 studies. The study-specific ORs for *XRCC3* T241M and *TP53* R72P are shown in Fig. 1. In addition, we observed a weak association for two other variants but among homozygote carriers only: *XPD* K751Q and *OGG1* S326C. *XPD* K751Q homozygote carriers conferred an OR (95% CI) of 1.19 (1.02-1.39) based on 6,463 cases and 6,603 controls from 9 studies. There was evidence of heterogeneity for *OGG1* S326C homozygote ($P = 0.02$), which was no longer present after stratification on ethnicity. *OGG1* S326C/326C genotype was shown to be associated with lung cancer risk in non-Hispanic Whites (homozygote OR, 1.34; 95% CI, 1.01-1.79) based on 2,569 cases and 4,178 controls from 4 studies (10-13) but not in Asians. The other 14 variants did not appear to have main effects on lung cancer risk.

Heterogeneity among studies was observed for *XRCC2* R188H (heterogeneity P value for the 188R/188H < 0.0001) and *ERCC1* T354C (heterogeneity $P = 0.002$ for 354T/354C and 0.001 for 354C/354C). This heterogeneity was not explained by either ethnicity, control source, or genotyping methodology but was accounted for by a single outlying study (14). Exclusion of this study did not change the conclusion of the summary estimates but reduced the heterogeneity to the P values of 0.52, 0.42, and 0.80 for *XRCC2* R188H

Table 2. Frequency distribution of demographic variables and putative risk factors of lung cancer in ILCCO pooled data set for DNA repair pooled analysis

	Cases, n (%)	Controls, n (%)
Total	8,454	9,344
Study ID name (genotyping method)		
Norway (TaqMan and APEX; ref. 14)	334 (3.95)	413 (4.4)
GenAir (TaqMan and PE/DHPLC; ref. 10)	116 (1.4)	1,077 (11.5)
Kyushu (RFLP; ref. 34)	190 (2.3)	108 (1.2)
University of California at Los Angeles (RFLP; ref. 29)	497 (5.9)	902 (9.7)
Lung Cancer of Young (MassArray-PE; ref. 32)	270 (3.2)	222 (2.4)
NCI-Maryland (TaqMan; ref. 30)	490 (5.8)	678 (7.3)
INSERM-CEPH (BeadArray; ref. 11)	151 (1.8)	172 (1.8)
M. D. Anderson (TaqMan; ref. 24)	342 (4.0)	362 (3.9)
Harvard (TaqMan; ref. 31)	2,253 (26.6)	1,418 (15.2)
German Cancer Research Center (LightCycler and RFLP; ref. 33)	1,020 (12.1)	425 (4.6)
IARC (TaqMan and Amplifluor; ref. 12)	2,210 (26.1)	2,845 (30.5)
NCI-China 2 (TaqMan; ref. 35)	119 (1.4)	114 (1.2)
Singapore 1 (TaqMan; ref. 36)	125 (1.5)	162 (1.7)
Hawaii-P (RFLP; ref. 13)	337 (4.0)	446 (4.8)
Sex		
Male	5,332 (63.1)	5,698 (61.0)
Female	3,122 (36.9)	3,646 (39.0)
Age (y)		
<40	163 (1.9)	431 (4.6)
40-49	1,152 (13.6)	1,539 (16.5)
50-59	2,362 (27.9)	2,838 (30.4)
60-69	2,693 (31.8)	2,854 (30.5)
70-79	1,835 (21.7)	1,568 (16.8)
≥80	249 (2.9)	114 (1.2)
Smoking status		
Never	878 (10.4)	3,326 (35.6)
Ever	7,547 (89.3)	5,999 (64.2)
Former	2,688 (31.8)	3,211 (34.4)
Current	4,788 (56.6)	2,721 (29.1)
Education		
Low	1,249 (18.7)	1,715 (21.0)
Medium	3,421 (51.2)	3,761 (46.0)
High	2,006 (30.0)	2,698 (33.0)
Ethnicity		
Non-Hispanic Whites	7,404 (87.6)	8,033 (86.0)
Asians	600 (7.1)	605 (6.5)
Native Hawaiian/other Pacific Islander	85 (1.0)	102 (1.1)
Black or African American	251 (3.0)	359 (3.8)
Hispanic or Latino	96 (1.1)	213 (2.3)
American Indian or Alaska Native	5 (0.1)	3 (0.03)
Other	8 (0.1)	27 (0.3)
Histology		
Squamous cell carcinoma	2,399 (28.4)	
Small cell carcinoma	1,052 (12.4)	
Large cell carcinoma	498 (5.9)	
Adenocarcinoma	2,923 (34.6)	
Others, not otherwise specified	1,416 (12.9)	

NOTE: Education level: low, no education to junior high school; medium, high school or technical school level; high, university level and above. PE, primer extension; APEX, arrayed primer extension.

heterozygote and *ERCC1* T354C heterozygote and homozygote, respectively.

Table 4 shows the stratified estimates of *XRCC3* T241M and *TP53* R72P by ethnicity, smoking, histology, and gender. There were no differences in the stratum-specific estimates when stratified by ethnicity and gender. However, both variants appeared to confer a stronger association with lung cancer risk among smokers. In terms of the effect on the histologic subtypes, *XRCC3* T241M allele showed a more prominent effect on risk of small cell carcinoma (heterozygote OR, 0.84; 95% CI, 0.66-1.08 and homozygote OR, 0.66; 95% CI, 0.44-0.98), whereas *TP53* R72P allele showed a more prominent effect on risk of squamous cell carcinoma (heterozygote OR, 1.26; 95% CI, 1.04-1.52 and homozygote OR, 1.52; 95% CI, 1.19-1.94).

Haplotype analysis suggested that subjects who carried the *XPD* 312N-751Q haplotype had an increased risk of lung cancer with an OR (95% CI) of 1.19 (1.03-1.37) when the subjects carried two copies of such haplotype. We did not observe any haplotype-specific association for *APEX1*, *XRCC1*, and *ERCC1* (data not shown).

Discussion

We have established the foundation of international collaboration in the area of molecular and genetic epidemiology of lung cancer. Here, we pooled the genotype data for 18 sequence variants that are commonly investigated for lung cancer from studies in the United

Table 3. Summary estimates of the main effects of the genetic variants

Gene variant (dbSNP no.)	No. studies (reference)	Case no. GG/Gg/gg	Control no. GG/Gg/gg	Heterozygotes OR (95% CI)	Homozygotes OR (95% CI)	Per allele OR (95% CI)	P_{trend}	$P_{\text{heterogeneity}}$
APEX1 D148E (rs3136820)	6 (10-12, 31, 33, 35)	1,499/2,480/1,101	1,596/2,903/1,361	0.89 (0.81-0.99)	0.91 (0.78-1.06)	0.95 (0.88-1.02)	0.14	0.34
OGG1 S326C (rs1052133)*	4 (10-13)	1,648/805/116	2,628/1,389/161	0.95 (0.84-1.07)	1.34 (1.01-1.79)	1.02 (0.93-1.13)	0.63	0.82
XRCC1 R194W (rs1799782)	5 (10-12, 14, 35)	2,495/363/26	3,947/596/28	0.92 (0.79-1.08)	1.57 (0.76-3.26)	0.98 (0.85-1.13)	0.77	0.54
XRCC1 R280H (rs25489)	4 (12, 14, 32, 35)	2,568/273/16	3,123/351/9	1.01 (0.75-1.36)	2.06 (0.83-5.09)	1.06 (0.83-1.37)	0.62	0.21
XRCC1 R399Q (rs25487)	9 (10-12, 14, 31-35)	2,768/2,871/793	2,824/2,934/792	0.98 (0.88-1.08)	0.93 (0.75-1.14)	0.97 (0.89-1.05)	0.39	0.26
XRCC2 R188H (rs3218536) [†]	3 (10-12)	2,126/281/10	3,324/470/18	1.01 (0.84-1.21)	0.90 (0.35-2.28)	0.99 (0.84-1.17)	0.90	0.74
XRCC3 T241M (rs861539)	8 (10-12, 14, 33-36)	1,719/1,386/362	2,144/2,248/629	0.89 (0.79-0.99)	0.84 (0.71-1.00)	0.91 (0.84-0.98)	0.01	0.50
ERCC1 T354C (rs11615) [†]	3 (10, 12, 31)	1,730/2,052/684	2,034/2,373/811	1.04 (0.94-1.15)	1.03 (0.89-1.19)	1.02 (0.95-1.09)	0.56	0.64
ERCC1 C8092A (rs3212986)	3 (12, 14, 31)	2,660/1,719/309	2,569/1,694/288	1.00 (0.91-1.11)	1.06 (0.88-1.29)	1.02 (0.94-1.09)	0.66	0.61
XPD D312N (rs1799793)	9 (10, 12, 14, 24, 30-33, 35)	2,875/2,988/1,009	2,948/3,124/1,011	0.96 (0.86-1.06)	1.09 (0.97-1.23)	1.03 (0.97-1.08)	0.37	0.83
XPD K751Q (rs13181)	9 (10-12, 24, 31, 33-36)	2,592/2,869/1,002	2,650/2,995/958	0.99 (0.89-1.10)	1.19 (1.02-1.39)	1.07 (0.98-1.18)	0.15	0.05
XPF R415Q (rs1800067)	3 (11, 12, 14)	2,201/306/13	2,208/390/21	1.00 (0.84-1.19)	0.72 (0.24-2.13)	0.96 (0.82-1.13)	0.65	0.66
XPG H1104D (rs17655)	5 (11, 12, 29, 24, 35)	1,852/1,155/209	2,485/1,510/286	1.07 (0.91-1.25)	1.00 (0.81-1.25)	1.03 (0.93-1.13)	0.62	0.32
XPA G23A (rs1800975)	3 (12, 14, 33)	1,181/1,260/362	1,422/1,603/427	0.85 (0.66-1.10)	1.06 (0.82-1.37)	0.97 (0.82-1.15)	0.70	0.08
MGMT L84F (rs12917)	3 (10, 14, 35)	366/143/19	1,121/391/33	0.92 (0.60-1.41)	1.53 (0.76-3.08)	1.02 (0.73-1.42)	0.91	0.13
MGMT H43V (rs2308321)	3 (11, 12, 14)	1,696/538/45	2,594/675/51	1.06 (0.94-1.20)	1.05 (0.91-1.20)	1.19 (0.71-1.99)	0.50	0.31
MGMT K178R (rs2308327)	3 (12, 14, 35)	1,800/528/108	2,342/621/112	1.08 (0.93-1.26)	1.27 (0.63-2.54)	1.07 (0.91-1.25)	0.41	0.31
TP53 R72P (rs1042522)	6 (10, 12, 13, 30, 33, 36)	1,764/1,480/366	2,733/2,079/481	1.14 (1.00-1.29)	1.20 (1.02-1.42)	1.10 (1.02-1.18)	0.01	0.40

NOTE: OR, individual OR adjusted for age, sex, cumulative tobacco smoking (as pack-years), and country when applicable. GG/Gg/gg, major allele homozygotes/heterozygotes/minor allele homozygotes.

*Estimates in non-Hispanic Whites only due to the significant heterogeneity by ethnicity and limited data on Asians.

[†]Norway study was excluded as it conferred an outlying estimate and contributed to the majority of the heterogeneity.

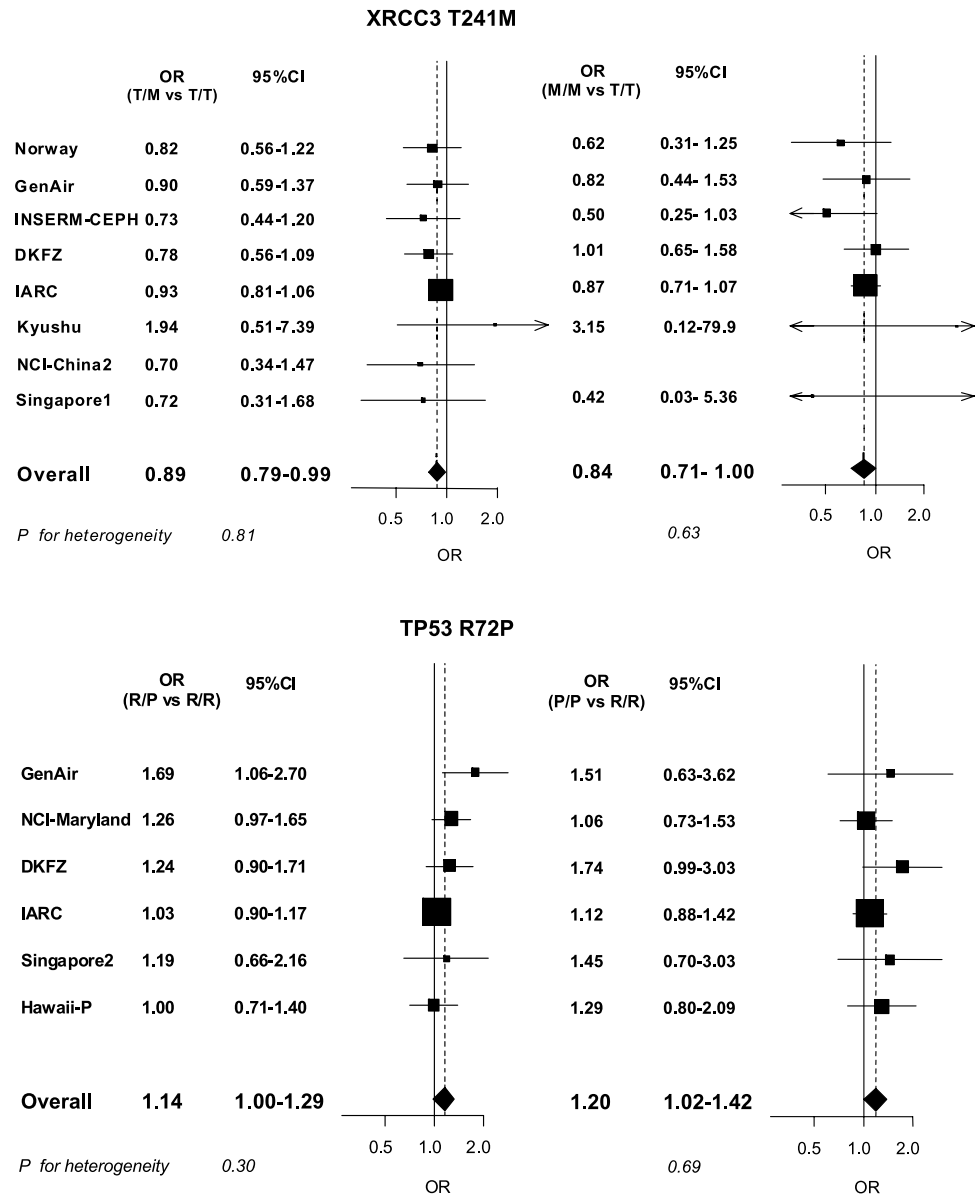


Figure 1. Study-specific estimates and summary OR for *XRCC3* T241M and *TP53* R72P.

States, Europe, and Asia. None of the variants appeared to have a large effect on lung cancer risk, although we did observe a modest association of *XRCC3* T241M and *TP53* R72P polymorphisms and lung cancer risk overall as well as the effect of *XPD* haplotype 312N-751Q and *OGG1* 326C/326C genotype on lung cancer risk among non-Hispanic Whites. The potential associations between lung cancer risk and the other 14 variants were refuted based on this large analysis.

TP53 gene is one of the most studied human genes due to its critical role as tumor suppressor gene, and we observed an increased risk of lung cancer among *TP53* 72Pro allele carriers. The 72Pro allele has been suggested to be less efficient in suppressing cell transformation and to induce apoptosis with slower kinetics (15, 16). Several studies have investigated the association between this variant and lung cancer risk; however, the results have been inconsistent as reviewed by Matakidou et al. (17).

We observed a more apparent effect of 72Pro allele among smokers, which agrees with the stronger association with the risk of squamous cell carcinoma. These results suggest that the effect of *TP53* 72Pro allele on lung cancer risk is mainly present in an environment challenged by tobacco-related carcinogens. It has also been shown previously that *TP53* somatic mutations increase the risk of lung squamous cell carcinoma when compared with adenocarcinoma (18).

We have observed a modest protective effect conferred by *XRCC3* 241Met allele carriers. Again, the association is mainly present among smokers and tobacco-related histology, such as small cell carcinoma and squamous cell carcinoma with no evidence of heterogeneity across ethnicities. *XRCC3* is a protein of Rad51-related family, which participates in homologous recombination repair of the double-strand breaks (19). Previous studies showed that the 241Met allele was shown to increase

Table 4. Stratified analysis for XRCC3 T241M and TP53 R72P by ethnicity, smoking status, and histology

Gene variant	No. studies	No. cases	No. controls	Heterozygotes OR (95% CI)	Homozygotes OR (95% CI)	Per allele OR (95% CI)	<i>P</i> _{trend}	<i>P</i> _{heterogeneity}
XRCC3 T241M								
Ethnicity								
Non-Hispanic Whites*	5	3,042	4,644	0.89 (0.80-0.99)	0.84 (0.71-1.00)	0.91 (0.84-0.98)	0.01	0.54
Asian*	3	425	377	0.82 (0.49-1.37)	0.92 (0.12-6.98)	0.84 (0.48-1.49)	0.55	0.22
Smoking								
Never	6	477	1,951	1.03 (0.78-1.36)	1.28 (0.86-1.92)	1.10 (0.91-1.33)	0.32	0.50
Ever*	7	2,985	3,062	0.85 (0.76-0.96)	0.78 (0.65-0.94)	0.87 (0.80-0.95)	0.002	0.73
Former*	5	686	1,410	0.79 (0.63-0.98)	0.77 (0.55-1.09)	0.86 (0.73-1.00)	0.05	0.93
Current*	5	2,229	1,587	0.91 (0.78-1.05)	0.80 (0.54-1.20)	0.90 (0.79-1.03)	0.11	0.32
Histology								
Squamous cell*	5	1,350	3,832	0.88 (0.71-1.11)	0.78 (0.62-0.99)	0.90 (0.74-1.08)	0.24	0.14
Small cell*	4	414	3,426	0.84 (0.66-1.08)	0.66 (0.44-0.98)	0.83 (0.70-0.98)	0.03	0.82
Adenocarcinoma*	6	935	4,741	0.88 (0.74-1.05)	1.04 (0.80-1.34)	0.98 (0.87-1.10)	0.72	0.87
Gender								
Male [‡]	7	2,546	3,348	0.87 (0.76-0.99)	0.83 (0.69-1.01)	0.90 (0.82-0.98)	0.02	0.49
Female [‡]	7	851	1,664	0.91 (0.74-1.13)	0.90 (0.64-1.26)	0.94 (0.81-1.10)	0.44	0.44
TP53 R72P								
Ethnicity								
Non-Hispanic Whites*	5	3,159	4,605	1.14 (0.94-1.39)	1.19 (0.97-1.45)	1.12 (0.99-1.26)	0.07	0.20
Asian*	2	232	332	1.30 (0.87-1.95)	1.46 (0.87-2.47)	1.23 (0.95-1.59)	0.92	0.85
Smoking								
Never	6	424	2,082	1.16 (0.91-1.48)	0.96 (0.64-1.43)	1.03 (0.87-1.22)	0.69	0.59
Ever*	5	3,181	3,203	1.17 (0.96-1.41)	1.37 (1.05-1.77)	1.18 (1.03-1.35)	0.017	0.11
Former*	5	929	1,804	1.23 (1.03-1.48)	1.37 (1.02-1.85)	1.19 (1.05-1.36)	0.007	0.42
Current*	4	2,251	1,397	1.11 (0.83-1.49)	1.42 (0.83-2.42)	1.17 (0.91-1.50)	0.20	0.03
Histology								
Squamous cell*	5	1,296	4,466	1.26 (1.04-1.52)	1.52 (1.19-1.94)	1.22 (1.10-1.35)	<0.001	0.59
Small cell*	3	376	3,635	0.81 (0.63-1.05)	1.27 (0.68-2.37)	0.96 (0.80-1.15)	0.64	0.41
Adenocarcinoma*	6	1,061	5,294	1.05 (0.90-1.22)	1.00 (0.77-1.29)	1.02 (0.91-1.14)	0.75	0.60
Gender								
Male [‡]	5	2,495	3,307	1.09 (0.96-1.23)	1.30 (1.05-1.60)	1.12 (1.02-1.22)	0.01	0.49
Female [‡]	6	1,115	1,987	1.14 (0.95-1.36)	1.10 (0.83-1.46)	1.08 (0.95-1.22)	0.25	0.75

*OR adjusted for age, sex, tobacco cumulative exposure (pack-years), and country when applicable.

†OR adjusted for age, sex, and country when applicable.

‡OR adjusted for age, tobacco cumulative exposure (pack-years) and country when applicable.

the DNA adduct level but had no effect on the repair of UV light-induced damage (20, 21). Two meta-analysis were conducted previously and reported a null association (21, 22). However, neither of them was able to adjust the results by a standard set of covariates across studies nor stratification by histology or smoking status, which may at least partially explain the differences in the conclusions from the present analysis.

The major strength of the pooled analysis in the consortium was to increase statistical power for common sequence variations with possibly modest effects, particularly for analysis in subgroups of interests such as rare histology, never smokers, or familial cases. In addition, pooling individual data has the advantage of being able to conduct analysis based on a standard approach as well as including multiple markers such as haplotype analysis.

There are several limitations of the present pooled analysis. First, because the studies were conducted in different populations and did not follow a standard protocol, the validity of the pooled estimate can be also threatened by the heterogeneity of the studies. In addition to single outlying estimates, heterogeneity can result from differences among study populations, study design, and often methodologic aspects including genotyping methodology. Apart from using random-effects models to allow for study heterogeneity to be taken into account, we also conducted stratified analysis by study design when there was evidence of heterogeneity.

However, we did not observe different effects by control source or genotyping methodology (data not shown). Second, the pooled data set contains subjects with different ethnic ancestry, mainly European decedents and Asians, which might lead to bias from population stratification or simply population mixing and mask the true association. We have conducted stratified analysis by ethnicity whenever appropriate but did not observe any differential effect by ethnicity, except for *OGG1* S326C, for which we reported the results of non-Hispanic Whites only, as they contributed the majority of the data for this variant. Third, the pooled analysis is limited to existing data available in at least three of the studies in ILCCO. Therefore, we were not able to conduct comprehensive investigations using tagging single nucleotide polymorphisms of specific genes of interest. For example, another TP53 variant of 16-bp repeats located in intron 3, which is in linkage disequilibrium with the R72P allele, has been hypothesized to increase lung cancer risk (23, 24). However, we were not able to disentangle the effect conferred by these two linked variants in the present analysis due to lack of relevant data in the participating studies. This limitation can be overcome in the consortium by coordinated genotyping instead of simply data pooling, and this is currently under way in ILCCO.

Future Research Priorities and Consortium Values. ILCCO provides an opportunity for leading researchers

of lung cancer epidemiology to share results, plan pooled analyses, and discuss replication studies. This study shows the value of consortia for clarifying putative risk associated with complex diseases (25). Other ongoing research activities in ILCCO include pooled analysis of risk factors for rare histologic types of lung cancer, pooled analysis of data on family history, occupational exposures, and indoor air pollution.

Future prospects of ILCCO include multistage validation and fine mapping for possible causative genetic regions identified from ongoing genome-wide association studies (26-28). In this respect, consortia and international collaborations are developing into an ideal way to maximize study efficiency and overcome the limitations (particularly in terms of statistical power) of individual studies. We anticipate that ILCCO will be a major step toward improving our understanding of the causes and mechanisms of lung cancer and the beginning of a long-standing cooperation.

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