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Radon, secondhand smoke, glutathione-*S*-transferase M1 and lung cancer among women

Matthew R. Bonner[1]*, William P. Bennett[2], Wenying Xiong[2], Qing Lan[1], Ross C. Brownson[3], Curtis C. Harris[4], R. William Field[5,6], Jay H. Lubin[7] and Michael C.R. Alavanja[1]

[1]*Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, US Department of Health and Human Services, Rockville, MD*
[2]*Division of Molecular Medicine, Beckman Research Institute at the City of Hope, Duarte, CA*
[3]*Department of Community Health, School of Public Health, St. Louis University, St. Louis, MO*
[4]*Laboratory of Human Carcinogenesis, Division of Cancer Biology, National Cancer Institute, National Institutes of Health, US Department of Health and Human Services, Rockville, MD*
[5]*Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, IA*
[6]*Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA*
[7]*Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, US Department of Health and Human Services, Rockville, MD*

Tobacco smoke and ionizing radiation induce oxidative stress by transmitting or generating reactive oxygen species (ROS). We hypothesized that glutathione-*S*-transferase M1 (*GSTM1*) null homozygotes would have decreased ability to neutralize ROS that might increase their susceptibility to lung cancer. A case-only design was used with lung cancer cases pooled from 3 previously completed case-control studies using archival tissue samples from 270 lung cancer cases to genotype *GSTM1*. Radon concentrations were measured with long-term α-track radon detectors. Secondhand smoke (SHS) was measured with questionnaires and interviews. Unconditional logistic regression was used to calculate the interaction odds ratios (OR) and 95% confidence intervals (95% CI). Radon concentrations >121 Bq m^{-3} were associated with a >3-fold interaction OR (OR = 3.41; 95% CI = 1.10, 10.61) for *GSTM1* null homozygotes compared to *GSTM1* carriers; the linear trend was significant (p trend = 0.03). The SHS and *GSTM1* interaction OR was also elevated (OR = 2.28; 95% CI = 1.15–4.51) among never-smokers. This may be the first study to provide evidence of a *GSTM1* and radon interaction in risk of lung cancer. Additionally, these findings support the hypothesis that radon and SHS promote neoplasia through shared elements of a common pathway.

Key words: radon; secondhand smoke; glutathione-*S*-transferase; lung cancer; epidemiology

Cigarette smoking is the leading cause of lung cancer,[1] but radon[2] and secondhand smoke[3] play important roles—especially among nonsmokers. Traditionally, radon and secondhand smoke (SHS) have been considered very different carcinogens: SHS has many chemical mutagens, while radon emits high linear energy transfer (LET) radiation in the form of an α-particle. However, recent evidence suggests that both may damage lung epithelia by generating reactive oxygen species.[4] Incinerated tobacco produces large quantities of reactive oxygen species (ROS),[4–6] and evidence is accumulating that high LET radiation need not traverse a cell's nucleus in order to damage DNA.[7,8] Instead, α-particles that pass through the cytoplasm may generate oxygen anions and hydrogen peroxide that produce mutations and other DNA lesions.[8] Further, neighboring, nonirradiated cells may be damaged *via* a "bystander effect,"[9,10] whereby, cellular signals from an irradiated cell may induce oxidative stress in adjacent nonirradiated cells.[8,11]

If radon and SHS operate, at least in part, through oxidative stress to induce lung carcinogenesis, then variable expression of xenobiotic metabolizing enzymes that quench ROS may affect lung cancer risk. Glutathione-*S*-transferases (*GST*s) are important in quenching and detoxifying ROS and their derivatives.[12] Genetic polymorphisms in genes that code for the *GST*s involved in the metabolism of endogenous and exogenous compounds are of primary interest in this regard. Glutathione-*S*-transferase M1 (*GSTM1*) is notable because 38–62% of Caucasians carry a "null" allele (*i.e.*, homozygous deletion) and do not express this enzyme.[13] These ideas suggest that individuals with the *GSTM1* null genotype may have greater risks of cancer because they are unable to detoxify certain types of reactive compounds. However, an association between *GSTM1*and lung cancer has not been clearly demonstrated among either smokers[14] or nonsmokers.[15]

Previously, we found that *GSTM1* null genotype more than doubled risk of lung cancer among never-smokers who were exposed to SHS (OR = 2.6, 95% CI = 1.1–6.1)[16] compared to *GSTM1* carriers. Since that initial report,[16] we have collected additional cases from 2 other case-control studies of lung cancer in Missouri[17] and Iowa.[18] The expanded series includes smokers as well as detailed measurements of exposure to residential radon. We report here our updated findings on SHS in never-smokers, as well as initial observations linking residential radon, *GSTM1* and risks of lung cancer.

Material and methods

Study population

We used a case-only study design that assumes exposure and genotype are independent in the controls, *i.e.*, the exposure must not be associated with the genotype in the control series.[19] Lung cancer cases were pooled from 3 case-control studies: 2 from Missouri (Missouri-I[20] and Missouri-II[17]) and 1 from Iowa.[18] All 3 studies used similar methods to measure exposure to secondhand smoke, radon and potential confounders. The methods for each of these studies has been described in detail elsewhere.[17,18,20] Briefly, lung cancer cases were identified from the Missouri and Iowa cancer registries. Cases ranged in age from 30 to 84 years. In-person interviews were used in Missouri-II and Iowa, while a telephone interview was primarily used in Missouri-I. In total, 1,755 lung cancer cases were recruited into these 3 studies. We received permission to retrieve archived tumor blocks from all the cases in all

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The first two authors contributed equally to this work.

Abbreviations: CI, confidence interval; GSTM1, glutathione-*S*-transferase M1; LET, linear energy transfer; OR, odds ratio; Rn, radon; ROS, reactive oxygen species; SHS, secondhand smoke.

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***Correspondence to:** Department of Social and Preventive Medicine, School of Public Health and Health Profession, University at Buffalo, Buffalo, NY 14214-8001, USA. Fax: +716-829-2979.
E-mail: mrbonner@buffalo.edu

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3 studies. However, we were only able to retrieve tumor blocks for 15% of the cases because most of the blocks were not retained by the pathology departments. Study protocols were approved by the appropriate institutional review boards and informed consent was obtained from all study participants.

Laboratory methods

Formalin-fixed, paraffin-embedded (FFPE) lung cancer tissues from therapeutic resections or diagnostic biopsies were collected from hospital pathology laboratories for 270 cases. Histologic sections were prepared using protocols to minimize contamination by tissue carry-over.[21] Nontumor tissues were microdissected manually for germline analysis. Genomic DNA was isolated using standard methods including enzymatic digestion of proteins followed by extraction with organic solvents and precipitation with ethanol.[22] Homozygous deletion of *GSTM1* coding sequences was assayed by manual methods using updated versions of protocols that were reported previously.[16] In brief, DNA samples from nontumor tissues were amplified in multiplex PCR reactions, and the products were examined by electrophoresis on agarose gels. Genotypes were inferred from patterns of DNA fragments in control samples. Samples collected since a previous report[16] were assayed with newly designed PCR primers, which were developed using modern algorithms[23] for selecting priming sites and genomic sequences of all known isomers. Thirty samples were tested with both assays: the results showed good agreement, and details are presented below. The *GSTM1* assay used heminested primers and 2 rounds of amplification to examine small samples from tissue biopsies. Primers were screened for overlap (*i.e.*, dimerization) and for homology to common repeat elements.

Genotyping assay

Homozygous deletion of *GSTM1* coding sequences is commonly assayed by a multiplex PCR reaction, which includes primers for a positive control gene (*i.e.*, *XRCC1*). Using this design, the absence of a product can be interpreted as *GSTM1* null so long as appropriate bands from the multiplexed control are observed. However, this strategy cannot discriminate the presence of 1 or 2 alleles of *GSTM1*. The external *GSTM1* primers amplified a 134 nucleotide segment extending from exon 5 to intron 5: forward, 5′-ggcatgatctgctacaat-3′ and reverse, 5′-gtaccattcatcctccaa-3′. The heminested second round used the same external forward primer and a nested reverse primer (5′-gacagagtttggatttgg-3′) to produce a 71 nucleotide product. The positive control gene used was *XRCC1* and external *XRCC1* primers amplified a 154 nucleotide segment of exon 10: forward, 5′-tgccaacacccccaagta-3′ and reverse, 5′-attgcccagcacaggataag-3′. The second round used nested (over-lapping) primers that produced a 142 nt product: forward, 5′-ccccaagtacagccaggtc-3′ and reverse, 5′-gcccagcacaggataaggag-3′.

Reaction mixtures

For the first round of amplification, genomic DNA (25 ng) was added to a PCR mix (20 μl total volume) composed of primers (0.4 μM, final concentration), deoxynucleoside triphosphates (200 μM, final concentration), 1.0 unit polymerase (Amplitaq, PerkinElmer, Norwalk, CT) in Amplitaq buffer (1×, final concentration). The second round reaction used nested or heminested primers plus the same PCR mix, except that 1.0 μl of the first round product was added as DNA template.

Thermocycling protocols

The PCR mixtures were assembled on wet ice and loaded onto a thermocycler (PE GeneAmp PCR System 9600). The thermocycling protocol used tiered (*i.e.*, "step-down" or "touch-down") annealing temperatures to accommodate differences and to reduce spurious priming.[24] The first round had an initial denaturation step at 95°C for 2 min, then a total of 19 cycles of denaturation (94°C for 30 sec), annealing (45 sec) at 4 tiered temperatures, and extension (72°C for 60 sec) followed by a terminal extension reaction (72°C for 5 min). The tiered annealing temperatures were 56°C for 3 cycles, 54°C for 3 cycles, 52°C for 3 cycles and 50°C for 10 cycles. The second round was similar except for additional cycles: an initial denaturation step at 95°C for 2 min, then a total of 32 cycles of denaturation (94°C for 30 sec), annealing (45 sec) at 4 tiered temperatures, and extension (72°C for 60 sec) followed by a terminal extension reaction (72°C for 5 min). The tiered annealing temperatures were 56°C for 4 cycles, 54°C for 4 cycles, 52°C for 4 cycles and 50°C for 20 cycles.

Comparison of old and new genotyping assays

We developed a new genotyping assay for *GSTM1* to test the new samples collected since our initial report.[16] We did this because modern primer design software showed that our previous assay—originally reported in 1997[25]—formed stable primer dimers, which reduce the sensitivity of PCR-based assays. To compare the 2 assays, we collected 30 samples that were analyzed by the old method, and we analyzed them with the new assay. When we compared the results, we found 90% concordance between the assays: there was complete agreement in 7 *GSTM1* present cases and 11 *GSTM1* null cases, but 2 formerly null cases showed evidence of at least 1 *GSTM1* allele in the new assay. Furthermore, the new protocol provided genotypes for 9 of 10 samples that had failed to amplify previously. These findings indicate that the new assay is equally specific, but more sensitive than the previous protocol.

Exposure assessment

Radon dosimetry for these studies has been described elsewhere.[17,18,20] Briefly, radon concentrations were measured in Missouri-I and Iowa using α-track radon detectors placed in these residences for a 1 year period. Iowa included only cases who resided at their current address for 20 or more years, while the Missouri-I study attempted to measure radon in all previous addresses in the 30 years prior to diagnosis. The Missouri-II study used 2 methods to measure residential radon. Similar to the Iowa and Missouri-I studies, 2 air-based α-track detectors were placed in current residences for 1 year. Annual time-weighted averages were calculated as the sum of the mean radon concentration of all the homes weighted by the years of residence spent in each home using the air-based α-track detector measurements. In addition to these air-based measurements, Missouri-II derived radon concentrations using the glass-based dosimeters.[26,27] This method measures decay of polonium-210, a decay product of radon that can embed in glass objects. The main advantages of glass-based dosimetry are (*i*) it provides a time-weighted estimate of radon exposure that is proportional to the age of the glass object and (*ii*) it eliminates the need to collect air samples from 30 years worth of domiciles. The time-weighted average concentration of radon was reported as Becquerel per cubic meter (Bq m^{-3}) where 37 Bq m^{-3} is equivalent to 1 pCi/L.

Exposure to secondhand smoke was assessed by telephone interviews for Missouri-I and in person interviews for Missouri-II and Iowa studies. For Missouri I and II, exposure to SHS was estimated quantitatively, whereby pack-years of exposure to cigarette smoke was calculated as the product of the number of packs of cigarettes a spouse smoked in confined spaces and the number of years this occurred. In Iowa, this quantitative metric was not used; however, SHS was estimated qualitatively as ever or never exposed to SHS.

Statistical analysis

The case-only design was used to examine whether there were interactions between exposure to residential radon, *GSTM1* and lung cancer. We also examined SHS exposure, *GSTM1* and lung cancer. Student's *t*-tests were used to determine statistical differences in continuous variables between *GSTM1* null and *GSTM1* present cases, while the χ^2 statistic was used for categorical variables. Unconditional logistic regression was used to calculate the

TABLE I – SELECTED CHARACTERISTICS OF LUNG CANCER CASES BY TUMOR BLOCK STATUS[1]

	FFPE[2] block (n = 267)	No FFPE block (n = 1,485)	p value[3]
Age	68.0 ± 10.6	67.9 ± 10.0	0.84
Radon (Bq m^{-3})	64.1 ± 48.6	82.6 ± 89.6	0.002
Smoking (pack-years)	20.1 ± 31.5	33.7 ± 32.4	<0.001
Secondhand smoke (pack-years)[4]	18.2 ± 26.4	22.6 ± 32.7	0.12
Education (years)			
<12	86 (33.2)	467 (32.2)	0.35
12	108 (41.7)	658 (45.5)	
>12	65 (25.1)	322 (22.3)	
Smoking status			
Never	160 (60.6)	388 (25.9)	<0.001
Former	35 (13.3)	597 (40.0)	
Current	69 (26.1)	508 (34.0)	
Histology			
Adenocarcinoma	164 (64.8)	540 (44.4)	<0.001
Nonadenocarcinoma	89 (35.2)	677 (55.6)	

[1]Values are number (%) unless otherwise indicated.–[2]FFPE, formalin fixed, paraffin embedded.–[3]Differences in means across categories assessed with Student's t-test; differences in categorical variables assessed with χ^2.–[4]Among never active smokers only.

TABLE II – SELECTED CHARACTERISTICS OF LUNG CANCER CASES BY GLUTATHIONE-*S*-TRANSFERASE (*GSTM1*) GENOTYPE[1]

	GSTM1 null (n = 138)	*GSTM1* present (n = 129)	p value[2]
Age	68.3 ± 10.2	67.7 ± 10.9	0.63
Radon (Bq m^{-3})	70.3 ± 55.5	55.5 ± 37.0	0.01
Smoking (pack-years)	20.1 ± 32.2	20.1 ± 30.8	0.99
Study			
Missouri-I	73 (53.3)	59 (46.5)	0.50
Missouri-II	55 (40.2)	61 (47.2)	
Iowa	10 (6.6)	9 (6.3)	
Education (years)			
<12	45 (33.1)	41 (33.3)	0.45
12	58 (42.7)	50 (40.7)	
>12	33 (24.3)	32 (26.0)	
Smoking status			
Never	88 (64.2)	72 (56.7)	0.45
Former	16 (11.7)	19 (15.0)	
Current	33 (24.1)	36 (28.4)	
Histology			
Adenocarcinoma	91 (67.9)	73 (61.3)	0.28
Nonadenocarcinoma	43 (32.1)	46 (38.7)	

[1]Values are number (%) unless otherwise indicated.–[2]Differences in means across categories assessed with Student's t-test; differences in categorical variables assessed with χ^2.

interaction odds ratios (OR) and 95% confidence intervals (95% CI). In a traditional logistic regression approach, the probability of being a case is modeled for a dichotomous dependent variable (*i.e.*, case or control). Because of the case-only study design, we used a logistic model to estimate the probability of being a *GSTM1* null homozygote where *GSTM1* null homozygotes are analogous to cases and *GSTM1* carriers are analogous to controls in a traditional logistic model. The interaction OR measures the degree to which the joint effect of the environmental exposure and the gene depart from what would be expected by the product of the gene OR alone and the exposure OR alone. In the absence of a gene-environment interaction, the interaction OR would be equal to one.[19]

Exposure to radon was categorized into quartiles based on the distributions among those with the null *GSTM1* genotype. The fourth quartile of radon was further divided at the median. SHS exposure was categorized into tertiles among the *GSTM1* null cases with SHS exposure; the referent group consisted of cases with no exposure to spousal SHS. In addition, SHS was dichotomized as ever or never exposed residential SHS.

Multivariable statistical models were adjusted for age. When radon was treated as the exposure variable, the models were further adjusted for SHS exposure (ever/never) and pack-years of active smoking. When SHS was treated as the exposure variable, the analysis was restricted to never active smokers and the models were further adjusted for radon exposure. In addition, we stratified both SHS and radon exposure models by histology to assess potential effect modification. The p for trend (two-sided) was determined by the p-value for the coefficient of the continuous exposure variable, while adjusting for covariates.

Results

Archival, formalin-fixed tissue samples were collected for 270 lung cancer cases, and *GSTM1* genotyping results were determined for 267 (99%). Among the 267 genotypes, 106 were reported previously in a study of SHS and nonsmokers,[16] and 161 are new findings from new samples using a new assay. Overall, 52% of cases had homozygous deletion (*GSTM1* null).

A comparison of participants for whom we retrieved tumor blocks and those for whom we could not, revealed similarities between age, education and secondhand smoke exposure (Table I). There was a statistically significant difference with regards to residential radon exposure. Although the difference was small, only 18.5 Bq m^{-3}, the biological effect on risk would be minimal. That is, if 100 Bq m^{-3} increases risk only 11% then a difference of 18.5 Bq m^{-3} would correspondingly increase risk only 2.0% and this excess risk is unlikely to substantially bias the interaction ORs. Participants with tumor blocks were more likely to be never smokers, have adenocarcinoma, and have fewer pack-years of smoking. These differences arose because never-smokers were purposely selected to facilitate the examination of SHS exposure and *GSTM1* genotype on lung cancer risk.

Descriptive characteristics of the study cases are shown in Table II. There were no statistical differences between *GSTM1* null and *GSTM1* present lung cancer cases, with the exception of radon concentration. In this instance, the *GSTM1* null cases' mean exposure was 70.3 Bq m^{-3}, while *GSTM1* present cases' mean exposure was 55.5 Bq m^{-3}. There was little difference between the subjects included in this study and those included in the previous analyses conducted by Bennett *et al.*[16]

Radon exposure greater than 121 Bq m^{-3} was associated with greater than a 3-fold increase in the interaction OR (OR= 3.41; 95% CI = 1.10–10.61) for those with a *GSTM1* null genotype (Table III). The test of linear trend was significant (p trend = 0.03). The overall association between radon and *GSTM1* null genotype was similar when we only considered the air-based radon measurements (p for trend = 0.03), although the trend was not monotonic. However, because the air-based measurements were lower on average than the glassed-based measurements, cases in the upper exposure categories tended to shift downward and the resulting interaction OR in the highest exposure category (OR = 2.89; 95% CI = 1.13–7.40) was slightly attenuated compared to the glass-based radon measurements (data not shown).

To further control for potential confounding by smoking, we stratified by active smoking status. The exposure-interaction patterns generally increased with increasing radon exposure and were similar between never, former and current smokers, although the estimates were unstable within strata because of small numbers (data not shown). In addition, the cases were stratified by histology (adenocarcinoma and other) to determine whether radon exposure was associated with specific histologic subtypes. An interaction between radon and *GSTM1* null genotype was most evident with adenocarcinoma, although the interaction estimates were unstable because of small sample size. The interaction estimates for the other histologies combined were difficult to interpret because of small numbers (data not shown). When stratified by study, results were similar between the 2 Missouri studies. Iowa, on the other hand, only contributed 14 cases and the sample size was too small to evaluate the relationship between radon, *GSTM1* and lung cancer in that study separately.

TABLE III – CASE-ONLY ANALYSIS OF RADON EXPOSURE: ASSOCIATION WITH GLUTATHIONE-*S*-TRANSFERASE (*GSTM1*) GENOTYPE, AMONG ALL SUBJECTS

Radon		*GSTM1* genotype			
Category	Bq m^{-3}	Absent	Present	OR[1]	OR[2]
1	<0–33	31	34	1.0	1.0
2	34–61	31	39	0.87 (0.44–1.72)[3]	0.87 (0.44–1.74)
3	62–91	30	27	1.22 (0.60–2.49)	1.15 (0.55–2.42)
4	92–121	14	7	2.19 (0.78–6.14)	1.90 (0.67–5.45)
5	>121	16	5	3.51 (1.15–10.71)	3.41 (1.10–10.61)
p for trend				0.02	0.03

[1]Crude odds ratio.–[2]Adjusted for age, SHS exposure (ever/never) and pack-years of smoking.–[3]Values in parentheses are 95% CI.

TABLE IV – CASE-ONLY ANALYSIS OF SECONDHAND SMOKE (SHS) EXPOSURE BY PACK-YEARS OF SMOKING BY SPOUSE: ASSOCIATION WITH GLUTATHIONE-*S*-TRANSFERASE (*GSTM1*) GENOTYPE, AMONG NEVER SMOKERS

SHS exposure		*GSTM1* genotype			
Quartile	Pack-years	Absent	Present	OR[1]	OR[2]
1	0	33	44	1.0	1.0
2	>0–21	18	8	3.00 (1.16–7.74)[3]	2.78 (1.04–7.40)
3	22–49	10	11	1.21 (0.46–3.19)	1.30 (0.48–3.57)
4	>49	17	5	4.53 (1.52–13.54)	3.95 (1.16–13.46)
p for trend				0.01	0.02
Any SHS		53	27	2.61 (1.37–5.00)	2.28 (1.15–4.51)

[1]Crude odds ratio.–[2]Adjusted for age and radon exposure.–[3]Values in parentheses are 95% CI.

The interaction between ever having been exposed to SHS and having a *GSTM1* null genotype was 2.28 (95% CI = 1.15–4.51) (Table IV). Further, there was a statistically significant test of linear trend with SHS exposure (p trend = 0.02), although the trend was not monotonic across the categories of SHS exposure. Results were similar when the analysis was restricted to the newly accrued never smoking cases (n = 40) and found similar results (data not shown). The interaction OR for any SHS exposure and *GSTM1* null genotype was 2.2 (95% CI 0.6–8.0) compared to nonexposed. However, a monotonic exposure interaction gradient was not evident (p for trend = 0.43).

We attempted to examine the joint effect of SHS and residential radon exposure; however, the small sample size in several strata resulted in unstable estimates (data not shown).

Discussion

The risk of lung cancer from relatively low exposure to residential radon was greater in *GSTM1* null individuals than it was in *GSTM1* carriers. This observation is plausible because recent advances in radiation biology suggest one possible mode of action is that α-particles promote carcinogenesis by producing ROS (in cytoplasm or nucleus), and the increased oxidative stress causes DNA damage.[7,8,28] Therefore, a cell's natural defense against oxidative stress could play an important role in determining the effect of exposure to residential radon. For example, individuals with deficient expression of *GSTM1* may be at greater risk than those carrying intact *GSTM1* alleles.

We considered sources of bias, and we found that the interaction between radon and *GSTM1* was consistent across strata of smoking status; this suggested that smoking did not appreciably confound or modify the interaction between radon and *GSTM1* on lung cancer risk. In addition, an exposure-interaction gradient was evident in those cases obtained from the 2 Missouri studies separately. There were too few cases from the Iowa study to examine the exposure-interaction separately. Further, there was no indication of confounding by study because when we included study in the model there was little effect on the interaction estimates.

It has been clear for some time that high concentrations of radon cause lung cancer in underground miners. Conversely, the results from individual population-based studies investigating residential radon exposure have not been definitive because of weak point estimates and relatively wide CI. This could be a result of the difficulty in measuring long-term residential radon exposure and low statistical power to detect relatively small ORs. To address some of the difficulties in studying low-dose residential radon exposure and lung cancer risk, a pooled analysis of North American radon studies[29] combined 3,662 cases and 4,966 controls, and found evidence that for every 100 Bq m^{-3} (2.7 pCi/L) increase in radon concentration, the excess risk of lung cancer increased 11% (95% CI = 0.00–0.28). Similarly, a pooled analysis of 13 European studies[30] found 8.4% increase in the risk of lung cancer for every 100 Bq m^{-3} in radon concentration.

We also observed a gene-environment interaction between SHS exposure and *GSTM1* null genotype. These additional data support our earlier observation[16] from a smaller series of never-smokers, although the original series of 106 cases accounts for two-thirds of the current group of 157. However, our finding was replicated in two case-control studies from Japan[31] and Detroit.[32] The Japanese study found a 2-fold joint effect for nonsmoking, *GSTM1* null women who were married to heavy smokers.[31] The Detroit series of 94 female and 70 male never-smokers also found a 2-fold joint effect for *GSTM1* null individuals who were exposed to at least 20 years of SHS.[32] Significant, but less direct, confirmation came from 2 studies of indoor air pollution from stoves burning coal or wood.[33,34] Two negative European studies[33,35] might be explained by their small numbers of never-smokers (*i.e.*, low statistical power).

We continued to find evidence of an interaction between SHS exposure and *GSTM1* genotype on the risk of lung cancer. The association was also evident among the newly acquired cases, although the number of these cases was too small for a separate analysis on these cases alone. Our finding of a 2.28-fold interaction for never *vs.* ever-exposure to SHS and *GSTM1* null genotype is much higher than risk estimates for active smoking. For example, a recent meta-analysis[14] surveyed 43 case-control studies with more than 18,000 subjects and found a 1.17-fold risk for active smokers with the *GSTM1* null genotype. This comparison of risks from active and passive smoking raises a question: how can a single genetic trait magnify a low-dose exposure, but seemingly neutralize a heavy one? A similar paradox of diminishing risk with

increasing exposure has been observed in the dose-response between cancer and cigarette smoking: risk tends to plateau above a dose of 20–25 cigarettes per day. Several explanations have been proposed: (*i*) recall bias: heavy smokers may under-report their smoking if they get cancer, (*ii*) behavior modification: shallow inhalation by heavy smokers and (*iii*) genetic heterogeneity: genetic variants in certain genes may render individuals more susceptible to low-dose effects from environmental exposures thereby increasing the likelihood of developing lung cancer. This last theory has been tested by applying traditional biochemical principles to published studies of lung and bladder cancer. By modeling the kinetics of metabolic enzymes, such as glutathione and acetyltransferases, Vineis *et al.*[36] found that subtle differences in substrate affinity could produce a counter-intuitive dose-response at low levels of exposure. They concluded that the differential risks conferred by genetic variants will be most apparent at low intensity exposures, such as SHS. Although cancer susceptibility is more complicated than enzyme kinetics, our finding of a genetic subpopulation with unexpectedly high risks from passive smoking is a plausible manifestation of a low dose effect.

The interaction OR generated from the analysis of a case-only study measures the degree to which the joint effect of the environmental exposure and gene depart from the expected product of the gene OR and exposure OR. In this study, we found evidence of gene-environment interactions between *GSTM1* and residential radon as well as SHS. However, a limitation of the case-only design was that we were unable to determine the relative joint effect of these factors on lung cancer risk because the case-only design cannot estimate these measures of association.

Several limitations may have biased our results. Tumor blocks were available for 15% (270/1,755) of the total case series from all 3 studies. The relatively small proportion of the total cases may have introduced a selection bias, although this seems unlikely given that the selected characteristics (*i.e.*, age and education) of the cases with tumor blocks were not very different from the total case group. Residential radon concentration, however, was slightly lower among those with tumor blocks than those without tumor blocks. Although statistically significant, the observed difference is small and the effect on the risk estimates would be minimal. For instance, Krewski *et al.*[29] estimate that risk increases 11% for every 100 Bq m^{-3} increase in radon concentration. Given that the difference between cases with tumor blocks and those without was only 18.5 Bq m^{-3}, we estimate that risk would be only 2.0% higher among those without tumor blocks and unlikely to bias the interaction ORs. Another potential limitation is the potential for exposure misclassification of long-term residential radon despite the rigorous protocol used in the 3 studies to estimate exposure. However, technical advances in dosimetry have increased risk estimates by giving better measures of historic exposures and reducing misclassification errors. The Missouri[17,20] and Iowa[18] studies collected the standard measurements, as well as more advanced retrospective dosimetry. This provided direct comparisons of the old and new methods, and both the modern detectors[17,20] and the new procedures[18] found greater risks.

Similarly, misclassification of *GSTM1* status could also influence our risk estimates. However, the validity of genotyping assays from FFPE samples has been addressed by our research team[25] and by other groups.[37–39] In general, there is good evidence of >95% concordance between FFPE and frozen tissues in genotyping assays such as *GSTM1*. In our validation study,[25] the concordance rate was 96%; one individual was misclassified as *GSTM1* null in FFPE and as *GSTM1* carrier using DNA from fresh frozen tissues. Overall, the rates of misclassification of FFPE samples were low. In summary, these validation studies show low error rates for genotyping FFPE samples. Small errors in *GSTM1* should not result in meaningful bias since the polymorphism is common in our study population. Moreover, significant genotype misclassification seems unlikely because our genotype frequencies are consistent with those reported in the literature.

In addition, we conducted a sensitivity analysis by examining the effect that each assay protocol had on the interactions between exposure (radon and SHS) and *GSTM1* null lung cancer separately. Further, the product terms for exposure and protocol were added to the models to formally test for heterogeneity of the interaction between the 2 protocols. The interaction ORs for each genotype protocol were similar for both radon and SHS exposure and the test for heterogeneity between the 2 protocols were not significant (p heterogeneity = 0.84 and 0.88 for radon and SHS, respectively), suggesting that aggregating the *GSTM1* genotypes from the 2 protocols did not appreciably bias the interaction OR estimates.

In addition to exposure misclassification and selection bias, the case-only study design assumes that exposure and the genotype, in this context radon and *GSTM1* null, must be independent in the control series. We have no *a priori* reason to suspect that residential radon concentrations would be associated with *GSTM1* genotype. However, biospecimens were not collected from the control series in these 3 studies and we are unable to verify that exposure and gene are independent in the controls. Another limitation of the case-only design is that the main effects of exposure and genotype cannot be estimated; although the main effects of secondhand smoke, residential radon exposure and *GSTM1* null genotype on lung cancer have been extensively investigated.[5,14,29]

In summary, exposure to residential radon interacted with *GSTM1* null genotype to increase the risk of lung cancer. In addition, we continued to find an association between SHS and *GSTM1* null genotype in never smokers. It has been hypothesized that radon and tobacco smoke operate through oxidative DNA damage to potentiate lung cancer.[4] Our study supports this hypothesis that SHS and radon may be operating through similar pathways that include *GSTM1*.

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