p53 Mutations in Lung Cancer Associated with Residential Radon Exposure[1]

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Abstract

Unusual mutation patterns in lung tumors among underground miners have been indicated, suggesting radon-specific alterations in the genome, but the data are not consistent. To investigate the association between residential radon exposure and *p53* mutations in lung tumors, we performed a study on cases from a nation-wide population-based investigation in Sweden. Our study included 83 nonsmoking lung cancer cases and 250 smoking lung cancer cases, diagnosed 1980–1984, with a time-weighted average radon exposure over 140 Bq/m^3 or up to 50 Bq/m^3. Radon was measured in dwellings occupied by the study subjects at some time since 1947. Information on smoking habits and other risk factors was obtained from questionnaires. After exclusions because of the initiation of treatment or insufficient material, the p53-status of 243 tumors was determined using PCR-single-stranded conformation polymorphism analysis and sequencing determination of exons 5–8. The overall mutation prevalence was 23.9%. An increased mutation prevalence was suggested among those with high exposure to residential radon [odds ratio (OR), 1.4; 95% CI, 0.7–2.6], especially among nonsmokers (OR, 3.2; 95% CI, 0.7–15.5), but no specific mutational pattern was indicated. Furthermore, the mutation prevalence seemed to be higher among smoking lung cancer cases than among nonsmoking cases (OR, 2.1; 95% CI, 0.9–5.0), and particularly among those smoking less than 10 cigarettes per day. It may be concluded that residential exposure to radon seems to contribute to a higher mutation prevalence of the *p53* gene in lung tumors.

Introduction

Epidemiological studies on underground miners show that the inhalation of radon progeny is associated with an increased risk of lung cancer (1). Animal experiments confirm that lung tumors can be induced by exposure to radon and its daughters (2). Radon in dwellings is the dominating source of exposure to ionizing radiation in most countries (3). A recent meta-analysis including all of the major epidemiological studies on residential radon and lung cancer indicated an association between estimated exposure and excess risk (4). In the largest study (5), the interaction between radon exposure and smoking with regard to lung cancer exceeded additivity and was closer to a multiplicative effect. Furthermore, there seemed to be a stronger association between estimated radon exposure and lung cancer for small cell carcinoma and adenocarcinoma.

The *p53* tumor suppressor gene is commonly mutated in human lung cancer (6). Mutations predominantly occur in exons 5–8, which are highly conserved through evolution and important for the function. The most common mutation in *p53* in lung cancer is G:C→T:A transversions (7), which are associated with smoking (8, 9). Two investigations have indicated unusual mutation patterns in the *p53* gene in lung tumors among underground miners. In one study (10), 7 of 19 patients had mutations in exon 5 and 6, but none was a G→T transversion. In another study (11), 16 of 29 mutated cases had identical G→T transversions in codon 249 (AGG→ATG). Later studies in underground miners have not confirmed these results (12–14). Almost all of the lung tumors under investigation appeared in smokers, which made it difficult to assess in detail the role of radon exposure.

The aim of our study was to assess mutations in the *p53* gene in lung tumors related to residential radon exposure and the joint effects of tobacco with radon. It was based on cases included in the nation-wide Swedish radon epidemiological study (5) and focused particularly on nonsmokers.

Subjects and Methods

Study Subjects. The nation-wide Swedish radon epidemiological case-control study on residential radon and lung cancer included 586 women and 774 men with primary cancer of the bronchus or lung, 35–74 years of age, who were diagnosed 1980–1984. The study was population based and cases were identified from the Swedish Cancer Registry, which includes 98% of all of the cancer cases with a histologically confirmed diagnosis (15). In the residential radon study, a total of 1145 (84.2%) lung cancer cases had a histopathological classification based on biopsy or autopsy. From this group, all of the non-smoking lung cancer cases were selected with a time-weighted average radon exposure exceeding 140 Bq/m^3 ($n = 34$) or up to 50 Bq/m^3 ($n = 49$). Furthermore, the intention was to randomly select up to 50 smoking cases from the two exposure categories (>140 Bq/m^3 and ≤50 Bq/m^3) for each if the major histological types (squamous cell carcinoma, small cell carcinoma, and

adenocarcinoma). Each of the three histological groups with radon exposure over 140 Bq/m^3 contained less than 50 smoking cases, and consequently, all of the subjects in these categories were included ($n = 111$). Additionally, 50 squamous cell carcinoma (58% of the cases in this category) and 50 small cell carcinoma cases (76%) were selected randomly among smoking lung cancer cases with radon exposure up to 50 Bq/m^3 as well as all of the adenocarcinoma cases ($n = 39$) in this exposure category. Patients treated with chemotherapy or radiation before the tissue sample was taken were excluded from the study ($n = 27$), and for 22 and 41 cases the tumor material was missing or insufficient, respectively, leaving 243 of the 333 originally selected cases for detailed analysis.

Exposure Information. All of the study subjects or the next-of-kin were sent a questionnaire asking about smoking habits of the subjects, their spouses, and parents. The subjects' lifetime occupational history and their residential addresses since 1947 were also investigated. Radon measurements were intended for all of the dwellings in which the subject had lived during a period of 2 years or more from 1947 up to 3 years before the end of the follow-up. In each dwelling, one detector was placed in a bedroom and another in the living room over a period of 3 months during the heating season. Radon was measured by solid-state α track detectors processed at the Swedish Radiation Protection Institute. Cumulative radon exposure since 1947 was estimated for each subject by adding the products of the measured radon level and the duration of residence in each dwelling. Time-weighted means radon concentrations were calculated by dividing the cumulative radon exposure by the total residential time in dwellings for which radon measurements were available.

Smoking habits were classified according to the time-weighted mean consumption of tobacco during the subject's lifetime. Conversions were made for pipe tobacco, cigarillos, and cigars. Subjects who stopped smoking 2 or more years before the end of the follow-up period were classified as ex-smokers. The occupation of each subject was grouped in one of four categories based on earlier evidence of occupational risks of lung cancer (16–18). Subjects who lived for 10 years or more in any of the three largest cities in Sweden at some time between 1947 and the end of follow-up were classified as urban dwellers because of reported excess risks of lung cancer in these cities.

Molecular Analysis. Microscopical slides and lung tumor samples were obtained from the pathology departments where the cancer cases were diagnosed. Paraffin blocks containing tumor tissue were selected from the original histological sections. Each block was then cut into (*a*) one section for demonstration of tumor tissue; (*b*) one section for IHC; (*c*) three 10-μm-thick sections for PCR analysis; and (*d*) one final section, again to confirm the presence of tumor. To avoid contamination between samples, the sectioning was performed with disposable knives and by cutting empty paraffin blocks between each new tumor.

The histopathological typing was reviewed and revised by one of us (A. H.). For immunohistochemical screening of abnormal p53 expression, sections of the tumors were mounted on glass slides, deparaffinized, placed in citrate buffer, and treated with microwaves for antigen retrieval. The immunohistochemistry staining was performed using a Tech Mate 500 immunostainer. DO-7 (Dako, code M 7001) was used as primary antibody (negative control Dako code H 0960) and developed using ChemMate Detection kit Peroxidase/DAB (Dako, code K 5001). The degree of staining of the nuclei of the cells was estimated on a scale from 0 to 3, and the distribution of the staining was classified as focal (more than 10 cells in a cluster and up to 20% of the tumor cell population) and general (more than 20% of the tumor tissue), respectively. The outcome was then classified as "reactive" and "nonreactive" regarding the binding of antibodies to the cell nuclei. No antibody binding (representing degree 0) and poor antibody binding (representing degree 1) in combination with focal distribution was considered as nonreactive.

Deparaffinized tumor tissues were digested with proteinase K, and genomic DNA was purified by phenol/chloroform extraction. Intronic primers for exons 5–8 of the *p53* gene were used to amplify genomic tumor DNA. For SSCP[3] analysis (19), PCR products were labeled by including [^{32}P]dATP in a secondary PCR-amplification for two cycles. Radiolabeled PCR products were diluted 20-fold with 0.1 mM EDTA/0.1% SDS, denatured, and loaded on 6–8% polyacrylamide gels containing 5% glycerol and 0.5 × mutation detection enhancement gels (FMC Bio-Products, Rockland, ME). DNA strands were separated for 16–20 h at 6–8 W and autoradiographed. Mutations were detected as shifts in the mobility of the bands of separated single strands in the autoradiogram. PCR products showing altered mobility were eluted from the gels and reamplified for sequence determination. Sequencing was performed using Thermo Sequenase with ^{32}P-radiolabeled dideoxynucleotides from Amersham Life Sciences. All of the analyses were performed in duplicates from at least two independent PCR amplifications.

Confirmative, direct solid-phase DNA sequencing of separately generated PCR products was performed (20). Primers used for DNA amplification were situated in intronic sequences covering exons 5–8 of the *p53* gene and labeled with biotin to facilitate direct solid-phase sequencing of PCR products by the use of paramagnetic beads. The biotinylated amplified fragments were immobilized onto streptavidin-coated solid support, and strand-specific alkali elution produced a clean template for sequencing. Solid-phase sequencing was performed by a robot with fluorescence-labeled primers, and an automated laser fluorescence instrument was used for sequence analysis. Alterations in the *p53* gene were analyzed by comparing sequence data from the tumor tissues with wild-type sequence data.

Parallel analyses were performed using the SSCP combined with traditional DNA sequencing and direct solid-phase sequence analysis of the first 49 samples for quality assessment. For the rest of the tumors, only those samples showing confirmed altered mobility in polyacrylamide and/or mutation detection enhancement gels in the SSCP analysis were further analyzed by the solid-phase methodology.

Statistical Analysis. The data were analyzed with the SAS software for PCs and STATA statistical software (Release 5.0; StataCorp, College Station, TX). Associations between exposure to radon and tobacco consumption and the presence of *p53* mutations were described with maximum-likelihood estimates of relative risks and 95% confidence intervals based on logistic regression analyses. All of the ORs presented are adjusted for age (three categories: <60, 60–69, and >69 years), gender, and smoking (three categories: nonsmoker, current smoker of <10 cigarettes/day, or at least 10 cigarettes per day) and/or residential radon exposure (three categories: <50 Bq/m^3, 140–400 Bq/m^3, and >400 Bq/m^3).

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[3] The abbreviations used are: SSCP, single-stranded conformation polymorphism; OR, odds ratio; CI, confidence interval; IHC, immunohistochemistry analysis.

Results

Detailed characteristics of the lung cancer cases included in the study are given in Table 1. Sixty-one *p53* mutations were found in the 243 lung tumors after SSCP and sequence analyses. Three patients had two *p53* mutations. The overall mutation prevalence was 23.9% (58 of 243). A higher mutation prevalence was indicated for small cell carcinomas and squamous cell carcinomas, OR = 2.5 (95% CI, 1.1–5.9) and 2.6 (95% CI, 1.1–6.4), respectively, compared with adenocarcinomas. Subjects exposed to more than 140 Bq/m^3 tended to have a higher mutation prevalence than those exposed to lower levels of radon (OR, 1.4; 95% CI, 0.7–2.6). A dose-response relation was suggested, with an OR of 2.8 (95% CI, 0.8–9.3) for radon exposure of at least 400 Bq/m^3. The mutation rate appeared higher among smoking cases than among nonsmoking cases (OR, 2.1; 95% CI, 0.9–5.0). Unexpectedly, the mutation prevalence was especially high among those smoking less than 10 cigarettes per day (OR, 3.4; 95% CI, 1.3–8.9 compared with nonsmokers). An association between *p53* mutations and environmental tobacco smoke was suggested among nonsmokers (OR, 3.1; 95% CI, 0.5–18.3), however, this was based on only nine cases with mutations.

The age distribution was the only characteristic of those mentioned in Table 1 that differed significantly between the patients included and not included in the study (*P* = 0.03). Those not included had a larger proportion of subjects between 60 and 69 years and a smaller proportion over 69 years, but the mean age in the two groups did not differ markedly (63.0 and 63.5 years, respectively).

Mutations of *p53* in relation to histological type, radon exposure, and smoking are further elucidated in Table 2. Squamous cell carcinomas showed the highest excessive risk for *p53* mutations in relation to residential radon exposure (OR, 3.6; 95% CI, 1.0–12.8). On the other hand, the strongest association between *p53* mutations and smoking was seen for small cell carcinomas, in which all of the 24 mutations were detected among smoking lung cancer cases.

The increased mutation rate associated with radon exposure was particularly pronounced among nonsmokers (OR, 3.2; 95% CI, 0.7–15.5; Table 3). In smokers, a negative interaction with radon exposure was found for those smoking 10 cigarettes per day or more, whereas the situation was reversed for those smoking less than 10 cigarettes per day.

Results based on analyses of radon and smoking according to IHC and IHC + SSCP, respectively, indicated the same associations as results based on SSCP. An increased mutation prevalence was suggested among those with high exposure to residential radon (OR, 1.7; CI, 0.7–2.6) for IHC and (OR, 1.6; CI, 0.9–2.7) for IHC + SSCP, especially among those exposed to more than 400 Bq/m^3 [OR, 2.2 (CI, 0.7–7.0) for IHC and OR, 7.1 (CI, 1.5–35.5) for IHC+SSCP]. Smokers seemed to have a higher mutation prevalence than nonsmokers [OR, 1.4 (CI, 0.7–2.7) for IHC and OR, 1.6 (CI, 0.9–3.1) for IHC+SSCP].

Missense mutations accounted for 37 (60.7%) of 61 mutations, whereas silent base substitutions (3.3%), deletions (19.7%), insertions (1.6%), splice mutations (8.2%), and mutations leading to a stop codon (6.6%) accounted for the rest (Table 4). A total of 43 (70.5%) mutations were base substitutions, mostly G→T transversions and G→A transitions. All of the G→T transversions were detected in smokers and especially in the low radon exposure category. Three identical splice mutations were detected in intron 8, CGAGgt→CGAGtt, all in smoking cases highly exposed to residential radon. Four mu-

Table 1 *p53* mutations in relation to some characteristics among 243 lung cancer cases diagnosed 1980–1984 in Sweden

	No. of subjects with *p53* mutation[a]	Total no. of subjects
Sex		
Female	20 (19.2)	104
Male	38 (27.3)	139
Age		
<60 years	8 (15.1)	53
60–69 years	39 (30.7)	127
>69 years	11 (17.5)	63
Histology		
Small cell carcinoma	24 (32.9)	73
Squamous cell carcinoma	19 (32.2)	59
Adenocarcinoma	12 (14.0)	86
Other	3 (12.0)	25
Radon exposure		
≤50 Bq/m^3	29 (20.9)	139
>140 to 400 Bq/m^3	23 (25.6)	90
>400 Bq/m^3	6 (42.9)	14
Smoking status		
Never smoked	9 (13.8)	65
Current smoker <10 cigarettes/day	26 (37.7)	69
Current smoker ≥10 cigarettes/day	23 (21.1)	109
Occupation[b]		
Low risk	4 (28.6)	14
Medium risk	36 (24.8)	145
High risk	17 (22.7)	75
Unknown	1 (11.1)	9
Urban dwellers[c]		
Yes	7 (22.6)	31
No	51 (24.1)	212
Total	58 (23.9)	243

[a] Per cent in each category after SSCP analysis and sequence analyses.
[b] The occupation of each subject was classified into one of four categories based on earlier evidence of occupational risks of lung cancer. Subjects working for 2 years or more in a job assigned to either of the two highest-risk categories were considered to have a high risk; those working only in jobs assigned to the category with lowest risk were considered to have a low risk; and the remaining were considered to have a medium risk.
[c] Subjects who lived for 10 years or more in any of the three largest cities in Sweden (Stockholm, Göteborg, or Malmö) at some time between 1947 and the end of follow-up were classified as urban dwellers.

tations were detected in codon 249, but none was of the same type as the hot spot mutation indicated by Taylor *et al.* (11).

Six of the 43 base substitutions were G:C→A:T transitions and occurred at CpG sites.[4] These base substitutions may result from spontaneous deamination of cytosine and 5-methylcytosine residues, which most often occur at CpG dinucleotides. Nine codons (*i.e.,* 158, 175, 179, 193, 245, 249, 273, 278, and 287) contained more than one mutation.

In the IHC, 100 tumors were classified as reactive, of which only 33 showed altered mobility in the SSCP analysis (in two independent analyses; Table 5). Twenty-five mutations detected in the SSCP analysis and direct sequencing were not observed in the immunohistochemistry staining. The concordance between the two methods was estimated to 62% and did not seem to be affected to any major extent by smoking or radon exposure. Assuming that the SSCP analysis in combination with DNA sequencing constituted the standard, the sensitivity and specificity of the immunostaining were estimated to

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[4] CpG site is a shorthand notation for a cytosine followed by a guanine. Cytosine bases within CpG sites are frequently methylated and susceptible to spontaneous deamination, which leads to a base substitution of cytosine to thymidine.

Table 2   *p53* mutations[a] in relation to residential radon exposure, smoking status, and histological type among 243 lung cancer cases diagnosed 1980–1984 in Sweden

Histological type	Low radon exposure		High radon exposure		Total	
	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker
Adenocarcinoma	3/27 (11.1)	3/28 (10.7)	2/11 (18.2)	4/20 (20.0)	5/38 (13.2)	7/48 (14.6)
Small cell carcinoma	0/4	16/40 (40.0)	0/4	8/25 (32.0)	0/8	24/65 (36.9)
Squamous cell carcinoma	0/5	6/23 (26.1)	4/9 (44.4)	9/22 (40.9)	4/14 (28.6)	15/45 (33.3)
Other	0/2	1/10 (10.0)	0/3	2/10 (20.0)	0/5	3/20 (15.0)
Total	3/38 (7.9)	26/101 (25.7)	6/27 (22.2)	23/77 (29.9)	9/65 (13.8)	49/178 (27.5)

[a] After SSCP analysis and sequence analyses. Numbers in parentheses represent percentages.

Table 3   OR of *p53* mutations[a] in lung cancer cases diagnosed 1980–1984 in Sweden, according to time-weighted mean residential radon exposure since 1947 and smoking status

	Nonsmoker	Current smoker <10 cigarettes/day	Current smoker ≥10 cigarettes/day
Low radon exposure ≤50 Bq/m^3	1	4.5[b] (1.0–19.6)	4.2 (1.1–16.3)
High radon exposure >140 Bq/m^3	3.7 (0.8–16.8)	11.2 (2.5–49.3)	2.3 (0.5–9.8)

[a] After SSCP analysis and sequence analyses.
[b] ORs and 95% CI are shown after adjustment for age and gender.

0.57 and 0.64%, respectively, and the positive and negative predicted values were 33 and 83%, respectively. Overall, the results regarding the association between radon exposure, smoking status, and occurrence of *p53* mutations were consistent between the immunohistochemical staining and the SSCP analysis, as indicated above.

Discussion

The overall mutation prevalence, after SSCP analysis and subsequent sequence analyses, is lower than usually reported in the literature, 24% compared with 30–90%, depending on the histological type of lung cancer (6, 21). There could be several reasons why we observed relatively few mutations: (*a*) a comparatively large number of nonsmoking lung cancer cases was included. Tobacco smoking has been associated with *p53* mutations in previous studies (22–25), and this association is also seen in our study; (*b*) almost one-half of the cases in the present study were women. Adenocarcinomas are relatively more common in females compared with males (26) and contain fewer *p53* mutations than other histological types of lung cancer (6), which was also indicated in our study; and (*c*) differences in detecting *p53* mutations between studies may contribute, including selection of tumor samples and analyzing techniques. In the present study, all of the mutations have been confirmed in at least two independent PCR-products. For 194 of the tumor samples, only those showing altered mobility in the SSCP analysis were sequence-analyzed, which may have affected the sensitivity. The relatively lower proportion of *p53* mutations may also depend on technical problems such as DNA quality extracted from paraffin blocks and the lack of detailed microdissection. This should not, however, influence the estimated association between radon exposure and *p53* mutations.

In the present study, mutations in the *p53* gene seemed to be associated with residential radon exposure. In most previous studies on lung cancer, radon exposure, and *p53* mutations, cases with very high radon exposure in mines have been included, to identify possible radon-associated mutation hot spots (12–14, 27). In two studies, the *p53* gene mutation prevalence

in lung cancer cases among uranium miners was reported to be 37 and 56%, respectively (10, 11), and in a third study, it was estimated to be at least 40% (12). Lung cancer cases exposed to approximately 80 Bq/m^3 of domestic radon showed a *p53* mutation prevalence of 28%, and those exposed to more than 300 Bq/m^3 for more than 20 years had a prevalence of 41% (28). Although the association between radon exposure and mutation prevalence in our study did not reach statistical significance, an association between *p53* mutation prevalence and residential radon exposure at levels occurring in Sweden seems plausible.

We found *p53* mutations to be associated with smoking status. A dose-response relation between tobacco consumption and *p53* mutation prevalence was not seen, although the proportion of *p53* mutations has been found to increase with the amount of tobacco smoking (23, 29). In contrast, high tobacco consumption in combination with high radon exposure seemed to contribute to a decreased risk for *p53* mutations compared with nonsmokers and smokers of less than 10 cigarettes per day with high radon exposure, indicating a possible antagonism of heavy tobacco consumption on the occurrence of radon-induced mutations. One explanation may be that heavy smokers have a thicker mucous membrane, preventing penetration of α-particles to target cells (30). It is also possible that lung cancer subjects smoking more than 10 cigarettes per day require a higher tobacco consumption to develop their lung cancer and, therefore, are less susceptible for *p53* mutations, or that they to a larger extent develop their lung cancer via other mechanisms not involving the *p53* gene. Interestingly, an association was suggested between passive smoking and *p53* mutations among nonsmokers.

Compared with the p53 database, we detected a larger fraction of splice mutations (8 compared with 2% in the database) and deletions (20 and 9%, respectively) but fewer missense mutations (61 and 75%, respectively; Ref. 7). In the p53 database, 23% of the mutations among smoking lung cancer cases are G→T transversions, which is similar to our study where 20% of the mutations among smokers were G→T transversions. For nonsmokers, 20% of the mutations reported in the database are G→T transversions compared with our study, in which no such transversions were detected among nine mutations in nonsmokers. Our study included a total of only 61 mutations, and the proportion of different mutations is uncertain. Furthermore, it is difficult to assess the quality of the information in the p53 database, and several sources of bias have been suggested, resulting from the selection of study subjects and analytical procedures (31).

Three identical CGAGgt→CGAGtt splice mutations in intron 8 were detected, all of which were among smokers with high radon exposure (two of the three mutations occurred among those exposed to over 400 Bq/m^3). The three mutations

Table 4 Types of *p53* mutations in relation to smoking status and time-weighted mean residential radon exposure since 1947 among 243 lung cancer cases diagnosed 1980–1984 in Sweden

Type of mutation	Nonsmoker		Smoker		Total	
	≤50 Bq/m³	≥140 Bq/m³	≤50 Bq/m³	≥140 Bq/m³	≤50 Bq/m³	≥140 Bq/m³
Base change	1	4	21	17	22	21
G→T	0	0	7	3	7	3
G→A	1	2	4	3	5	5
C→T	0	1	2	3	2	4
Other	0	1	8	8	8	9
Deletions	1	2	7	2	8	4
Splicing	0	0	1	4	1	4
Insertion	1	0	0	0	1	0
Total	3	6	26	23	29	29

Table 5 *p53* mutations among 243 lung cancer cases diagnosed 1980–1984 in Sweden, according to SSCP analysis and immunohistochemical staining

	Low radon exposure				High radon exposure			
	Nonsmoker		Smoker		Nonsmoker		Smoker	
	Positive in SSCP	Negative in SSCP	Positive in SSCP	Negative in SSCP	Positive in SSCP	Negative in SSCP	Positive in SSCP	Negative in SSCP
Reactive[a]	1	10	13	26	4	8	15	23
Nonreactive	2	25	13	49	2	13	8	31
Concordance	0.68		0.61		0.63		0.60	

[a] The percentage of reactive tumors according to the IHC was 28.9 among nonsmokers and 38.6 among smokers with low radon exposure, and 44.4 among nonsmokers and 49.4 among smokers with high radon exposure.

are notable, inasmuch as only one mutation of this type has been detected in intron 8 among the more than 1000 reported mutations in lung cancer, suggesting a possible radon-specific mutation (7).

There are some methodological issues involved in a case-only study such as ours (32, 33). Bias resulting from the selection of lung cancer cases is unlikely because we used a sample from a consecutive series of incident cases in a defined population base. In certain exposure categories, all of which were nonsmoking lung cancer cases, and a stratified sample of the smoking lung cancer cases were selected from the nationwide Swedish case-control study on residential radon and lung cancer. However, only cases with available and sufficiently large tumor samples were possible to analyze, which could lead to bias if treatment, availability (surgery and autopsy), or tumor size are related to the occurrence of *p53* mutations. Confounding by other possible risk factors for *p53* mutations, such as smoking status, age, and gender, was adjusted for in the analysis. There are substantial uncertainties in the estimation of radon exposure because of errors in radon measurements, duration of measurements, number of rooms measured, the measurement of radon instead of unattached and attached radon progeny, and measurements made recently to estimate exposure decades ago (34). These uncertainties are likely to dilute a possible association between residential radon exposure and *p53* mutations. Next-of-kin constituted the source of questionnaire information for most of the cases, which may raise concerns regarding data quality. However, several Swedish studies (35–37) show that information of high quality about a deceased subject's residential history and smoking habits can be obtained from a next-of-kin with the methods used.

There were some discrepancies in results on *p53* mutations in individual tumors between the IHC staining and the SSCP analysis followed by DNA sequencing, although the overall picture in relation to residential radon and smoking was consistent. The concordance between p53 protein nuclear accumulation and *p53* gene mutation data was estimated to be 63%, which is in the range (53–79%) of what previous studies have reported (10, 38–43). It is probable that the mutations detected by repeated SSCP and sequence analysis are true mutations. Although tissue samples were selected to contain tumor tissue to a considerable extent, relative paucity of tumor cells may contribute to explaining the negative SSCP analysis despite positive results from the IHC. Another possibility may be accumulation of wild-type p53 in tumor cells, at levels sufficient to be detected in the IHC. On the other hand, splice mutations or deletions, which have been reported to cause accumulation of the p53 protein to a lesser extent than missense mutations, may partly explain the negative results in the IHC but positive results in the SSCP and sequence analyses.

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