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Residential radon, genetic polymorphisms in DNA damage and repair-related*

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ABSTRACT

Objectives: To analyze the relationship of GSTT1, GSTM1, XRCC1 (rs25487), ERCC1 (rs11615, rs3212986), ERCC2 (rs13181), XRCC3 (rs861539), OGG1 (rs1052133), and Alpha-1-Antitrypsin mutations (AAT) with the risk of lung cancer in never-smokers, and ascertain if there is an effect modification between these polymorphisms and residential radon exposure.

Material and methods: We designed a multicenter hospital-based case-control study in a radon-prone area. 322 cases and 338 controls, all never-smokers, were included. They were selected using a frequency sampling based on sex and age distribution of the cases. Participants donated 3 ml. of whole blood used to determine genotype for polymorphisms. They placed a radon detector to measure residential radon exposure in their dwelling.

Results: The OR for deleted GSTM1 patients was 3.46 (95% CI = 1.52–7.89) at residential radon exposures above 200 Bq/m^3. The ERCC1 rs3212986 polymorphism was the most associated with the risk of developing lung cancer, both for low and high radon exposures. The ERCC1 rs321986 GT and TT genotypes (at radon concentrations > 200 Bq/m^3) were more significantly associated with higher lung cancer risk (OR = 2.40, 95% CI = 1.29–4.45; OR = 4.45, 95% CI = 1.26–15.7, respectively).

Conclusions: These findings support the hypothesis that certain polymorphisms in genes involved in DNA-repair and carriers of GSTM1 deletion have an increased risk of lung cancer in never-smokers exposed to residential radon.

Abbreviations: WHO, World Health Organization; USEPA, United States Environmental Protection Agency; LET, linear energy transfer; SNP, single nucleotide polymorphism; ETS, environmental tobacco smoke; OR, odds ratio

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Residential radon, genetic polymorphisms in DNA damage and repair-related genes and lung cancer risk in never-smokers

1. Introduction

Lung cancer is the leading cause of cancer mortality in the developed world, with more than 1.4 million deaths annually [1,2]. Tobacco is the most important risk factor for lung cancer; however, between 15–25% of all lung cancer cases are diagnosed in never-smokers [3]. The incidence of lung cancer in never-smokers has been increasing [4], mainly due to the progressive reduction of ever-smokers as a result of tobacco control policies. If we consider lung cancer in never-smokers as a specific cancer type, it would be ranked as the seventh cause of cancer mortality in the world [5]. In fact, it has been proposed that lung cancer in never-smokers should be classified as a different disease from that arising in ever-smokers [6,7].

The World Health Organization (WHO) and the United States Environmental Protection Agency (USEPA) classify residential radon exposure as the main cause of lung cancer in never-smokers [8,9] and WHO reports that 3–15% of all lung cancer worldwide is caused by indoor radon exposure [10]. The molecular and cellular mechanisms of radon-induced carcinogenesis in causing lung cancer is not completely understood, although DNA damage generated by high Linear Energy Transfer (LET) ionizing radiation plays an essential role. Since only a minority of highly radon-exposed individuals develop this cancer, we have hypothesized that genetic susceptibility might play an important role in the occurrence of the disease. However, to date, few studies have been published analyzing the possible role of genetic variation in genes repairing damaged DNA and therefore modifying lung cancer risk [11,12].

Cytosolic glutathione S-transferase family is a large family of isozymes (phase II) involved in detoxification of harmful electrophilic endogenous and exogenous compounds. Polymorphisms in this group of genes could produce an accumulation of carcinogens. There is evidence that the deletions of GSTM1 and GSTT1 genotypes (occurring approximately in 50% and 20% of Caucasians, respectively) are associated with a higher risk of lung cancer [13,14]. Furthermore, it has been observed that for a similar radon concentration, those individuals with some of these genes deleted pose a higher lung cancer risk compared to those with these genes present [12]. Most of these studies have not distinguished between one or two copy numbers of these genes [15].

Polymorphisms in genes involved in DNA repair capacity may also contribute to lung cancer susceptibility because an impaired capacity to repair DNA damage. Thus, individuals with a lower capacity for DNA repair would have an increased risk of lung cancer. The published studies have focused on genes involved in base excision repair (XRCC1, OGG1), nucleotide excision repair (ERCC1, ERCC2) and double-strand break repair (XRCC3) [16–18]. To date, there is no study associating exposure to residential radon with these polymorphisms involved in DNA repair.

The aim of this study, using the resources of a case-control study performed exclusively in never-smokers in a radon-prone area, was to analyze the association of multiple genetic polymorphisms with the risk of lung cancer and ascertain if there is an effect modification between these polymorphisms and residential radon exposure. Polymorphisms in the following genes were examined: GSTT1, GSTM1, XRCC1 (rs25487), ERCC1 (rs11615, rs3212986), ERCC2 (rs13181), XRCC3 (rs861539), OGG1 (rs1052133) and Alpha-1-Antitrypsin mutations (AAT).

2. Material and methods

2.1. Design, subjects and settings

A multicenter hospital-based case-control study was carried out involving nine hospitals; seven of which are located in Galicia (Northwest of Spain), one in Asturias and one in Northern Madrid. The study area has been previously characterized as a radon-prone area [19,20]. The general population is fully covered by the National Health Service and lung cancer diagnosis is exclusively performed at the participating hospitals. Cases and controls were recruited between January 2011 and March 2015. All participants were never smoking individuals, defined according to the following WHO definition for never-smokers: 1) individuals reporting less than 100 cigarettes smoked in lifetime or, 2) had smoked less than a cigarette per day for less than six months. We included patients who were at least 30 years with no upper age limit. Subjects with a previous history of cancer were excluded. All cases had a confirmed biopsy of primary lung cancer. Controls were individuals undergoing minor, non-oncological, ambulatory surgery. In order to increase the comparability between cases and controls, controls were selected using a frequency sampling based on sex and age distribution of the cases. The study protocol was approved by the Galician Committee of Research Ethics (reference 2010/295). All participants signed a written informed consent.

2.2. Data collection and radon measurements

All participants were interviewed by trained researchers using a detailed questionnaire asking about different aspects of their lifestyle including exposure to environmental tobacco smoke. The interviewer gave the participants a radon detector to take home along with detailed instructions to place the detector correctly on their own. They also received a prepaid envelope to send back the detector to the coordinating center once the measurement period finished. Detectors were placed for a minimum of three months, mainly in the main bedroom and away from doors, windows, and electric devices, at a height of 60–180 cm off the floor, and more than 15 cm away from walls. All participants were followed-up, through phone calls, to ensure that the detector was correctly positioned and to resolve any doubts or questions they might have. A final phone call was made to remind them to send back the detector. The devices were read at the Galician Radon Laboratory (Santiago de Compostela, Spain) which has taken part in intercomparison exercises with excellent results [21,22]. Detectors were of the alpha-track type (CR-39 Radosys Inc.,Budapest, Hungary). Radon results were sent to the participants, with specific recommendations according to radon concentration obtained at each dwelling.

2.3. Laboratory methods

Participants donated 3 ml of whole blood which was used to determine genotype for polymorphisms in the following genes: AAT, GSTM1, GSTT1, XRCC1, ERCC1, ERCC2, XRCC3 and OGG1. All blood samples were stored at -84 °C until they were analyzed at the Centro Nacional de Genotipado-CeGen at the University of Santiago de Compostela. This facility is one of the two existing in Spain and it has the most advanced techniques combined with rigorous quality control procedures. Genetic analysis determined the complete deletion of GSTM1 and GSTT1 genes, 1 polymorphism in XRCC1 (Arg399Gln), 1 polymorphism in OGG1 (Ser326Cys), 2 polymorphisms in ERCC1 (Asn118Asn and C8092A), 1 polymorphism in ERCC2 (Lys751Gln), 1 polymorphism in XRCC3 (Thr241Met) and alpha-1-antitrypsin deficiency. Agena Bioscience´s MassARRAY system was the technology used, which allows the analysis of punctual variants of DNA, single nucleotide polymorphisms (SNPs), as well as of small insertions and deletions. After PCR, excess nucleotides are dephosphorylated by shrimp alkaline phosphatase and the iPLEX single base extension reaction is performed. Reactions were carried out in 384-well plates and the extension products (analytes) are transferred to a SpectroCHIP® Array for MALDI-TOF MS.

2.4. Statistical analysis

We performed a bivariate descriptive analysis to determine the distribution of the study variables according to the case or control status. Then, we used a multiple logistic regression where the dependent variable was the case or control status and the independent variables were presenting polymorphisms of the following genes: AAT mutations, GSTT1 deletion, GSTM1 deletion, XRCC1 (rs25487), ERCC1 (rs11615, rs3212986), ERCC2 (rs13181), XRCC3 (rs861539) and OGG1 (rs1052133). Results were adjusted for age (continuous), sex, environmental tobacco smoke (ETS) and residential radon exposure (continuous) in one model, and genotypes of all polymorphisms were included in a second model (full multivariate model). Genotype data were analyzed with the homozygote of the common allele (wild-type) as the reference group.

To assess whether genetic polymorphisms modified the effect of radon exposure on lung cancer, we stratified radon exposure into two categories (≤ 200 Bq/m^3 and > 200 Bq/m^3) and three variant genotypes for each polymorphism where necessary. This radon stratification was chosen because previous research showed lung cancer risk departing from 200 Bq/m3 in never-smokers [23,24]. Age (continuous), sex and ETS were introduced in the model as adjustment variables. The same analysis was then performed including only adenocarcinoma cases. We could not combine the AAT variable with radon exposure because of the low sample size in some categories. Results are expressed as odds ratios (OR) with their 95% confidence intervals and all statistical tests were two-side. The statistical analysis was performed with IBM SPSS v22 (IBM, Armonk, NY, USA).

3. Results

660 never-smokers were included, 322 cases and 338 controls. Residential radon exposure was available for 288 cases (88.5%) and 275 controls (81.4%). Radon exposure was significantly higher among cases compared to controls. 45.3% of cases were exposed to radon concentrations higher than 200 Bq/m^3 vs 30.2% among controls. This difference increased to 20.4% of cases vs 8.36% of controls for those exposed to 400 Bq/m^3 or more. Age and sex distribution between cases and controls was similar. Cases and controls lived a median time of 30 and 36 years in the measured dwellings, respectively. The most incident histological type was adenocarcinoma (78.5%), followed by squamous cell carcinoma (9.3%). A sample description is shown in Table 1.

The distribution of the different genotypes in cases and controls and their association with lung cancer risk is summarized in Table 2. Distribution of wild type (+/+), heterozygous (+/-) and homozygous (-/-) of GSTM1 was 14.1%, 34.0% and 51.9% in cases and 15.4%, 32.4% and 52.2% in controls. Compared to individuals with wild type (+/+) genotype, the OR of lung cancer was 1.37 (95% CI = 0.71–2.65) for the heterozygous genotype (+/-) and 1.22 (95% CI = 0.66–2.27) for the homozygous genotype (-/-). For the GSTT1 polymorphism, the frequency of the GSTT1 homozygous deletion (-/-) was higher in cases (22.8%) than in controls (19.9%) although after adjustment for age, sex, environmental tobacco smoke, indoor radon exposure and the other genotypes, this was not associated with lung cancer risk (OR = 0.77; 95% CI = 0.42–1.42).

Six SNPs in five genes involved in DNA repair were investigated. No significant association with lung cancer risk was observed for any of these variants (Table 2). As regards to AAT mutations, those individuals carrying SS homozygous genotype had a higher risk of developing lung cancer compared to those carriers of the MM genotype (OR = 4.20; 95% CI = 0.78–22.5). However, no association was observed with lung cancer for the other AAT-deficient genotypes.

Table 3 shows the association of different polymorphisms with lung cancer risk for residential radon exposure by exposure level (≤ 200 Bq/m^3, > 200 Bq/m^3). Lung cancer risk increases when radon concentration increases for all polymorphisms analyzed. For the same radon concentrations, carriers of certain variant genotypes present an increased risk of lung cancer compared to those individuals carrying the wild-type genotype. At radon concentrations lower than 200 Bq/m^3, those carriers of ERCC1 rs3212986 GT and TT genotypes, and carriers of heterozygous (+/-) and homozygous (-/-) genotypes of GSTM1 had a higher risk of lung cancer compared to those having wild-type genotypes. This increased risk is also appreciated for certain polymorphisms when radon concentrations exceed 200 Bq/m^3, as observed in the case of XRCC1 rs25487 AA, ERCC1 rs3212986 TT, OGG1 rs1052133 GC and GG genotypes, and for the complete deletion of GSTM1 (-/- genotype). The OR for completely deleted GSTM1 patients was 3.46 (95% CI = 1.52–7.89) whereas OR was 2.89 (95% CI = 1.03–8.11) for wild-type genotype for the highest radon levels. The ERCC1 rs3212986 polymorphism was the most associated with the risk of developing lung cancer, both for low and high radon exposures. The ERCC1 rs321986 GT and TT genotypes (at radon concentrations > 200 Bq/m^3) were more significantly associated with higher lung cancer risk (OR = 2.40, 95% CI = 1.29–4.45; OR = 4.45, 95% CI = 1.26–15.7, respectively). In the homozygous genotype TT, this risk was accentuated when restricted to adenocarcinoma cases (OR = 4.91, 95% CI = 1.34–18.1) (Table 4).

Table 4 shows the same analysis but only for adenocarcinoma cases. The results are quite similar for these patients compared to all lung cancer patients.

Table 1
Sample Description.

Variable	Cases	Controls
Number of patients	322	338
Median age (range)/25-75th percentile	70 (30-94)/ 61-78	70 (39-92)/63-79
Sex		
Female	259 (80.4)	263 (77.8)
Male	63 (19.6)	75 (22.2)
Education		
No formal studies	143 (45.1)	166 (49.1)
Primary School	119 (37.5)	138 (40.8)
High School	29 (9.1)	20 (5.9)
University degree	26 (8.2)	14 (4.1)
Participant´s habitat		
Urban	96 (40.2)	74 (22.6)
Rural	143 (59.8)	254 (77.4)
Residential radon exposure Bq/m^3		
≤100	68 (23.9)	73 (26.5)
101-147	41 (14.4)	61 (22.2)
148-199	47 (16.5)	58 (21.1)
≥200	129 (45.3)	83 (30.2)
Years (median) living in the measured dwelling (25-75th percentiles)	30 (15.0-43.8)	36 (20.0-51.5)
Exposure to ETS at home in the last 20 years		
No	180 (55.9)	184 (54.6)
Yes	142 (44.1)	153 (45.4)
Histological Types		
Adenocarcinoma	252 (78.5)	
Squamous cell carcinoma	30 (9.3)	
Small cell carcinoma	19 (5.9)	
Large cell carcinoma	8 (2.5)	
Other histological types	12 (3.7)	

Data are presented as n (%) unless otherwise stated. ETS: environmental tobacco smoke.

4. Discussion

To our knowledge, this is the first study conducted exclusively on lung cancer never-smokers to assess multiple genetic polymorphisms, including genes involved in metabolism and in DNA repair combined with radon exposure. The study sample size of 660 never-smokers is larger than that of Couraud et al. in BIOCAST research [25], also carried out in never-smokers. We have observed that at the same radon concentrations, carriers of certain susceptibility polymorphisms in genes

Table 2
Risk of Lung Cancer by each Polymorphism in Never-Smokers.

Polymorphism Allele	Cases n (%) n = 322	Controls n (%) n = 338	Crude OR (95% CI)	Adjusted OR[a] (95%CI)	Adjusted OR[b] (95%)
Alpha-1-antytripsin					
MM	147 (70.0)	207 (65.3)	1 (—)	1 (—)	1 (—)
MS	45 (21.4)	91 (28.7)	0.70 (0.46-1.06)	0.66 (0.41-1.05)	0.77 (0.47-1.28)
MZ	8 (3.8)	12 (3.8)	0.94 (0.37-2.35)	0.67 (0.24-1.91)	0.74 (0.25-2.20)
SZ	3 (1.4)	4 (1.3)	1.06 (0.23-4.79)	0.72 (0.14-3.67)	0.21 (0.02-2.07)
SS	7 (3.3)	3 (0.9)	3.29 (0.84-12.9)	4.09 (1.00-16.4)	4.20 (0.78-22.5)
XRCC1 rs25487					
GG	95 (44.8)	132 (41.8)	1 (—)	1 (—)	1 (—)
AG	85 (40.1)	144 (45.6)	0.82 (0.56-1.20)	0.74 (0.49-1.12)	0.70 (0.44-1.10)
AA	32 (15.1)	40 (12.7)	1.11 (0.65-1.90)	0.96 (0.53-1.74)	1.05 (0.55-2.00)
ERCC1 rs11615					
CC	39 (18.4)	44 (13.9)	1 (—)	1 (—)	1 (—)
CT	109 (51.4)	151 (47.6)	0.81 (0.50-1.34)	0.73 (0.42-1.26)	0.81 (0.40-1.62)
TT	64 (30.2)	122 (38.5)	0.59 (0.35-1.00)	0.55 (0.31-0.99)	0.63 (0.27-1.44)
ERCC1 rs3212986					
GG	110 (51.9)	188 (59.3)	1 (—)	1 (—)	1 (—)
GT	86 (40.6)	114 (36.0)	1.29 (0.89-1.86)	1.32 (0.88-1.99)	1.13 (0.65-1.94)
TT	16 (7.5)	15 (4.7)	1.82 (0.87-3.83)	1.94 (0.85-4.42)	1.54 (0.53-4.51)
ERCC2 rs13181					
TT	96 (45.3)	126 (39.6)	1 (—)	1 (—)	1 (—)
GT	93 (43.9)	152 (47.8)	0.80 (0.56-1.16)	0.79 (0.52-1.19)	0.62 (0.39-1.00)
GG	23 (10.8)	40 (12.6)	0.76 (0.42-1.35)	0.75 (0.40-1.43)	0.50 (0.23-1.08)
XRCC3 rs861539					
CC	83 (39.2)	115 (36.3)	1 (—)	1 (—)	1 (—)
CT	109 (51.4)	150 (47.3)	1.01 (0.69-1.47)	0.85 (0.56-1.29)	1.04 (0.66-1.64)
TT	20 (9.4)	52 (16.4)	0.53 (0.30-0.96)	0.46 (0.24-0.87)	0.53 (0.28-1.04)
OGG1 rs1052133					
CC	135 (63.7)	199 (62.8)	1 (—)	1 (—)	1 (—)
GC	68 (32.1)	97 (30.6)	1.03 (0.71-1.51)	1.13 (0.74-1.72)	1.16 (0.73-1.84)
GG	9 (4.2)	21 (6.6)	0.63 (0.28-1.42)	0.57 (0.23-1.39)	0.52 (0.19-1.47)
GSTM1					
+/+	29 (14.1)	46 (15.4)	1 (—)	1 (—)	1 (—)
+/-	70 (34.0)	97 (32.4)	1.15 (0.66-2.00)	1.03 (0.56-1.89)	1.37 (0.71-2.65)
-/-	107 (51.9)	156 (52.2)	1.09 (0.64-1.84)	1.05 (0.59-1.86)	1.22 (0.66-2.27)
GSTT1					
+/+	60 (30.5)	92 (30.5)	1 (—)	1 (—)	1 (—)
+/-	92 (46.7)	150 (49.7)	0.94 (0.62-1.43)	0.73 (0.46-1.16)	0.71 (0.44-1.16)
-/-	45 (22.8)	60 (19.9)	1.15 (0.69-1.91)	0.76 (0.43-1.34)	0.77 (0.42-1.42)

[a] Adjusted for age, sex, environmental tobacco smoke and indoor radon exposure.
[b] Adjusted for age, sex, environmental tobacco smoke, indoor radon exposure and for all polymorphisms.

involved in DNA repair and oxidative damage present an increased risk of lung cancer compared to those individuals carrying the wild-type variant. Furthermore, lung cancer risk increases when radon concentration increases for all the polymorphisms analyzed. A key aspect of our research is the fact of avoiding bias due to tobacco consumption since we have only selected a never-smokers' population.

Most of the published literature studying the glutathione transferase polymorphisms does not discern between one or two copies of GSTM1 and GSTT1 [12,26,27]. However, in the current study three genotypes were identified for both genes; homozygous wild-type (+/+), heterozygous (+/-) and homozygous null (-/-). In a meta-analysis published in 2005 [13] the GSTM1 null variant was observed to be associated with a small increase in lung cancer risk (OR = 1.22; 95% CI = 1.14–1.30). No significant association was found between lung cancer and GSTT1 deletion either overall or in Caucasians, though a positive association was found for Asians (OR = 1.28; 95% CI = 1.10–1.49) [14]. This could be related to a limited power of the included studies, since a lower frequency of the GSTT1 null genotype was observed among Caucasians (10–20%) compared with Asians (50–60%). Our previous research in ever-smokers on the association of GST polymorphisms supports the hypothesis that GSTM1 and GSTT1 null genotypes increase the risk of lung cancer in individuals exposed to residential radon, where we reported an elevated risk (OR = 2.64; 95% CI = 1.18–5.91) for GSTM1 null genotype compared to non-null genotype when residential radon concentrations are above 147 Bq/m^3 [12]. In the present study, the OR for carriers of GSTM1 null (-/-) exposed to residential radon concentrations above 200 Bq/m^3 is 3.46 (95% CI = 1.52–7.89). A very similar result to ours was obtained in a study published by Bonner et al. with an OR of 3.41 (95% CI = 1.10–10.61) for those subjects with a GSTM1 null genotype and exposed to radon levels greater than 121 Bq/m^3 [11].

Some studies have addressed the relationship between genes involved in DNA repair and lung cancer risk although we are not aware of any associating residential radon exposure with these polymorphisms [28–31]. One of the most assessed polymorphisms implied in DNA repair is the Arg399Gln polymorphism of XRCC1, which was associated to lung cancer with an OR of 1.3 (95% CI = 1.0–1.8) [32]. The most relevant aspect was its greater risk for never-smokers than in ever-smokers, since the results show how the risk increases as the number of pack-years decrease, obtaining an OR of 2.4 (95% CI = 1.2–5.0) in never-smokers [32]. Our research, carried out exclusively in never-smokers, supports these results obtaining a very similar OR for individuals carrying minor homozygote of XRCC1 rs25487 (OR = 2.68; 95% CI = 1.07–6.67) when they are exposed to residential radon concentrations higher than 200 Bq/m^3. An identical increased of risk was also seen in a study performed by Kiyohara et al. in 2012 (OR = 2.59, 95% CI = 1.36–4.95), which included both ever and never-smokers [17].

The polymorphism most associated with the risk of lung cancer in never-smokers is ERCC1 rs3212986, in which the mutant genotype is present in 7.5% among cases and in 4.7% among controls. These figures are similar to those obtained in the study by Zhou et al. with 7% among cases and 6% among controls [29]. The mentioned study revealed that the ORs for those carriers of the mutant genotype decreased

Table 3
Residential Radon Exposure and each Polymorphism Effect on lung Cancer Risk in Never-Smokers.

Polymorphisms	Cases, Controls; OR[a] (95% CI)	
	Indoor radon exposure (Bq/m^3)	
	≤200	> 200
XRCC1 rs25487		
GG	50, 77	38, 33
	1 (—)	1.90 (1.04-3.48)
AG	42, 84	37, 40
	0.73 (0.43-1.23)	1.47 (0.82-2.63)
AA	14, 26	15, 9
	0.82 (0.39-1.73)	2.68 (1.07-6.67)
ERCC1 rs11615		
CC	20, 25	16, 11
	1 (—)	1.97 (0.74-5.24)
CT	57, 96	43, 34
	0.73 (0.37-1.45)	1.69 (0.80-3.58)
TT	29, 67	31, 37
	0.56 (0.27-1.18)	1.15 (0.53-2.47)
ERCC1 rs3212986		
GG	51, 112	52, 48
	1(—)	2.61 (1.54-4.42)
GT	48, 67	30, 30
	1.62 (0.98-2.68)	2.40 (1.29-4.45)
TT	7, 8	8, 4
	1.89 (0.64-5.54)	4.45 (1.26-15.7)
ERCC2 rs13181		
TT	46, 71	42, 35
	1 (—)	1.95 (1.08-3.54)
GT	48, 91	39, 39
	0.83 (0.49-1.39)	1.76 (0.97-3.19)
GG	12, 26	9, 8
	0.74 (0.34-1.62)	1.73 (0.61-4.91)
XRCC3 rs861539		
CC	46, 67	34, 28
	1 (—)	1.90 (1.00-3.61)
CT	47, 86	50, 41
	0.79 (0.47-1.32)	1.81 (1.03-3.19)
TT	12, 35	6, 13
	0.48 (0.22-1.02)	0.74 (0.26-2.13)
OGG1 rs1052133		
CC	69, 116	56, 55
	1 (—)	1.81 (1.11-2.95)
GC	34, 58	29, 22
	1.00 (0.59-1.69)	2.46 (1.29-4.69)
GG	3, 14	5, 5
	0.36 (0.10-1.29)	1.97 (0.54-7.17)
GSTM1		
Wild Type (+/+)	12, 27	15, 12
	1 (—)	2.89 (1.03-8.11)
Hemizygous (+/-)	40, 51	25, 34
	1.63 (0.73-3.64)	1.71 (0.72-4.04)
Homozygous (-/-)	52, 101	47, 31
	1.15 (0.54-2.47)	3.46 (1.52-7.89)
GSTT1		
Wild Type (+/+)	38, 57	18, 16
	1 (—)	1.84 (0.82-4.11)
Hemizygous (+/-)	51, 92	36, 40
	0.83 (0.48-1.42)	1.38 (0.74-2.57)
Homozygous (-/-)	16, 30	23, 21
	0.80 (0.38-1.68)	1.76 (0.85-3.66)

[a] Adjusted for age, sex and environmental tobacco smoke.

Table 4
Residential Radon Exposure and each Polymorphism Effect on Adenocarcinoma Risk in Never-Smokers.

Polymorphisms	Cases, Controls; OR[a] (95% CI)	
	Indoor radon exposure (Bq/m^3)	
	≤200	> 200
XRCC1 rs25487		
GG	39, 77	28, 33
	1 (—)	1.80 (0.94-3.46)
AG	33, 84	30, 40
	0.74 (0.42-1.31)	1.54 (0.83-2.87)
AA	11, 26	12, 9
	0.82 (0.36-1.84)	2.83 (1.08-7.42)
ERCC1 rs11615		
CC	14, 25	12, 11
	1 (—)	2.26 (0.78-6.59)
CT	46, 96	33, 34
	0.86 (0.41-1.83)	1.88 (0.83-4.30)
TT	23, 67	25, 37
	0.66 (0.29-1.51)	1.35 (0.58-3.12)
ERCC1 rs3212986		
GG	41, 112	42, 48
	1(—)	2.58 (1.47-4.51)
GT	37, 67	21, 30
	1.54 (0.89-2.65)	2.12 (1.08-4.18)
TT	5, 8	7, 4
	1.62 (0.50-5.33)	4.91 (1.34-18.1)
ERCC2 rs13181		
TT	36, 71	35, 35
	1 (—)	2.09 (1.11-3.94)
GT	38, 91	28, 39
	0.84 (0.48-1.47)	1.63 (0.85-3.11)
GG	9, 26	7, 8
	0.72 (0.30-1.70)	1.75 (0.57-5.37)
XRCC3 rs861539		
CC	39, 67	27, 28
	1 (—)	1.88 (0.95-3.70)
CT	35, 86	40, 41
	0.71 (0.40-1.24)	1.75 (0.96-3.18)
TT	9, 35	3, 13
	0.43 (0.19-1.00)	0.42 (0.11-1.61)
OGG1 rs1052133		
CC	55, 116	44, 55
	1 (—)	1.78 (1.05-3.00)
GC	26, 58	23, 22
	0.96 (0.54-1.70)	2.48 (1.25-4.92)
GG	2, 14	3, 5
	0.29 (0.06-1.35)	1.47 (0.33-6.48)
GSTM1		
Wild Type (+/+)	8, 27	14, 12
	1 (—)	4.12 (1.34-12.6)
Hemizygous (+/-)	33, 51	19, 34
	2.05 (0.82-5.08)	2.00 (0.75-5.33)
Homozygous (-/-)	39, 101	34, 31
	1.31 (0.55-3.16)	3.83 (1.50-9.78)
GSTT1		
Wild Type (+/+)	29, 57	15, 16
	1 (—)	2.05 (0.87-4.84)
Hemizygous (+/-)	39, 92	24, 40
	0.84 (0.47-1.52)	1.23 (0.62-2.46)
Homozygous (-/-)	13, 30	21, 21
	0.88 (0.40-1.96)	2.15 (1.00-4.62)

[a] Adjusted for age, sex and environmental tobacco smoke.

significantly as pack-years increased, with an OR of 2.11 (95% CI = 1.03–4.31) for never-smokers.

We have used residential radon categorized into two groups with a cut-off point of 200 Bq/m^3. Our decision is based on the results obtained in a previous study [24] where we observed that residential radon increases the risk of lung cancer in never-smokers more than two-fold when they are exposed to concentrations above 200 Bq/m^3. In addition, legislation regarding residential radon concentration in other countries, such as Ireland, United Kingdom or Canada, has an action level of 200 Bq/m^3.

The present study has some limitations. The main one is the low sample size for some polymorphisms, which makes it difficult to assess precisely their cancer association. Another limitation related to sample size is that very few men were included in the study to perform a separate analysis in this subgroup. Only 19.6% among cases and 22.2% among controls were men. Finally, it should be noted that we have only made residential radon measurements in the current dwelling where participants are living. There is not information from dwellings where

they previously lived. We believe that this is not an important limitation since the Galician population uses to live for decades in the same dwelling, contrary to the populations of other countries where residential mobility is more frequent.

Our study has also several strengths. The first is the large number of polymorphisms analyzed, including genes involved in metabolism and in DNA repair. As we previously explained, this is, to our knowledge, the first study conducted on never-smokers. Another important advantage is that Galicia is a radon-prone area [19,20] achieving a median radon concentration of 99 Bq/m^3, making it the most radon-affected Spanish region. Galician population tends to reside in the same dwelling for a long period of time, in fact, in the current study the median number of years that cases and controls have been living in the same dwelling are 30 and 36 years, respectively. This point makes it easier to attribute the risk of lung cancer to residential radon. Lastly, the number of unreturned detectors was very small, with a return rate of 85%; this reduces the likelihood of bias in our exposure assessment.

These findings support the hypothesis that certain polymorphisms in genes involved in DNA-repair and carriers of GSTM1 deletion have an increased risk of lung cancer in never-smokers exposed to residential radon. Further studies should be conducted with larger sample size allowing for more detailed exploration of the interaction of exposure with all of the variant forms of these genes. It would be of particular interest to analyze the interaction of these genetic polymorphisms with very high radon concentrations (i.e. $>$ 400 Bq/m^3) on the possibility of lung cancer risk.

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Declaration of Competing Interest

The authors declare no conflict of interest

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