Genetic Susceptibility, Residential Radon, and Lung Cancer in a Radon Prone Area

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Introduction: Radon exposure has been classified as the second cause of lung cancer, after tobacco, and the first in never smokers. *GSTM1* and *GSTT1* genes deletion increase the risk of lung cancer. We aim to know whether the risk of lung cancer because of residential radon is modulated by these genetic polymorphisms.

Methods: Hospital-based, case-control study where cases had confirmed lung cancer. Cases and controls did not have previous neoplasm and were older than 30. Controls attended hospital for noncomplex surgery. We analyzed the results for the whole sample and separately for never/light smokers and moderate/heavy smokers.

Results: Seven-hundred and ninety-two participants were analyzed. GSTM1 and GSTT1 deletion conferred an odds ratio (OR) of 1.38 (95% confidence interval [CI] 0.93–2.04) and 1.13 (95% CI 0.70–1.82), respectively. Individuals with GSTM1 present and residential radon concentrations higher than 148 Bq/m^3 had an OR of 1.48 (95% CI 0.73–3.00), whereas those with GSTM1 deleted had an OR of 2.64 (95% CI 1.18–5.91) when compared with participants with GSTM1 present and radon concentrations below 50 Bq/m3. Similar results were observed for GSTT1 deletion. These results were basically the same for the moderate/heavy smokers' subgroup.

Conclusions: The absence of *GSTM1* and *GSTT1* genes increases the risk of lung cancer because of radon exposure. These genes might modulate the carcinogenic pathway of alpha radiation. Further studies are warranted analyzing this association in never smokers.

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Lung cancer is a major health problem in developed countries. It is the leading cause of cancer death in males and females in the United States, according to recent data. Lung cancer mortality in females doubles that caused by breast cancer.[1] Survival has hardly improved in the last 30 years, with a 13–17% 5-year survival rate.[1,2]

Lung cancer incidence in Spanish males has an intermediate position regarding other European Union countries. Standardized incidence rate (2012 estimations) was 66.3 cases/100,000 persons-year in Europe and 76.8 in Spain. Lung cancer incidence in females was one of the lowest (26.1 cases/100,000 persons-year in Europe versus 15.7 in Spain). Lung cancer is the second cause of mortality and the first cause of cancer death in Spanish males.[3]

Radon is the second cause of lung cancer after active smoking and the leading cause in never smokers.[4] The United States Environmental Protection Agency (USEPA)[5] and the World Health Organization[4] have published recommendations to aware citizens on radon exposure hazards.

Radon emits radiation in form of α-particles that damage lung epithelia by generating oxygen-anions and hydrogen that produce mutations and other DNA lesions.[6] Neighboring nonirradiated cells may also be damaged through a "bystander effect," whereby cellular signaling from an irradiated cell may induce oxidative stress in adjacent but nonirradiated cells.[7]

Genetic polymorphisms on genes participating in detoxification processes of environmental carcinogens can modulate lung cancer risk. Animal models suggest that several gene polymorphisms may cooperate in increasing the individual risk of lung cancer.[8]

Polycyclic aromatic hydrocarbons and aromatic amines are classes of compounds that cause cancer in humans. Microsomal epoxide hydrolase 1 (EPHX1) plays an important role in both the activation and detoxification of polycyclic aromatic hydrocarbons and aromatic amines. A meta-analysis has indicated that in white population, the high activity variant genotype of EPHX1 polymorphisms at exon 4 was associated with a modest increase in the risk of lung cancer.[9] Cytosolic glutathione S-transferase family are a large family

of isozymes involved in detoxification of many electrophilic substrates. The *mu* (GSTM1) and *theta* (GSTT1) members are susceptibility genes because of their ability to regulate the conjugation of carcinogenic compounds to excretable hydrophilic metabolites.[10] *GSTM1* and *GSTT1* genes are deleted among 50% and 20% of Caucasians, respectively,[11] and this results in the lack of an active enzyme.[10] Meta-analyses have indicated that the carriers of GSTM1 null or GSTT1 null genotypes have a higher risk of developing lung cancer compared with carriers of at least one functional allele.[12,13]

Despite the literature analyzing the effect of many genetic polymorphisms on the risk of lung cancer and their interaction with tobacco consumption, there are very few studies assessing residential radon exposure in combination with different variants of susceptibility genes. We have found only one case-only study[14] providing evidence of a GSTM1-radon interaction on the risk of lung cancer. There is also no information regarding whether the possible effect of radon exposure modulated by these genes could be different for never/light smokers or moderate/heavy smokers.

The aim of the present study is to assess, to our knowledge for the first time through a case-control study, whether there is any effect modification between polymorphisms in *GSTM1*, *GSTT1,* and *EPHX1* genes and residential radon exposure on the risk of lung cancer. A secondary objective is to assess whether this effect is different for never/light smokers or moderate/heavy smokers.

MATERIALS AND METHODS

Design, Subjects, and Settings

A hospital-based, case-control study was conducted in Northwest Spain (Galicia) between 2004 and 2008. Galicia is characterized for having high indoor radon concentrations because of the granitic nature of the earth crust.[15,16] Approximately, 19–21% of all dwellings are above the USEPA action level. This fact places Galician population on a natural experiment where research on radon-related health effects can take advantage. A further advantage is that previous studies have demonstrated that Galician population has low mobility. The median of years living in the same residence is 30,[17] and therefore radon effects can be attributed easier than in other populations.

We recruited cases and controls at two Galician hospitals with full capacity to diagnose and treat lung cancer. The participating hospitals were the Santiago de Compostela Clinic University Hospital and the Ourense Hospital Complex. Both had to be older than 30 and should have lived at least 5 years in their current residence. Individuals with previous cancer were excluded. Cases had an anatomopathologically confirmed lung cancer and were recruited through consecutive sampling. Cases were identified through checking at least twice per week the databases of the Pathologic Anatomy Department. They were interviewed immediately after diagnosis. Controls were recruited from individuals attending hospital for noncomplex surgery unrelated with tobacco consumption. We selected controls with this characteristic to avoid selection bias in order that controls can represent adequately tobacco consumption of the general population and not over represent it. Two days per week one researcher recruited controls from the presurgery unit of both participating hospitals that fulfilled the inclusion criteria. More than 90% of the controls underwent the following surgeries: orthopedic surgery, cataract surgery, or surgery for inguinal hernias. We did a frequency-based sampling of controls regarding cases on age and gender to guarantee that both were comparable for these two variables. The study protocol was approved by the Galician Clinical Research Ethics Committee (REF 2004/108) and informed written consent was obtained from all participants. The results of residential radon exposure on lung cancer have been published recently.[17]

Information Retrieval

All participants were interviewed by trained researchers using a detailed questionnaire with special attention on lifestyle habits. We collected detailed information regarding tobacco consumption. The questions on tobacco consumption were directed to the etiologic period, that is, 30 to 5 years previously to the time of the interview. For the purpose of this study, tobacco exposure was classified in four categories, never smokers, and smokers in tertiles according to their lifetime tobacco consumption: light smokers (1–33 packs/yr), moderate smokers (34–66 packs/yr) and heavy smokers (>66 packs/yr). We used these categories for adjusting the results by tobacco consumption.

Radon Measurements

A radon technician placed a detector in the participants' homes. The devices were of the alpha-track type (Radosys) and were away from doors, windows, or electrical devices and between 60 and 180 cm off the floor. The detectors were placed for a minimum of 6 months and radon concentrations were determined at the Galician Radon Laboratory at the Santiago de Compostela Clinic University Hospital. Seasonal adjustment was taken into account when the detectors were revealed. Quality controls are conducted periodically and our laboratory has been certified through intercomparison tests organized by the University of Cantabria with excellent results.[18]

Laboratory Methods

Total blood was collected in 5 ml EDTA tubes and was transferred to FTA classic cards (Whatman, Maidstone, United Kingdom). FTA cards were left to dry for a minimum of two hours and then stored in foil envelopes with a desiccant at room temperature. Before DNA extraction, a disc with a diameter of 2 mm was cut out from each of the FTA cards using a Harris Micro Punch tool and was placed into a clean Eppendorf. Discs were washed twice with FTA Purification Reagent and vortexed. Then, samples were washed twice in TRIS-EDTA (10 mM Tris, 0.1 mM EDTA, pH8) and were left to air dry for one hour.

Polymorphic deletion of the GSTM1 was determined by polymerase chain reaction (PCR) using β-interferon as an internal control. PCR conditions, including primer sequences were carried out following the protocol described previously by Khedhaier et al.[19] PCR products were amplified on the disc.