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Genetic modifiers of radon induced lung cancer risk - A genome-wide interaction study in former uranium miners

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Compliance with ethical standards

Supplementary materials and methods, supplementary figures I to III, supplementary tables I to IV and further discussion of the gene-radon interaction at 10p13 and 12p12.1can be found at Online Resource 1.

Ethical approval: The sampling of blood from the Wismut miners was approved by the Bavarian Medical Association (Bayerische Landesärztekammer) #08082 and the German Federal Commissioner for data protection and freedom of information. This research was approval from the Dartmouth Committee for Protection of Human Subjects on 7/30/2014 with id STUDY00023602.

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Abstract

Purpose—Radon is a risk factor for lung cancer and uranium miners are more exposed than the general population. A genome-wide interaction analysis was carried out to identify genomic loci, genes or gene sets that modify the susceptibility to lung cancer given occupational exposure to the radioactive gas radon.

Methods—Samples from 28 studies provided by the International Lung Cancer Consortium were pooled with samples of former uranium miners collected by the German Federal Office of Radiation Protection. In total 15,077 cases and 13,522 controls, all of European ancestries, comprising 463 uranium miners were compared. The DNA of all participants was genotyped with the OncoArray. We fitted single-marker and in multi-marker models and performed an exploratory gene-set analysis to detect cumulative enrichment of significance in sets of genes.

Results—We discovered a genome-wide significant interaction of the marker rs12440014 within the gene CHRNB4 (OR=0.26, 95%.CI: 0.11–0.60, p=0.0386 corrected for multiple testing). At least suggestive significant interaction of linkage disequilibrium blocks was observed at the chromosomal regions 18q21.23 ($p=1.2\times10^{-6}$), 5q23.2 ($p=2.5\times10^{-6}$), 1q21.3 ($p=3.2\times10^{-6}$), 10p13

($p=1.3\times10^{-5}$) and 12p12.1 ($p=7.1\times10^{-5}$). Genes belonging to the Gene Ontology term „DNA dealkylation involved in DNA repair" (GO:0006307; $p=0.0139$) or the gene-family HGNC:476 „microRNAs" ($p=0.0159$) were enriched with LD-block-wise significance.

Conclusion—The well-established association of the genomic region 15q25 to lung cancer might be influenced by exposure to radon among uranium miners. Further, lung cancer susceptibility is related to the functional capability of DNA damage signalling via ubiquitination processes and repair of radiation-induced double-strand breaks by the single-strand annealing mechanism.

Keywords

GWAS; radon progeny; occupational exposure; gene-environment interaction; DNA repair

Introduction

You cannot see it; you cannot hear it and you cannot smell it; but be aware it is omnipresent in indoor and outdoor air and contaminates many underground mines (Sethi et al. 2012). Radon is a radioactive noble gas released by the uranium decay chain. An increased risk for lung cancer (LC), the main cause of cancer-related death worldwide (Jemal et al. 2011; Siegel et al. 2016; Torre et al. 2016), caused by inhalation of radon has been consistently demonstrated in several studies of indoor-exposure in dwellings as well as for uranium miners (Darby et al. 2005; Grosche et al. 2006; National Research Council 1999; Sethi et al. 2012). It was estimated, that ionising radiation due to residential radon causes 3% to 15% of LC cases in the general population (Sethi et al. 2012). That is why radon is the second strongest risk factor for LC and among the top 4 environmental risks to public health in the United States (McColl et al. 2015; Sethi et al. 2012).

Pooled analyses of genome-wide association studies (GWASs) within the International Lung Cancer Consortium (ILCCO) have revealed that genomic variations at e.g. 5p15.33, 6p21–22 and 15q25 and further 42 LC susceptibility loci influence LC risk in European populations (Bosse and Amos 2018). In total 92 genes are postulated to be suspected causal genes for LC. Although the strongest genetic association with an odds ratio (OR) of 7.2 was reported for 15q25 in a familial form of LC, for sporadic LC an OR of only ~1.3 was observed, albeit highly significant ($p = 3.08 \times 10^{-103}$). However, "cumulative effects of loci have shown promising results to improve the discriminatory performance of risk prediction models" (Bosse and Amos 2018) Nevertheless, genes can be associated to several traits and contribute to the functional efficacy of multiple interlocked biological processes. One may assume that for example nicotine dependency or DNA repair play a role in an individual's susceptibility to developing LC (Brennan et al. 2011; Romero-Laorden and Castro 2017). For example, some genetic variants in CHRNA5 on chromosome 15q25.1 increase the risk for smoking-related disorders such as LC and chronic obstructive pulmonary disease (COPD) but are also associated with delayed smoking cessation (Amos et al. 2008; Chen et al. 2015b). Taken together, the harming mechanisms of smoking consist at least in parts of a complex interplay between tobacco exposure, previous diseases and genetics. However, smoking is the most important but an avoidable risk factor.

Exposure to radon is ubiquitous and not self-inflicted, but can be reduced in homes and buildings; the related biological defence mechanisms are complex (McColl et al. 2015). DNA damage, induced by radioactive alpha particles emitted by radon progenies, is considered as pivotal mechanism of carcinogenesis in the lung (Sethi et al. 2012). A heritable component in the capacity to repair DNA-damage was demonstrated (Rosenberger et al. 2012). Ionizing radiation induces oxidation of DNA bases and generates single-strand breaks (SSBs) and double-strand breaks (DSBs) (Rosenberger et al. 2012). An individual's capacity to repair DSBs is recognised as a risk factor or an effect modifier in LC (Ishida et al. 2014; Ridge et al. 2013). DSBs capacity determining genes are widely investigated as susceptibility genes for lung cancer.(Chen et al. 2015a; Kazma et al. 2012) The interaction of radon with some genes belonging to biological mechanisms other than DNA damage response was also investigated with candidate gene approaches in either high dose exposed uranium miners (SIRT1; P53; CDKN2A and MGMT; IL6) or low dose exposed humans in dwellings (GSTM, GSTT and EPHX1; P53) (Leng et al. 2013; Leng et al. 2016; Ruano-Ravina et al. 2014; Vahakangas et al. 1992; Yngveson et al. 1999). Nevertheless, it is still unclear which genomic dispositions make one susceptible to radiation-induced LC.

The uranium miners of the former German Wismut mining company, with about 400 000 employees, form a large population with documented radiation exposure. In 2009 the German Federal Office of Radiation Protection (Bundesamt für Strahlenschutz, BfS) started to build up the German Uranium Miners Bio- and Databank (GUMB) with DNA from blood and/or tissue samples from LC cases and healthy controls of former uranium miners of this company. Exposure estimations and data are captured in the same way as for a large cohort study of the same population and includes an estimate of the cumulative occupational exposure to radon progeny (Kreuzer et al. 2010b; Walsh et al. 2010).

This work was conducted as collaboration between the Transdisciplinary Research of Cancer in Lung and the International Lung Cancer Consortium (TRICL/ILCCO), the German Federal Office of Radiation Protection (BfS) and the University Medical Centre Göttingen. We merged phenotypic and genotypic information from TRICL/ILCCO and from BfS. Genotypes were yielded by the OncoArray, to perform a genome-wide search for radon x gene interaction –without restricting the investigation to any presumed mode of action.

Materials and methods

The participating studies of TRICL/ILCCO are individually described in the supplement of McKay et al. (2017), Table 1 and Supplementary Table I (Online Resource 1). The LC cases of the BfS sample collection were recruited for a study investigating indoor-radon exposure between 1990 und 1997 (Brüske-Hohlfeld et al. 2006). The cancer-free BfS controls are former uranium miners recruited from 2009–2012, who continuously participated in health surveillance program of the German Social Accident Insurance and are long term survivors (Pesch et al. 2015). This control samples stored in German Uranium Miners Biobank (GUMB) of the BfS were drawn from these miners, which were either very high (>750 Working Level Months, WLM) or low (≤50 WLM) radiation exposed in a targeted and no-representative ratio of 2:1 (Pesch et al. 2015). The method how radon exposures was

measured is given elsewhere (Kreuzer et al. 2010b) (see Scaling residential and occupational radon exposure, Online Resource 1).

Study population

The analysed sample consisted of 28 599 study participants with European ancestry and valid information on age at diagnosis/interview, sex and smoking status (15 077 cases : 13 522 controls); 463 thereof are former uranium miners of the Wismut mining company (61 cases : 402 controls), 949 are from the German Lung Cancer Study (471 cases : 478 controls), the remaining are from 25 studies of TRICL/ILCCO (14 545 cases : 12 642 controls) (see Table 1 and Supplementary Table I, Online Resource 1). 49 of 15 077 (0.3%) LC cases and 259 of 13 522 cancer-free controls (1.9%) had been occupationally exposed by a high cumulative dose exposure to radon and its progeny (WLM>50). It is unlikely that a cumulative lifetime exposure solely due to an exposure by indoor or other environmental radon sums up to more than 50 WLM. Thus, we categorised occupational radon exposure into ≤50 ("unexposed") and >50 WLM ("exposed"), a threshold for significant elevated relative LC-risk (Kreuzer et al. 2010a). All TRICL/ILCCO participants were assigned to the exposure categories ≤50 WLM. Misclassification would be conservative. A detailed justification is given in the supplement (see Online Resource 1).

Genotyping and QC

The Infinium OncoArray-500K was used for high-throughput genotyping. Quality control (QC) was performed following the approach previously described for the OncoArray (Amos et al. 2017). To validate the European ancestry of the participants the probability of being Caucasian based on a set of 159 ancestry- and PCA-informative markers was estimated (Huckins et al. 2014; Kosoy et al. 2009; Setsirichok et al. 2012) applying the program ADMIXTURE (Alexander et al. 2009). 407 117 markers entered the analysis, after excluding markers of low quality genotyping or a minor allele frequencies (MAF) <1%. These remaining markers could be clustered into 103 983 blocks (67 161 LD blocks and 36 822 hot spots; for definition see Online Resource 1).

Merging samples

The crude odds ratio (OR) for the occupational radon exposure within participants of the BfS sample collection was OR=2.25. Because naïvely adding the TRICL/ILCCO participants would biased this association to OR=0.17, we down-weighted the cases of TRICL/ILCCO by the factor 1 : 13.6. In this way we avoided this unjustified inversion of the crude association, and still use all available information for analysis. However, we have fixed the marginal risk of a radon exposure at the point estimate from the BfS sample collection (for a more detailed explanation see Online Resource 1).

Statistical analysis

We fitted two models to individual data and also carried out a gene-set analysis (GSA) to search for accumulated significance in pre-defined groups of genes for pathways and gene families of interest. All calculations, data handling and image acquire were performed using PLINK 1.9 (Purcell et al. 2007) and SAS 9.4 of the SAS Institute Inc., Cary. NC. USA.

Single-marker interaction analysis

We first performed single-marker interaction analysis fitting the log-additive model:

$$\ln(\mathrm{Odd}_D) = \ln\left(\frac{p_D}{1-p_D}\right) = \beta_o + \boldsymbol{\beta}_{1i} PC_i + \beta_{PS} PS + \beta_G G + \beta_E E + \beta_{G\times E}(G \cdot E) \quad [1]$$

where D is the disease status (D=1: LC patient; D=0: control); G is minor allele count at marker m; E is the exposure category (0: ≤50 WLM, 1: WLM>50); PS is a propensity score comprising the probability being a case explained by age, sex and smoking. To adjust for genomic population stratification we calculated the principal components (PC) of genotypes. Only the first four PCs were included in the statistical modelling, because the fifth PC was significantly correlated with the disease status. The remaining inflation factor (median of the χ^2-distribution for unadjusted association) was $\lambda \sim 1.1$, which is acceptable close to 1.0 (Yang et al. 2011).

The data at hand are not a representative data set of a well-defined source population. Thus the effect estimate of interaction, expressed as odds ratio $\widetilde{OR} = e^{\beta_{G\times E}}$, is potentially proportionally biased. Therefore, the tilde is added to indicate that a weighted sample was used for estimation (see Merging samples). However, estimating OR is not our main interest, rather than testing the null hypothesis $H_0: \beta_{G\times E} = 0$, which is still valid (Mukherjee et al. 2008; Stenzel et al. 2015).

With $\alpha = 5\%$ as global level of significance, we use $\alpha' = 0.05/103 \quad 983 \sim 0.5 \times 10^{-7}$ as Bonferroni-corrected, genome-wide level of significance. A suggestive level of significance was set to 1×10^{-5}. Significance was determined according the Hybrid 2-step (H2) method of Murcray et al. (2011). All markers were first grouped into four classes: (a) disease-gene (DxG) effect only, (b) environmental-gene (ExG) effect only, (c) both or (d) none. Correction for multiple testing was performed within these groups, however under accounting for a tuning parameter ρ that can take values between 0.5 to $1–10^{-20}$ (for a more detailed explanation see Online Resource 1).

Multi-marker interaction analysis

We also searched for the best fitting model of each LD block, allowing all markers of a block to enter the model (denoted as complete model). We then applied a backward selection with the best model chosen according to Akaike's information criteria (AIC), requiring at least one interaction with a marker (denoted as AIC-best model) (for a more detailed explanation see Online Resource 1).

Gene-set analysis

We applied a Gene-Set Enrichment Analysis (GSEA), based on the p-values obtained from the multi-marker interaction analysis (Subramanian et al. 2005). For GSA we assigned markers to genes according to ENSEMBL (Cunningham et al. 2015), and genes to gene sets according to Gene Ontology (GO) and the Human Genome Nomenclature Committee

(HGNC) (Ashburner et al. 2000; Gray et al. 2015). In addition, the gene set of homeobox (HOX) genes in regulatory networks with respect to LC was defined based on literature (Bhatlekar et al. 2014). In total 119 gene sets were considered for analysis. Due to the subjective and in parts data driven selection of gene sets, the GSA was performed as explorative data analysis. The global level of significance of α=0.05 was used. For a list of all investigated gene sets, along with literature references and further detailed explanations see Online Resource 1.

Results

Of 20 study participants each, 9 are from North America (43%), 9 from Europe (46%) and 2 from Israel or Russia (11%). 63% of the total sample were man, 37% were women. The median age was 63 years. 20% of the participants never smoked during their lifetime; 33% were former smokers and 42% were current smokers at the time the entered the particular study (see Supplementary Table I, Online Resource 1). The proportion of never smokers as well as of current smokers where higher in uranium miners (unexposed: 36%, respectively 51%; exposed: 26%, respectively 55%). However, investigating all available former Wismut employees Kreuzer et al. (2010a) concluded, that "... there was [only] a low correlation between smoking and cumulative radon exposure. Thus, it is unlikely that smoking is a major confounder [for the estimation of radon-related risk of lung cancer]". Radon exposure among the 308 exposed spread from 51 to 1479 WLM (mean 966 WLM). The second smallest observed value within exposed LC cases was 335 WLM, corresponding to about 2850 Bq/m^3, which is a very unlikely level of elevated indoor radon exposure.

Single-marker interaction analysis

For three markers we achieved suggestive significant gene-radon (GxE; gene-environment) interaction when applying a Bonferroni correction for multiple testing. Two of them, **rs6891344** and **rs11747272,** are near each other at chromosome **5q23.2** but belong to different LD blocks. We estimated an interaction effect of$\widetilde{OR}$ = 3.9 (95% CI: 2.2–7.0) and $\widetilde{OR}$ = 3.4 (95% CI: 2.0–5.7). Both can be assigned to the gene **CSNK1G3** (casein kinase 1 gamma 3), which encodes a member of a family of serine/threonine protein kinases that phosphorylate caseins and other acidic proteins. The third marker, **rs10911725,** is located in an **inter-genetic region** of chromosome **1q25.3** (see Table 2 and Supplementary Figure 1, Online Resource 1)

Applying the Hybrid 2-step (H2) method and choosing the parameter ρ=1–1×10^{-16} (the screening weight is almost completely set to the genetic-disease (GxD) marginal effects), we could detect a genome-wide significant interaction for marker **rs12440014.** The H2-corrected p-value of p_{mf}=0.03856 would correspond to a fictive uncorrected p-value of p^* = 0.03856 / 103 983 LD block = 3.7 × 10^{-7}with an estimated odds ratio $\widetilde{OR}$ = 0.26 (95% CI: 0.11–0.60). This marker, and five closely related markers with suggestive significance (rs6495309, rs28534575, rs1316971, rs17487514 and rs6495314), are located on chromosome **15q25.1**, nearby or within the gene **CHRNB4** encoding the cholinergic receptor nicotinic beta 4 subunit. This is a well-known LC region; however the strongest association was observed 69 kb upstream nearby the gene CHRNA5 (OR$_G$=1.29;

p=3.6×10^{-101}) (McKay et al. 2017). The marker **rs12440014** was found associated with LC by McKay, et al. **(**p=1.6×10^{-51}; OR=0.81), but no genetic (G) main effect was seen in our analysis ($\widetilde{OR}_G$=0.99, 95% CI: 0.88–1.12). Changing the tuning parameter ρ diminishes the significance of all these markers (see Supplementary Figure 2, Online Resource 1) (McKay et al. 2017).

Multi marker interaction analysis

The "inflation factor" of the χ^2-test statistics for unadjusted association was λ~1.0 for the complete as well as the AIC-best models, indicating no distracting influence of residual population stratification or model selection.

For one **block** (**no. 91734**) on chromosome **18q21.32** we observed a suggestive significant gene-radon (GxE) interaction (p=2.6×10^{-6}), when all five markers of the block (rs1346830, rs11659206, rs7237496, rs9946324) were included in the model. However, fitting the model results in a strong increase in the estimated association strength of the radon (E) main effect ($\widetilde{OR}$=197 instead of $\widetilde{OR}$~2.25). At the same time the GxE interaction of the marker rs1346830 was estimated with $\widetilde{OR}$=0.09 (95% CI: 0.03–0.22; p=2.0×10^{-7}). Potentially, strong collinearity between the marker and the exposure results in such extreme point estimates. Hence, the estimated ORs are untrustworthy and no marker can be highlighted. This block is also merely surrounded by two uncharacterized gens (LOC107985187, LOC105372156) and two pseudogenes (CTBP2P3 / ENSG00000267153, RP11–325K19.2 / ENSG00000267382).

Allowing for marker selection (AIC-best model) revealed a suggestive gene-radon (GxE) interaction within the **block no. 33137** on chromosome **5q23.2**. This interaction is related to the single marker **rs11747272** discussed above (see Table 3).

When the Hybrid 2-step (H2)-method was applied, genome-wide significance for the block **no. 91734** on chromosome 18q21 was achieved, consistent across a wide range of the tuning parameter ρ. Additional, we observed suggestive gene-radon (GxE) interaction of the **blocks no. 2271** on chromosome **1p21.3** and the blocks **no. 33135** and **no. 33137** on chromosome **5q23.2** (see Figure 1 and Supplementary Figure 3, Online Resource 1).

The block **no. 2271** on chromosome **1p21.3** contains in total 10 markers, 7 of these remained in the AIC-best model; three of these with a local significant GxE interaction, while no marker carried a genetic (G) main effect. The strongest LC risk increasing effect was observed for marker rs2029868, with an estimated OR=22.6 for each minor allele (95% CI: 1.7–109; p=0.0001). The block covers the gene **UBE2U** (ubiquitin conjugating enzyme E2 U), a member of the gene family UBE2.

Setting ρ=0.5 of the Hybrid 2-step (H2)-method, the block **no. 58899** on chromosome **10p13** (p_{mt}=0.1878) and the block **no. 69267** on chromosome **12p12.1** (p_{mt}=0.9875) advanced to suggestive significance (see Figure 1; more details are given in the Online Resource 1).

Gene-set analysis

In total **148 sets of genes** were considered for the analysis; 29 too small or duplicate sets were excluded (see Supplementary Table IV, Online Resource 1); hence 119 gene sets entered the GSA. Among them are 95 sets build according to GO terms, 23 HGNC gene families and one set was built on basis of the literature. These sets contained 6 to 3946 genes (median: 46) and cover 5 to 7237 LD blocks (median: 67).

For two gene sets we observed local significance (see Table 4), further two were borderline significant.

The most significant gene set was **„DNA dealkylation involved in DNA repair"** (GO: 0006307;p_{GS} = 0.0139). It consists of 10 genes and comprises genotyped markers in 90 LD blocks. For 15 of these 90 LD blocks (16%) at least local significant interactions were observed in the multi-marker analysis, in contrast to 6404 out of all remaining 90 768 LD-blocks (7%). This set hosts 7 „driving"-genes assigned to 21 „driving"-LD blocks. The most significant LD-block (no. 84619, p=0.0005 for gene-radon interaction) is located within the gene **FTO** (fat mass and obesity-associated protein) on chromosome 16q12.2, also known as **ALKBH9** (alpha-ketoglutarate dependent dioxygenase).

Within the GO hierarchy of terms, GO:0006307 is a direct subtopic of DNA repair (GO: 0006281) which yielded a p_{GS}=1.0, as well as of DNA dealkylation (GO:0035510), which was not tested. The second best subtopic of DNA repair (GO:0006281) was the double-strand break repair via single-strand annealing (GO:0045002) with a p_{GS} =0.1574, which was rank 8 within all tested gene sets. For comparison, e.g. double-strand break repair (GO: 0006302) attained rank 75 with p_{GS} =0.834.

The other significant set was the gene family **HGNC:476 „microRNAs**" (p_{GS}=0.0159), which consists by definition of 1776 very short, non-coding genes, but markers were genotyped for only 147 of these genes. This set hosts in total 38 „driving" genes assigned to 44 „driving" LD-blocks, spread over all chromosomes.

The gene sets „acyl-CoA metabolic process" (GO:0006637, p_{GS}=0.0538) and "Membrane" (GO:0016020, p_{GS}=0.0558) were borderline significant.

Discussion

Lung cancer has a complex disease mechanism, in particular with respect to the interaction of environmental and genetic factors. Environmental exposure to the radioactive noble gas radon is considered as the second strongest risk factor for LC in the general population; but the occupational exposure of former uranium miners, e.g. of the Wismut mining company, can be tens of times higher. We conducted a genome-wide gene-radon interaction analysis on LC using data of 28599 samples from 27 studies in men and women of European descent. Although heterogeneity in genetic susceptibility across histological subtypes of LC was demonstrated, the informative sample (n=463 miners, comprising 49 exposed LC cases) is too small to stratify the analysis by histological subtypes or by smoking behaviour (McKay et al. 2017). We performed three types of analyses: single-marker and multi-marker

interaction analyses and gene-set enrichment analysis on top of the latter. We determined significance according to the Hybrid 2-step (H2) method of Murcray et al. (2011). In brief, markers or regions with marginal effect in a disease-gene (DxG) or an environmental-gene (ExG) model are given a higher a-priori weight for the final test on gene-environmental (GxE) interaction on then disease (D).

We detected a genome-wide significant gene-radon interaction for marker **rs12440014** (p_{mt}=0.03856) located within the gene **CHRNB4** on chromosome **15q25.1**, a well-known LC susceptibility region (Bosse and Amos 2018; Sakoda et al. 2011). Previously this intronic marker was described as associated with LC ($p=2.8\times10^{-52}$; OR=0.80; 95% CI: 0.78–0.83) in Caucasians (McKay et al. 2017). In our analysis we observed no significant genetic main effect ($\widetilde{OR}$=0.99, 95% CI: 0.88–1.12), but a lower LC risk for carriers of the minor allele among the occupationally radon exposed miners (≥50 WLM), compared to non-carriers or not occupationally exposed individuals, respectively ($\widetilde{OR}$=0.26 per minor allele, 95% CI: 0.11–0.60). The region 15q25.1 hosts three genes (CHRNA5, CHRNA3 and CHRNB4) that encode nicotinic acetylcholine receptor (nAChR) subunits. Due to strong linkage disequilibrium in this region, the observed interaction may possibly only mark interactions of functional variants in neighbouring genes. It is also believed that the association of this region with lung cancer cannot be reduced to a single variant, but is modified by age and smoking (Sakoda et al. 2011). In vitro studies examining the functional role of the genes at 15q24–25.1 in human lung tissue notified an involvement of *CHRNA3* and *CHRNA5* in lung carcinogenesis. An up-regulation of CHRNA5 and a down-regulation of CHRNA3 in lung adenocarcinoma as compared with the normal lung was observed (Falvella et al. 2009). However CHRNA3 is not required to maintain cancer cell proliferation (Liu et al. 2009).

The marker rs16969968 assigned to CHRNA3 was most detailed discussed as associated with smoking quantity and nicotine dependence, suggesting that this variant confers risk of LC through its effect on tobacco addiction. Interestingly, no modification of risk was found across smoking categories or histological subtypes of LC (Bosse and Amos 2018; Sakoda et al. 2011). Also, evidence exists that nAChRs can be directly associated with lung carcinogenesis owing to the complexity of nAChR function in the brain (Papke 2014). This is of interest, since a sub-multiplicative interaction between radon and smoking in causing LC was speculated independently in several uranium miner cohorts and case-control studies (Leuraud et al. 2011; National Research Council 1999; Schubauer-Berigan et al. 2009). The estimated excess relative risk (ERR) per WLM was higher for never than for current smokers (e.g. ERR/WLM=0.012 for never and long-term ex-smokers vs. ERR/WLM=0.007 for short-term ex- and current smokers (Leuraud et al. 2011)). This resulted in a small decrease of the point estimate of the relative risk for current smokers compared to never smokers from RR=6.70 (unadjusted on radon exposure) to RR=6.41 (adjusted on radon exposure). However, the difference was statistically not significant. Accordingly, a protective effect of smoking against radon induced LC was hypothesized and justified by thicker mucus layer and increased mucus velocities. Contrary, Baias et al. (2010) calculated the local radiation dose due to inhaled radon progeny in bronchial target cells to be twice as high in heavy smokers compares to never smokers. However, the apparent “LC protection by

smoking" perhaps results from interaction in opposite direction of genes at chromosome **15q25.1** with smoking- respectively radon-induced LC.

Furthermore, the risk of LC for homozygous carriers of the minor allele of two markers within **15q25.1** (rs8034191, rs1051730) was estimated as at least five-fold higher in subjects who had a familial history of LC (Liu et al. 2008). We have discovered LC-risk stratification within this genomic region with respect to radon. Thus the observed familial risk of the region **15q25.1** may in part be caused by a common environmental radon exposure, albeit at a lower level than the occupational exposure of former uranium miners.

The most significant gene-radon interaction outside suspected LC susceptibility regions (Bosse and Amos 2018) was observed for **UBE2U** (1p21.3), a gene of the family of ubiquitin-conjugating enzymes UBE2, also known as E2 enzymes. The coded enzyme performs a central step in the ubiquitination reaction that targets a protein for degradation, a major factor for life and death of proteins (van Wijk and Timmers 2010). Protein ubiquitination is a pivotal regulatory reaction promoting the cellular responses to DNA damage (Guo et al. 2017; Kazma et al. 2012). **UBE2U** was recently identified as a positive regulator of TP53BP1, which promotes the formation of ionizing radiation-induced foci and thereby chromatin responses at DSBs in human cell lines (Guo et al. 2017). E2 ligases are in general involved in multiple biological processes, for example UBE2T (1q32.1) promotes efficient DNA repair; UBE2B (5q31.1) is involved in UV mutagenesis, and UBE2N (12q22) is implicated in post-replication DNA repair following UV and ionizing radiations. UBE2N was associated with LC by a candidate gene approach ($p=7\times10^{-6}$) (Kazma et al. 2012). The strong involvement of the human E2 ubiquitin- and ubiquitin-like conjugating enzymes in DNA damage signalling and DNA-repair processes confirms mechanistically the plausibility for the observed gene-radon interaction of UBE2U resulting in an increased radiations sensitivity for individuals bearing this genetic make-up.

In a review of DNA repair and cancer risk Romero-Laorden and Castro (2017) recently stated that defects in DNA repair genes are the genetic events most commonly involved in hereditary cancers. Once the DNA is damaged 16 or more repair mechanisms can be engaged, and a substantial cross-talk between these pathways exist (Ciccia and Elledge 2010). Exposure of a cell to a dose of 1 Gy of X-rays can cause more than 1000 base lesions, about 1000 single-strand breaks (SSBs) and 30–40 double-strand DNA breaks (DSBs) (Ward 1988). DSBs, the most harmful lesions, are repaired by an intricate network of multiple DNA repair pathways; inter alia single-strand annealing (SSA), non-homologous end-joining (NHEJ) or homologous recombination (HR) (Ciccia and Elledge 2010). Seven of the 93 genes suspected to affect susceptibility to LC are DNA repair genes: BRCA2, CHEK2, GTF2H4, MSH5, PMS1, RAD52, XRCC4. Only the first (HR and SSA), the second last (HR and SSA) and the last (NHEJ) belong to DSB repair pathways.

Because gene-radon interaction with a long-term occupational exposure to radon was investigated, we expected findings to be related to DNA repair, in particular DSB (Robertson et al. 2013). To our surprise we did not achieve cumulative significance overall on DNA-repair genes (GO:0006281, p=1.0), nor for DSB repair (GO:0006302, p=0.8340) or SSB repair (GO:0000012, p=0.9204). This missing significance may be attributed to the nature of

the applied test in the GSA. The power for broadly defined gene sets of interest is low, because these contain too many not associated genes. For the more specifically defined pathway SSA of DSB repair we achieved a stronger, albeit not significant signal for association (GO:0045002, p=0.1574). Local significance was achieved for genes involved in DNA dealkylation involved in DNA repair (G0:0006307, p=0.0139), a reaction to DNA damage caused by **free radicals and other reactive species** generated by metabolism which results in alkylated bases. Ionizing radiation induces this type of DNA damage by indirect radiation reactions through the induction of ROS. Bases can become oxidized, **alkylated**, or hydrolysed through interactions with these agents (Dexheimer 2013). These lesions are repaired through base excision repair.

To our knowledge, this is the first genome-wide investigation for radon exposure x gene interaction with respect to LC. We have combined samples of disparate sizes from several sources, resulting in an extreme relation of 1 exposed to about 90 unexposed individuals. The most informative subsample consists of only 463 former uranium miners but with carefully determined occupational exposure to radon. To have enough power for the genome-wide analysis, we had to include such a large amount of controls. This should be seen as a necessity rather than a disadvantage, given the small available sample of occupational radon-exposed lung cancer cases. We were further forced to make some assumptions, e.g. no participant of a TRICL/ILCCO study was substantial long-term exposed to radon ($WLM < 50$) However, even long-term but low-dose exposure to radon, occupational (Kreuzer et al. 2015) as well as residential (Darby et al. 2005), was previously associated with a small increase in lung cancer risk. Thus, the small risk of misclassifying few of the many participants of a TRICL/ILCCO study is more likely for cases than for controls. Hence the allocation made is conservative in terms of statistical testing.

We also needed to fix the marginal odds ratio for radon exposure to the value observed within the miners. Subgroup analysis by histological cancer type could not be performed owing to the small number of exposed cases, in particular those with reliable records.

The risk of confounding effect due to smoking on radon-associated risk for lung cancer was previously investigated in a case-control study nested in the cohort of German uranium miners. The estimation of radon-related lung cancer risks was robust against model fitting with and without smoking. Consequently, smoking does not act as a major confounder (Schnelzer et al. 2010). Potential confounding due to other mining related exposures was also examined within the German uranium miners cohort (Kreuzer et al. 2010a; Preston et al. 2003). The correlation between measured radon exposure with external gamma radiation, long-lived radionuclides or arsenic was low; the correlation with fine dust or silica dust was moderate. The influence of adjustment for these potential confounders on the exposure–response relationship was only modest. Hence major confounding by these other occupational risk factors can be excluded (Walsh et al. 2010).

The reported study was restricted to Caucasian populations to minimize population stratification. Although the miners came from a small area in the middle of Germany, no differing genetic background compared to the TRICL/ILCCO samples from Russia to Hawaii was found. The results may not be generalized to other ethnicities because of

different genetic background. It should also be noted that within the small sample of miners, controls are long-term survivors with a disproportionally high sampling of high radon exposed subjects. To discover further susceptibility genes for radon-related lung cancer or to assess the usefulness of determining the susceptibility of a subject, e.g. genetic testing requires further study.

Conclusion

We could demonstrate that the well-established association of the genomic region 15q25 might be influenced in parts by exposure to radon among uranium miners. Further, the susceptibility to lung cancer is related to the functional capability of DNA damage signalling via ubiquitination processes and repair of radiation-induced double strand breaks by the single-strand annealing mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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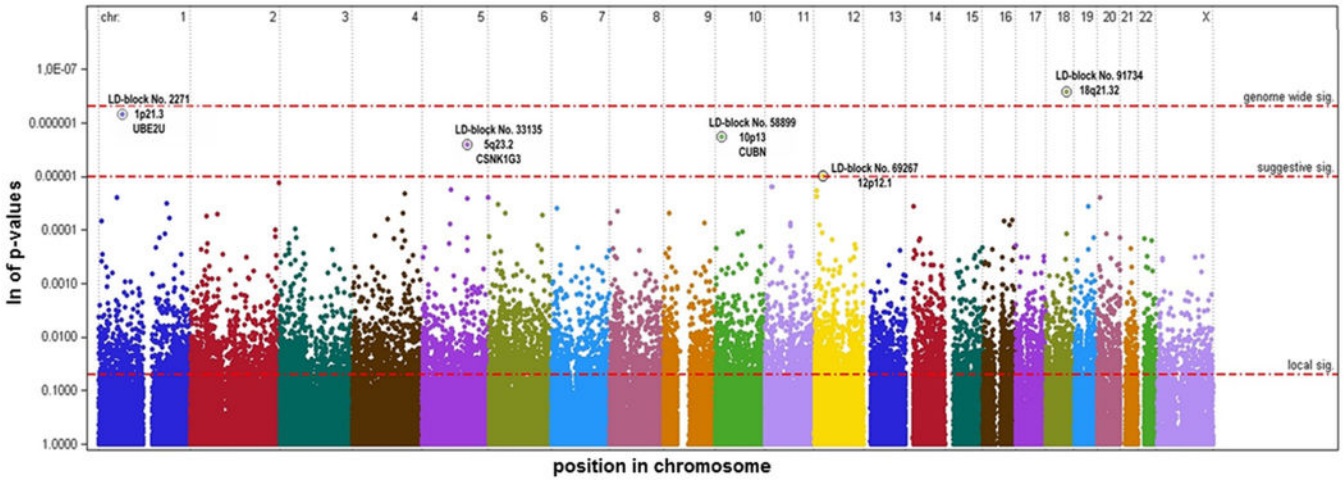

Figure 1: Manhattan plot of p-values of AIC-best models corrected with the Hybrid 2-step (H2) method with ρ=0.5

Each point represents the significance of a gene-radon (GxE) interaction within a LD block., p-value are modified according to Hybrid 2-step (H2) method of Murcray et al. (2011)

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Table 1

Source studies

Acronym	Study name	Institution	PI (principal investigator)	Country	Design	Participants in this analysis	Time span of recruitment
CARET	The Carotene and Retinol Efficacy Trial	Fred Hutchinson Cancer Research Center (FHCRC)	G. Goodman, J. Doherty, C. Chen	USA	Cohort	1065	Recruitment 1985–1996
BioVU	The Vanderbilt Lung Cancer Study	Vanderbilt University	M. Aldrich	USA	Hosp. CC	1 160	2007-ongoing
HLCS	Harvard Lung Cancer Study	Harvard School of Public Health, Mass General Hospital	D. Christiani	USA	Hosp. CC	1 605	1992–2004
ATBC	The Alpha-Tocopherol, Beta-Carotene Cancer Prevention	National Cancer Institute (NCI)	D. Albanes	Finland	Cohort	1 683	1985–1993
PLCO	The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial	National Cancer Institute (NCI)	N. Caporaso	USA	Cohort	2 231	1992–2001
MSH-PMH	Princess Margaret Hospital Early Detection Study	Mount Sinai Hospital (MSH), Princess Margaret Hospital (PMH)	R.J. Hung, G. Liu	Canada	Hosp. CC	2 295	2008–2012
LCRI-DOD	Population based case-control study of lung cancer in Appalachian Kentucky	Markey Cancer Center	S. Arnold	USA	Pop. CC	220	2012- ongoing
TAMPA	Tampa Lung Cancer Study	H. Lee Moffitt Cancer Center	P. Lazarus	USA	Hosp. CC	242	1999–2003
NELCS	New England Lung Cancer Study	Dartmouth College of Medicine	A. Andrew	USA	Pop. CC	329	2005–2007
TLC	Total Lung Cancer: Molecular Epidemiology of Lung Cancer Survival	Moffitt Cancer Center, Tampa	M.B. Schabath	USA	case only	419	2012-- ongoing
MEC	Multi Ethnic Cohort Study	University of Hawaii (USC)	L. Le Marchand, Ch. Haiman	USA	Cohort	430	Recruitment 1993–1996
Canada	Pan-Candadian screening study	University Health Network (UHN), British Columbia Cancer Agency (BCCA)	S. Lam, G. Liu	Canada	screening cohort	656	2004–2011, 2008–2013
EAGLE	Environment and Genetics in Lung Cancer Study Etiology	National Cancer Institute (NCI)	M.T. Landi	Italy	Pop.CC	3 494	2002–2005
Copenhagen	Copenhagen Lung Cancer Study	University of Copenhagen	S. E. Bojesen	Denmark		1 823	
CAPUA	Cancer de Pulmon en Asturias	University of Oviedo	A. Tardon	Spain	Hosp.CC	1 399	2002–2012
GLC	German Lung Cancer Study	University of Göttingen, Deutsches Krebsforschungszentrum Heidelberg (DKFZ)	H. Bickeböller, A. Risch	Germany	Mixed CC	1 014	1998–2013

Acronym	Study name	Institution	PI (principal investigator)	Country	Design	Participants in this analysis	Time span of recruitment
		DKFZ-part					
GLC-500K	German Lung Cancer Study	University of Göttingen, Helmholtz Zentrum München (HMGU), HMGU-part	H. Bickeböller, A. Risch, H.-E. Wichmann	Germany	Mixed CC	949	1998–2013
Nijmegen	The Nijmegen Lung Cancer Study	Radboud University Medical Centre	B. Kiemeney	The Netherlands	Pop.CC	816	2002–2008
ReSoLucent	Resource for the Study of Lung Cancer Epidemiology in North Trent	University of Sheffield,	M.D. Teare	UK	Mixed CC	750	2005–2014
Norway	Norway National Institute of Occupational Health Study	National Institute of Occupational Health (NIOH)	A. Haugen	Norway	Pop.CC	725	1986–2005
LLP-2008, LLP-2013	Roy Castle Lung Study (Liverpool Lung Cancer Project)	University of Liverpool	J.K. Field	UK	Cohort	200 675	1999–2007, 1999–2011
NSHDC	Northern Sweden Health and Disease Cohort	Umeå University	M. Johansson	Sweden	Cohort	473	1985-ongoing
MDCS	The Malmö Diet and Cancer Study	Lund University	H. Brunnsstöm	Sweden	Cohort	325	1991–1996
NICCC-LCA	Israel Lung Cancer Study	Carmel Medical Center & Technion	G. Rennert	Israel	Pop.CC	1149	2008-ongoing
L2	The IARC L2 Study	International Agency for Research on Cancer (IARC)	P. Brennan	Central Europe	Pop./Hosp. CC	2009	2005–2013
Wismut	Case-control study on lung cancer in former Wismut uranium miners (cases)	Helmholtz Zentrum München (HMGU)	H.-E. Wichmann, L. Kreienbrock,	Germany	case sample	58	1990–1995
GUMB	Biobank of healthy former Wismut uranium miners (controls)	Bundesamt für Strahlenschutz (BfS)	M. Gomolka	Germany	sample collection	405	2009-ongoing

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Table 2

Markers with genome-wide significance or suggestive significance for gene-radon (GxE) interaction

Marker	Chr.	Position	Block No.	$\widetilde{OR}$(95%-CI)*			p-value[1]	p_{mt}-value[2]
				G	E	GxE	GxE	
rs10911725	1	185395182	5078	1.02 (0.89–1.16)	6.70 (4.30–10.4)	0.21 (0.11–0.42)	5.3×10^{-6}	0.5515
rs6891344	5	123136656	33135	0.96 (0.84–1.10)	1.57 (0.96–2.55)	3.91 (2.18–6.99)	2.7×10^{-6}	0.2832
rs11747272	5	123179990	33137	0.97 (0.86–1.10)	1.23 (0.69–2.19)	3.35 (1.98–5.68)	4.3×10^{-6}	0.4504
rs6495309	15	78915245	82002	0.99 (0.87–1.13)	4.05 (2.75–5.98)	0.35 (0.16–0.76)	0.0072	0.2387
rs28534575	15	78923845	82002	1.00 (0.89–1.12)	4.15 (2.80–6.14)	0.36 (0.17–0.75)	0.0060	0.1964
rs12440014	**15**	**78926726**	**82003**	**0.99 (0.88–1.12)**	**4.43 (3.00–6.55)**	**0.26 (0.11–0.60)**	**0.0012**	**0.0386**
rs1316971	15	78930510	82005	0.97 (0.84–1.13)	4.09 (2.78–6.02)	0.32 (0.14–0.72)	0.0052	0.1722
rs17487514	15	78953785	82008	1.02 (0.89–1.17)	1.81 (1.06–3.07)	2.01 (1.19–3.39)	0.0071	0.2325
rs6495314	15	78960529	82008	1.02 (0.91–1.13)	1.62 (0.86–3.05)	1.87 (1.12–3.12)	0.0145	0.4779

[1] uncorrected p-value (genome-wide significant if $< 0.5\times10^{-7}$, suggestive significant if $< 1\times10^{-5}$);

[2] p-value corrected for multiple testing (genome-wide significant if < 0.05, suggestive significant if < 1); using the Hybrid 2-step (H2) method of Murcray et al. (2011) with $1\text{-}\rho=1\times10^{-16}$; chr: chromosome; position: position on the chromosome [bp]; G: genotypic, log-additive main effect; E: main effect of radon exposure; GxE: gene-radon interaction; OR: This is not an unbiased estimate owing to sampling and merging of samples, hence useful only to compare the strength of effects

Table 3

Regions with genome-wide or suggestive significant gene-radon (GxE) interaction

LD-block	Chr.	p-value[1]		Hybrid 2-step (H2) method			
		Gene	GxE	Min. p_{mt}-value[2]	ρ of min. p_{mt}-value	Modified min. p-value	Range of ρ with $p_{mt}<1$[3]
2271	**1p21.3**	UBE2U	3.2×10^{-6}	0.0563	0.9999	5.4×10^{-7}	0.5 to $1–10^{-17}$
33135	**5q23.2**	CSNK1G3	2.5×10^{-6}	0.2585	0.5	2.5×10^{-6}	0.5 to $1–10^{-17}$
58899	**10p13**	CUBN	1.3×10^{-5}	0.1878	0.5	1.8×10^{-6}	0.5 to 0.6
69267	**12p12.1**	SOX5	7.1×10^{-5}	0.9875	0.5	9.5×10^{-6}	0.5
91734	**18q21.32**	--	1.2×10^{-6}	0.0214	0.9999	2.1×10^{-7}	0.5 to $1–10^{-17}$

[1] uncorrected p-value for gene-radon (GxE) interaction of the AIC-best model (genome-wide significant if $< 0.5\times10^{-7}$, suggestive significant if $< 1\times10^{-5}$);

[2] p-value corrected for multiple testing (genome-wide significant if < 0.05, suggestive significant if < 1) with tuning parameter ρ; Chr: chromosome; GxE: gene-radon interaction;

[3] corresponding to suggestive significance

Table 4

Significant results of the gene-set enrichment analysis

Gene set ID	Description	Number of genes	Number of markers	Number of „driving"-genes	Number of „driving"-LD blocks	p_{GS}-value
GO:0006307	*DNA dealkylation involved in DNA repair*	10	90	7	21	0.0139
HGNC:476	*microRNAs*	1,776	147	38	44	0.0159
GO:0006637	*acyl-CoA metabolic process*	23	36	11	20	0.0538
GO:0016020	*membrane (cellular-component)*	1,896	5,903	90	178	0.0558