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Germline mutations in young non-smoking women with lung adenocarcinoma

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ABSTRACT

Objectives: Although the primary cause of lung cancer is smoking, a considerable proportion of all lung cancers occur in never smokers. Gender influences the risk and characteristics of lung cancer and women are overrepresented among never smokers with the disease. Young age at onset and lack of established environmental risk factors suggest genetic predisposition. In this study, we used population-based sampling of young patients to discover candidate predisposition variants for lung adenocarcinoma in never-smoking women.

Materials and methods: We employed archival normal tissue material from 21 never-smoker women who had been diagnosed with lung adenocarcinoma before the age of 45, and exome sequenced their germline DNA.

Results and conclusion: Potentially pathogenic variants were found in eight Cancer Gene Census germline genes: BRCA1, BRCA2, ERCC4, EXT1, HNF1A, PTCH1, SMARCBl and TP53. The variants in TP53, BRCA1, and BRCA2 are likely to have contributed to the early onset lung cancer in the respective patients (3/21 or 14%). This supports the notion that lung adenocarcinoma can be a component of certain cancer predisposition syndromes. Fifteen genes displayed potentially pathogenic mutations in at least two patients: ABCG10, ATP7B, CACNA1S, CFTR, CLIP4, COL6A1, COL6A6, GCN1, GJB6, RYR1, SCN7A, SEC24A, SP100, TN1 and USH2A. Four patients showed a mutation in COL6A1, three in CLIP4 and two in the rest of the genes. Some of these candidate genes may explain a subset of female lung adenocarcinoma.

1. Introduction

Lung cancer is the most common cancer in the world and the leading cause of cancer death [1]. It is highly fatal, with an overall mortality to incidence ratio of 0.87. The primary risk factor for lung cancer is cigarette smoke. Other established non-inherited risk factors include ionizing radiation, asbestos, different kinds of occupational exposures, air pollution and pulmonary diseases such as tuberculosis and chronic obstructive pulmonary disease [2]. Lung cancer and pulmonary fibrosis are driven by similar biological pathways [3], and a recent study found an eightfold higher incidence ratio of lung cancer among sufferers of idiopathic pulmonary fibrosis (IPF) when compared to the general population [4]. A family history of lung cancer has been estimated to double the risk of developing the disease [5,6], and the heritability estimate based on the largest twin cohort to date (Nordic Twin Study on Cancer) is 0.38 [7]. A number of susceptibility loci and genes have been identified including 5p15 [8,9], 6p2 [10,11], 15q25.1 [10,11], EGFR [12–14], HER2 [15], BRCA [16], BAP1 [17], and PARK2 [18]. Also, germline TP53 and DICER1 mutations causing Li-Fraumeni and DICER1 syndromes, respectively, are linked to an increased risk of lung cancer [19,20].

The two main histological subtypes of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC originates from neuroendocrine-cell precursors, while NSCLC originates from bronchial epithelial-cell precursors and can be further divided into three types: squamous cell-, adeno- and large cell carcinoma. All major histological subtypes of lung cancer are associated with smoking.

Epidemiological, pathological, and molecular evidence suggest that lung cancer in never smokers should be treated as a distinct disease entity [21]. Women are more likely to suffer from non-smoking-associated lung cancer than men, reflecting sex-based differences in exposure to risk factors and possibly also susceptibility [22]. A woman with an affected parent has a higher risk than a man of developing lung cancer, and the molecular profile and prognosis of the disease in...
women differs from that in men [23]. A key driver of lung adenocarcinoma tumorigenesis especially in never-smoker women is somatic mutation of \textit{EGFR} [24,25].

Here, we studied germline predisposition to lung adenocarcinoma (LUAD) in never-smoking women, first identifying the youngest patients within the population-based Finnish Cancer Registry (FCR) database. Young age at onset is a hallmark of hereditary susceptibility to many common cancers. Normal tissue archival samples from never-smoking female patients under 45 years of age were systematically collected as guided by the database. The FCR harbors data on all cancer cases diagnosed in Finland since 1953, totaling over one million cases.

2. Materials and methods

2.1. Ethics statement

This study was approved by the National Supervisory Authority for Welfare and Health (Valvira; 1423/06.01.03.01/2012), the National Institute for Health and Welfare (THL; 151/5.05.00/2017), and the ethics committee of the Hospital District of Helsinki and Uusimaa (HUS; 408/13/03/03/09).

2.2. Patients and samples

We included female never smokers listed in the FCR and diagnosed with LUAD between 1953 and 2013, and before the age of 45. Smoking histories were collected from patient records, and if there was no mention of smoking habits the patient was excluded from the study. Since the establishment of the FCR, altogether 105 women had been diagnosed with LUAD before the age of 45. Of these 49 were current or former smokers, 26 had no mention of smoking history or patient records were no longer available, and 30 were never smokers. Sufficient quality normal tissue formalin-fixed, paraffin-embedded (FFPE) samples were available for 21 of the 30 never-smoking patients. Median age at diagnosis was 39 for the exome sequenced samples (range 20–44).

We identified the first and second degree relatives of these patients from the Population Information System, and checked their cancer histories from the FCR. Family history of cancer as well as relevant clinical information on the 21 patients that underwent exome analysis are listed in Table 1. For 12 of the patients we collected tumor tissue blocks in addition to the normal tissue blocks for loss of heterozygosity (LOH) analysis. In addition, we collected normal tissue FFPE blocks from six cancer-affected family members of LUAD11 (Fig. 1A), the parents of LUAD14, and the son of LUAD24 (Fig. 1B) who suffered from IPF.

2.3. Exome sequencing

We extracted genomic DNA from normal tissue archival tissue samples with the conventional phenol-chloroform method and prepared the DNA samples for exome sequencing with the KAPA Hyper Prep Kit (Kapa Biosystems Inc., Wilmington, MA) and SeqCap EZ System (Roche Nimblegen Inc., Madison, WI). The sources of the FFPE tissue and clinical information on the 21 patients that underwent exome analysis are listed in Table 1. For 12 of the patients we collected tumor tissue blocks in addition to the normal tissue blocks for loss of heterozygosity (LOH) analysis.

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The quality of raw sequencing data was examined using FastQC version 0.10.0 (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/) and Qualimap version 2.1 (http://qualimap.bioinfo.cipf.es/). 3' ends of reads with high adapter similarity were removed with Trim Galore! version 0.3.07 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and trimmed reads were mapped to 1000 genomes Phase 2 reference genome (GRCh37 / hg19) with Burrows-Wheeler Aligner (BWA)-MEM version 0.7.12 [26]. Overlapping read pairs were clipped using BamUtil version 1.0.13 (http://genome.sph.umich.edu/wiki/BamUtil#Releases) ClipOverlap. Duplicate reads were removed using Samtools version 1.0 (http://www.htslib.org/) rmdup on both paired-end and single-end reads. Aligned reads were locally realigned using the GATK version 3.5 [27] IndelRealigner and base scores were recalculated with GATK BaseRecalibrator. After realignment the final SNV and indel calls were made with the GATK HaplotypeCaller using a variant quality threshold of 1.0.

2.4. Data analysis

The variants were verified by Sanger sequencing of DNA extracted from FFPE blocks with the GeneRead DNA FFPE Kit (Qiagen). Primers were designed with Primer3web version 4.0.0 [31] and capillary sequencing was performed at the Institute for Molecular Medicine Finland (FIMM) using the BigDye v.3.1 sequencing reaction and ABI3730xl DNA Analyzer electrophoresis (Applied Biosystems, Foster City, CA). Sequences were analyzed manually using FinchTV 1.5. LOH was determined by comparing peaks between normal and tumor (> 50% tumor tissue) samples when tumor tissue was available (12 cases). Each reaction was performed in triplicate to ensure consistency of the observations and exclude mono-allelic amplification. Normal tissue DNA from a total of eight relatives of LUAD11 and LUAD14 was also sequenced to see whether candidate variants in the patients segregated with malignancy.

3. Results and discussion

More than 100 cancer predisposition genes have been identified to date. A recent pan-cancer study that included more than 10,000 cases across 33 different cancers discovered plausible susceptibility variants in 8% of all patients [32]. However, the genetic architecture of lung cancer susceptibility is not well known. In the previously mentioned study [32], probable predisposing variants were found in more than 6% of LUAD. Another study, that analyzed the amount of rare germline truncating variants in cancer predisposition genes across 12 cancer types, found a similar rate (7%) for LUAD [33]. Here we performed a population based search through the FCR to identify young female patients diagnosed with the malignancy, as early age at onset is a hallmark of inherited cancer susceptibility. Family history was also looked into, but this was not a criteria for inclusion or exclusion.

We looked into genes listed in the Cancer Gene Census (CGC), available in the Catalogue of Somatic Mutations in Cancer (COSMIC) database [34], to see whether the patients could be suffering from known cancer predisposition syndromes. We observed eight potentially pathogenic heterozygous mutations (MAF < 0.001) in eight CGC known cancer predisposition syndromes. We observed eight potentially pathogenic heterozygous mutations (MAF < 0.001) in eight CGC germline genes (Table 3). The variants in TP53, BRCA1, and BRCA2, are in light of current knowledge likely to have contributed to the early-onset lung cancer in our patients. The TP53-nonsense mutation p.Gln52Ter was not found in any controls and has, to our knowledge, not previously been reported as a germline variant. Due to its truncating nature, it is likely to have an effect on the protein function. Constitutional mutations in TP53 predispose to multiple different cancers in the carriers. The gene is mutated in approximately 70% of patients with Li-Fraumeni syndrome (LFS) and 40% of patients with Li-Fraumeni-Like syndrome (LFL) [35]. These conditions are characterized by predisposition to a wide spectrum of neoplasia, including soft-tissue sarcoma, osteosarcoma, leukemia, breast cancer, brain tumors, and adrenocortical carcinoma, often at early age at onset. Although lung cancer is not a classic LFS spectrum cancer, it has been linked to LFS by Li-Fraumeni patients with Li-Fraumeni syndrome (LFS) and 40% of patients with Li-Fraumeni-Like syndrome (LFL) [35]. These conditions are characterized by predisposition to a wide spectrum of neoplasia, including soft-tissue sarcoma, osteosarcoma, leukemia, breast cancer, brain tumors, and adrenocortical carcinoma, often at early age at onset. Although lung cancer is not a classic LFS spectrum cancer, it has been linked to LFS by Li-Fraumeni
The **BRCA1** variant p.Cys47Arg was found in a patient diagnosed with LUAD at the age of 35. The variant could not be found among the controls, nor has it been reported as a germline variant or to be somatically mutated. Residue 47 is a zinc coordinating residue located in the conserved RING motif of BRCA1. It is critical for the binding of BARD1. Functional studies have shown that another variant of this residue, Cys47Gly, is defective in homology-directed recombination (HDR) [38]). BARD1 binding and HDR are critical for the tumor suppressive function of BRCA1. Furthermore, the Cys47Gly variant has been found to cause centrosome duplication [39]. **BRCA1** is a well-established breast and ovarian cancer susceptibility gene. Considering the rarity of the discovered variant and its critical location, it is plausible that it was involved in the carcinogenesis of LUAD in the patient in question. The variant was inherited from the father who was diagnosed with gastric cancer at the age of 62. A number of studies have found an increased risk of gastric cancer in **BRCA1** mutation carriers [40–42]. Of the other cancer affected family members of whom we were able to attain tissue material, the variant was also found in an uncle with lymphoma at the age of 83 as well as a female cousin with breast cancer at the age of 47 (Fig. 1A).

A plausible susceptibility variant was also found in **BRCA2**: p.Arg2784Trp. The mouse equivalent of this residue, p.Arg2705, forms hydrogen bonds with the side chains of the residues p.Asn2702 (human p.Asn2784) and p.Ser2728 (human p.Ser2807), as well as the p.Asp41 hydrogen bonds with the side chains of the residues p.Asn2702 (human p.Asn2784Trp. The mouse equivalent of this residue, p.Arg2705, forms tertiary interactions is a well-established breast and ovarian cancer susceptibility gene. Considering the rarity of the discovered variant and its critical location, it is plausible that it was involved in the carcinogenesis of LUAD in the patient in question. The variant was inherited from the father who was diagnosed with gastric cancer at the age of 62. A number of studies have found an increased risk of gastric cancer in **BRCA1** mutation carriers [40–42]. Of the other cancer affected family members of whom we were able to attain tissue material, the variant was also found in an uncle with lymphoma at the age of 83 as well as a female cousin with breast cancer at the age of 47 (Fig. 1A).

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Altogether 15 genes harbored a rare (MAF < 0.001) potentially pathogenic variant in at least two patients within the series. The 33 variants are listed in Table 4. None of the recurrently mutated genes were among the CGC germline genes. The most frequently mutated candidate gene in the sample set was **COL6A1**. Three patients harbored one, and one patient harbored two heterozygous missense variants in the gene. The protein product **COL6A1** has three von Willebrand factor type A (VWA) domains, and all the variants were located in these domains. VWA domains are likely to mediate protein-protein interactions by coordination of divalent cations [46]. Exon skipping, nonsense and some missense mutations in **COL6A1** cause Bethlem myopathy 1 [47], and the gene has been found to be overexpressed in neoplastic lung tissues [48]. The second most frequently mutated gene with a variant in three patients was **CLIP4**. Little is known about the function of its protein product.

**SCN7A** was heterozygously mutated in two patients, including LUAD24, who shared the c.1133C > T variant with her son who suffered from IPF. The family had a notable history of lung disease in general (Fig. 1B). Other potentially pathogenic variants shared by LUAD24 and her son are listed in Table 5. **SCN7A** is a sodium channel which is altered by sodium concentrations instead of membrane depolarization. The channels contribute to sodium absorption by cells when extracellular sodium levels are high, and channels in the circumventricular organs are also likely to be involved in the control of water and salt uptake behavior and thus body fluid homeostasis. **SCN7A** is expressed in specialized neurons, specialized ependymal and glial cells, non-myelinating Schwann cells, and lung alveolar type II (AT2) cells [49]. AT2 is the main cell type responsible for the secretion of surfactant proteins SFTPC and SFTPA2. Interestingly, mutations in the genes SFTPC and SFTPA2 have been found to cause familial interstitial pneumonia [50], a hereditary form of IPF. The other variant, c.1003T > C, was found in LUAD21. We did not have tumor tissue from patient LUAD24, but Sanger sequencing of tumor-derived DNA from LUAD21 showed a decrease in the WT allele signal.

The remaining 13 shared genes (Table 4) were heterozygously mutated in two patients. **CFTR** is the most prominent candidate gene with regards to known function. Homozygous or compound heterozygous mutations in **CFTR** cause cystic fibrosis. A variant (c.1558G > T) affecting the same nucleotide as one of the two variants found in this study is listed as a cystic fibrosis causing mutation in The Clinical and Functional Translation of **CFTR** (CFTR2) database (http://cftr2.org). The relationship between cystic fibrosis and lung cancer is unclear, but both frequent hypermethylation [51] and mutation [52] of **CFTR** have been observed in NSCLCs. A recent study found lower expression of **CFTR** in tumors and showed that the expression levels correlated with disease stage, lymph node metastasis, poor prognosis, and progression-free survival [53]. Knockdown of **CFTR** enhanced the malignant behavior of NSCLC, whereas its overexpression suppressed cancer progression in vitro and in vivo. The two patients in this study carrying **CFTR** variants were of Southeast Asian ancestry.

Somatic loss of heterozygosity was observed at 4/25 tested loci in 12 tumor samples. We analyzed the variants listed in Tables 3 and 4 in the respective patients in the cases in which tumor tissue material was available (LUAD3, LUAD5, LUAD10-11, LUAD13-15, LUAD18-21, and LUAD26). The results of the LOH analysis are listed along with the variants in Tables 3 and 4. A decrease of the wild type allele signal was observed for **SMARCB1** in LUAD3, and both **SP100** and **SCN7A** in LUAD21. **SMARCB1** is part of the SWF/SNF complex and a tumor suppressor gene, loss-of-function mutations in it cause rhabdoid predisposition syndrome, while non-truncating splice-site and missense mutations have been found to cause familial schwannomatosis. The variant here has to our knowledge not previously been found in the
germline, but twice as a somatic mutation in colorectal cancer, one being a de novo mutation in a lung metastasis [34]. Its role in the carcinogenic process in our patient remains elusive, however.

During the review process of this manuscript, a study on the mutational profile of 36 young LUAD patients was published [54]. Besides characterizing unique somatic mutational features of this patient subgroup, potential predisposing mutations were also identified. Such variants were found in TP53 and BRCA2 supporting some of the findings in our study, and showing the value of multiple independent efforts to elucidate the molecular basis of this devastating disease.

### 4. Conclusions

This population-based study on a highly selected sample set, together with prior reports on germline variants in known cancer predisposition genes in lung cancer, calls for a broadening of the cancer spectrum of cancer syndromes caused by TP53, BRCA1, and BRCA2 mutations, and highlights genetic susceptibility as a relevant cause for LUAD in young females. This study also provides a set of candidate predisposition genes, some of which may explain a subset of female LUAD, for validation in other similar materials. Symptoms of lung

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**Table 4**

Genes mutated more than once within the patient series.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant location</th>
<th>Base change</th>
<th>Variant effect</th>
<th>rs-code</th>
<th>Population MAF</th>
<th>Patient</th>
<th>LOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL6A1</td>
<td>chr21:47421948</td>
<td>c.2030G &gt; A</td>
<td>p.(Arg677His)</td>
<td>rs753731596</td>
<td>N/A</td>
<td>LuAd20</td>
<td>None</td>
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<td>p.(Ser378Asn)</td>
<td>rs177512434</td>
<td>N/A</td>
<td>LuAd29</td>
<td>N/A</td>
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<td>TP53</td>
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<td>p.(Thr440Ile)</td>
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<td>rs767662986</td>
<td>N/A</td>
<td>LuAd26</td>
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</tr>
<tr>
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<td>c.1133C &gt; T</td>
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<td>N/A</td>
<td>LuAd24</td>
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<td>p.(Thr440Ile)</td>
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<td>LuAd21</td>
<td>WT loss</td>
</tr>
<tr>
<td>TTCN</td>
<td>chr2:179425967</td>
<td>c.8492G &gt; A</td>
<td>p.(Arg28298Trp)</td>
<td>rs779582232</td>
<td>4.7E-04**</td>
<td>LuAd13</td>
<td>None</td>
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**Table 5**

Variants shared between LuAd24 and IPF affected son.

<table>
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<tr>
<th>Gene</th>
<th>Variant location</th>
<th>Base change</th>
<th>Variant effect</th>
<th>rs-code</th>
<th>gnomAD Finnish MAF</th>
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<td>ACTR2</td>
<td>chr21:60495807</td>
<td>c.1139G &gt; A</td>
<td>p.(Arg343Glu)</td>
<td>rs77512364</td>
<td>1.94E-4</td>
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<td>N/A</td>
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<td>None</td>
</tr>
<tr>
<td>SCN7A</td>
<td>chr2:167313537</td>
<td>c.1133C &gt; T</td>
<td>p.(Ser378Asn)</td>
<td>rs77512434</td>
<td>N/A</td>
<td>LuAd24</td>
<td>None</td>
</tr>
<tr>
<td>SEC24A</td>
<td>chr5:134032990</td>
<td>c.2071G &gt; A</td>
<td>p.(Arg690His)</td>
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<td>9.03E-05</td>
<td>LuAd14</td>
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</tr>
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<td>SP100</td>
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<tr>
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<td>p.(Arg28298Trp)</td>
<td>rs779582232</td>
<td>4.7E-04**</td>
<td>LuAd13</td>
<td>None</td>
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</tbody>
</table>

**Table 5**

Variants shared between LuAd24 and IPF affected son.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant location</th>
<th>Base change</th>
<th>Variant effect</th>
<th>rs-code</th>
<th>gnomAD Finnish MAF</th>
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<tr>
<td>ACTR2</td>
<td>chr21:60495807</td>
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<td>RAR1</td>
<td>chr19:38566047</td>
<td>c.250G &gt; T</td>
<td>p.(Arg83Arg)</td>
<td>rs767662986</td>
<td>N/A</td>
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<tr>
<td>SCN7A</td>
<td>chr2:167313537</td>
<td>c.1133C &gt; T</td>
<td>p.(Ser378Asn)</td>
<td>rs77512434</td>
<td>N/A</td>
</tr>
</tbody>
</table>
cancer often appear only at an advanced stage, and most cases are di-
gnosed when treatment options are limited. Identifying individuals
with high genetic risk is important, since such knowledge provides
opportunities for early diagnosis and improved management, including efforts towards cancer prevention in the at-risk relatives.

Conflict of interest statement
None declared.

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References

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

[16] J. Malhotra, M. Malvezzi, E. Negri, C. La Vecchia, P. Bo, Lung carcinogenesis and
subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

[18] J. Malhotra, M. Malvezzi, E. Negri, C. La Vecchia, P. Bo, Lung carcinogenesis and
subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

[22] J. Malhotra, M. Malvezzi, E. Negri, C. La Vecchia, P. Bo, Lung carcinogenesis and
subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

[23] J. Malhotra, M. Malvezzi, E. Negri, C. La Vecchia, P. Bo, Lung carcinogenesis and
subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39

subtypes and clinical features: the Spanish REASON study, Cancer Epidemiol. 39
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