Genes Associated with Susceptibility to Lung Adenocarcinoma Among Never Smokers Suggest the Mechanism of Disease

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Abstract. Global statistics estimate that 15% of all cases of lung cancer in men and 53% in women are not attributable to smoking, and these data indicate that worldwide, approximately 25% of patients with lung cancer are never smokers. The etiology of lung cancer is disputed. The present study reviews the genes associated with susceptibility to lung cancer among never smokers and suggests possibilities for the involvement of metabolic syndrome. The environment appears to have changed the genes susceptible to lung cancer. Classical genes associated with lung cancer are decreasing and novel emerging genes may reflect changes in lifestyle. We provide evidence that the genes associated with susceptibility to lung cancer in never smokers are very similar to those reported in patients with metabolic syndrome, and that simply quitting smoking is not sufficient as the primary means of preventing lung cancer.

The purpose of this article is to review the genes associated with susceptibility to lung adenocarcinoma among never smokers, including a description of the mechanism of disease. Lung cancer is a leading cause of death in both developed and developing countries. It comprises of two morphological groups, small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC includes squamous cell lung cancer (SC) and adenocarcinoma (AC) (1, 2). The increasing incidence of lung AC in men surpassed the incidence of lung SC in the 1960s and 1970s in the USA and Europe, and in the 1980s and 1990s in Japan (1, 2). Among never smokers with lung cancer, over 65% of patients had AC (3). The absolute numbers of female patients with lung AC were not high compared to the numbers of male patients with lung AC, but a high percentage of AC in females was observed (1-8).

Global statistics estimate that 15% of all lung cancer cases in men and 53% in women are not attributable to smoking, and these data indicate that worldwide, approximately 25% of patients with lung cancer are never smokers (4). More never-smoker patients with lung AC were female, younger and showed better prognosis (5-8). NSCLC in never smokers is currently on the rise (7), and there is a question as to whether it might be a whole different disease altogether (3, 5).

The reason why AC is increasing remains unknown. The use of cigarette filters, and air pollution have been pointed-out as possible reasons (9). Santoro et al. explained that the proportion of never smokers with lung cancer is expected to increase in parallel with successful cessation programs (10). If patients were never smokers, the following exposures have been considered: second-hand smoke, radon, outdoor air pollution, cooking oil fumes, coal fumes and asbestos (7, 9, 10). Cessation of smoking is absolutely necessary but by itself is not sufficient for total prevention of lung cancer, because obesity, lack of physical activity, heavy alcohol consumption and consuming food with a high fat content all lead to metabolic syndrome, which causes a high percentage of cancer (9, 11). Very recently Mazieres et al. examined 140 women with AC (63 never-smokers and 77 former/current smokers) and found that the never smokers were characterized by a higher frequency of lipidic features (60.3% vs. 37.7%) compared with smokers (12).

The present review analyzes recent work on genes associated with susceptibility to lung AC among never
smokers, and demonstrates that this information on susceptibility genes suggests the etiology of recent emerging lung cancer in never smokers.

**Classical Genes Associated with Susceptibility to Lung Cancer**

**Xenobiotic genes.** Our previous article (9) showed that genes related to lung cancer susceptibility had changed over the last 20 years in Japan (all referenced articles are in English with peer review). The odds ratios (ORs) of the rare alleles of cytochrome P450 1A1 (CYP1A1)*2A, CYP1A1*2B, and glutathione S-transferase M1 (GSTM1) null-type genes were investigated by a meta-analysis method. The incidence of lung cancer comparing “before 2000” with that “after 2001” for the population with CYP1A1*2A (m2/m2) was much greater than that with CYP1A1*2A (m1/m1) (averaged OR of 2.493 vs. 0.899, respectively). There were no statistical differences in ORs between CYP1A1*2B (Val/Val) and CYP1A1*2B (Ile/Ile), or between GSTM1 null and positive types (9).

A recent report by Lopez-Cima et al. (13) showed that negative data in the CYP1A1, GSTM1, glutathione S-transferase T1 (GSTT1), glutathione S-transferase P1 (GSTP1) metabolic genes with no gene-gene association between them in 789 cases (SC=313; AC=246) and 789 controls (described later in detail) was also found in the same GWAS (26). C3orf21 encodes a UDP-xylose:α-xylosidetrix, 3-xylosyl-transferase, which plays an important role in the formation of notch epidermal growth factor (NOTCH EGF) repeats (described in detail below).

**DNA damage-repair genes.** Several DNA damage repair-genes have been reported in association with lung cancer, e.g., human 8-oxoguanine DNA glycosylase (hOGG1), nucleotide excision repair (NER) genes [ERCC1, XPB/ERCC3, XPG/ERCC5, CSB/ERCC6 and XPC], NAD(P)H: quinoneoxidoreductase (NQO1), cytosine DNA-methyltransferase-3B (DNMT3B), O6-alkylguanine DNA alkyltransferase (AGT), X-ray repair cross complementing group 1 (XRCC1), O6-methylguanine-DNA methyltransferase (MGMT) (9). Among heavy smokers with SC, these gene polymorphisms had significantly high ORs for their association with lung cancer (9). This is reasonable because tobacco smoke contains carcinogenic chemicals which damage DNA, and if these gene polymorphisms negative affect repair of damaged DNA or prevent the formation of DNA-adducts, the resultant reduced DNA-repair capacity would play a role in the carcinogenesis of tobacco-related cancer (9).

**Susceptibility Genes Found by GWAS**

15q25, *Nicotine acetylcholineesterase genes.* GWAS for lung cancer reported by Amos et al. (27), Hung et al. (28) and Thorgeirsson et al. (29) indicated an association between chromosome 15q variants (in particular rs16969968 and
### Table I. Genes associated with susceptibility to lung cancer in never smokers (2005-2013).

<table>
<thead>
<tr>
<th>Author(s) (Year)</th>
<th>Gene Cases/controls</th>
<th>Comments</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Classical Genes, never smokers</strong></td>
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<tr>
<td>Lopez-Cima et al. (2012)</td>
<td>CYP1A1/GSTM1/GSTP1/GSTT1 (789/789)</td>
<td>No association</td>
<td>13</td>
</tr>
<tr>
<td>Chen et al. (2011)</td>
<td>CYP1A1 (meta-analysis of 71 case-control studies)</td>
<td>No association</td>
<td>14</td>
</tr>
<tr>
<td>Osawa et al. (2007)</td>
<td>CYP1A2<em>1F A/A, CYP1A2</em>1C G/G and NAT2 slow</td>
<td>OR 4.95, 3.31</td>
<td>20</td>
</tr>
<tr>
<td>Larsen et al. (2006)</td>
<td>CYP1A1 Ile462Val and MPO G-463A (1,103/627)</td>
<td>OR 3.72 (AC)</td>
<td>19</td>
</tr>
<tr>
<td>Chion et al. (2005)</td>
<td>NAT2 fast acetylator (162/208)</td>
<td>OR 2.56 (female), no effect (male)</td>
<td>21</td>
</tr>
<tr>
<td><strong>Tumor-suppressor genes: TP53, TP63</strong></td>
<td></td>
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<tr>
<td>Hosgood et al. (2012)</td>
<td>TP63 (3,467/3,787, Taiwan, China, South Korea, Singapore)</td>
<td>OR 0.80</td>
<td>25</td>
</tr>
<tr>
<td>Le Calvez et al. (2005)</td>
<td>TP53 mutation (40 never smokers/27 former/64 current)</td>
<td>47.5%, 55.6%, 77.4</td>
<td>77</td>
</tr>
<tr>
<td><strong>DNA damage and repair genes</strong></td>
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<tr>
<td>Bock et al. (2005)</td>
<td>NQO1 T allele (161/173)</td>
<td>OR 0.57 (ETS exposure), 0.98 (without exposure)</td>
<td>78</td>
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<tr>
<td><strong>2. Genes found by GWAS</strong></td>
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<tr>
<td>Lan et al. (2012)</td>
<td>15q25 (5,510 never-smoking female cases/4,510)</td>
<td>No association</td>
<td>38</td>
</tr>
<tr>
<td>Ito et al. (2012)</td>
<td>15q25 (716/716, Japanese)</td>
<td>No association</td>
<td>31</td>
</tr>
<tr>
<td>Wang et al. (2011)</td>
<td>15q25 (rs12914385) (2,405/7,622, UK)</td>
<td>OR 1.17 (rs2736100), 2.48 (rs402710)</td>
<td>31</td>
</tr>
<tr>
<td>Timofeeva et al. (2011)</td>
<td>15q25 (rs16969968) (894/1,805, Sweden)</td>
<td>OR 1.18</td>
<td>77</td>
</tr>
<tr>
<td>Wang et al. (2010)</td>
<td>15q25 (rs12914385) (239/553, UK)</td>
<td>OR 1.18</td>
<td>36</td>
</tr>
<tr>
<td>Lips et al. (2010)</td>
<td>15q25 (rs16969968) (342/3,273)</td>
<td>OR 1.18</td>
<td>78</td>
</tr>
<tr>
<td>Shiraishi et al. (2009)</td>
<td>CHRNA (rs8034190, rs16969968, rs1051730)</td>
<td>OR 2.3, 2.4, 2.7</td>
<td>35</td>
</tr>
<tr>
<td>Wu et al. (2010)</td>
<td>15q25 (rs12914385) (239/553, UK)</td>
<td>OR 1.39 (rs2036534), 1.49 (rs667282), 1.42 (rs12910984), 1.40 (rs6495309)</td>
<td>36</td>
</tr>
<tr>
<td>Lips et al. (2010)</td>
<td>15q25 (rs16969968) (342/3,273)</td>
<td>OR 1.34 (rs2736100, TERT)</td>
<td>80</td>
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<tr>
<td>Lan et al. (2012)</td>
<td>10q25.2 (rs7086803) (5,510/4,544, 328/407, 92/161, 91/439)</td>
<td>OR 1.69</td>
<td>56</td>
</tr>
<tr>
<td>Zhang et al. (2012)</td>
<td>C3orf21 (200/200, Chinese)</td>
<td>0.6219 (rs2131877)</td>
<td>26</td>
</tr>
<tr>
<td>Alm et al. (2012)</td>
<td>18p11.22 (434/1,000, Koreans)</td>
<td>OR 0.69 (rs11663246), 0.69 (rs11080466)</td>
<td>54</td>
</tr>
<tr>
<td>Li et al. (2010)</td>
<td>15q25.1 (377/377, 328/407, 92/161, 91/439)</td>
<td>OR 1.69</td>
<td>79</td>
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<tr>
<td><strong>TERT and CLPTM1L</strong></td>
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<tr>
<td>Ito et al. (2012)</td>
<td>Sp15 (716/716, Japanese)</td>
<td>OR 1.17 (rs2736100), 2.48 (rs402710)</td>
<td>31</td>
</tr>
<tr>
<td>Pande et al. (2011)</td>
<td>Sp15.33 (rs451360) (1,681/1,235, USA)</td>
<td>OR 0.62</td>
<td>49</td>
</tr>
<tr>
<td>Hsiung et al. (2010)</td>
<td>Sp15.33 (rs2736100) (2,768/3,100, Chinese and Taiwanese)</td>
<td>OR 1.54</td>
<td>39</td>
</tr>
<tr>
<td>Jin et al. (2009)</td>
<td>Sp15.33 (1,221/1,344 Chinese)</td>
<td>OR 1.59 (rs2736100), 1.09 (rs402710)</td>
<td>47</td>
</tr>
<tr>
<td>McKay et al. (2008)</td>
<td>Sp15.33 (3,259/4,159)</td>
<td>OR 1.25 (402710), 1.15 (rs2736100)</td>
<td>79</td>
</tr>
<tr>
<td>Landi et al. (2009)</td>
<td>Sp15.33 (362/1,402)</td>
<td>OR 1.34 (rs2736100, TERT)</td>
<td>80</td>
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<tr>
<td><strong>Other genes found by GWAS</strong></td>
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<tr>
<td>Lan et al. (2012)</td>
<td>10q25.2 (rs7086803) (5,510/4,544, 328/407, 92/161, 91/439)</td>
<td>OR 1.32</td>
<td>38</td>
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<td>Li et al. (2010)</td>
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<td>OR 1.69</td>
<td>79</td>
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<td><strong>3. Driver genes</strong></td>
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<tr>
<td>Li et al. (2013)</td>
<td>EGFR mutation in female (47.5% vs. 15.0% in males), never-smokers (42.3% vs. 13.9% in smokers), AC (44.2% vs. 8.0% in AC)</td>
<td>62</td>
<td></td>
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<tr>
<td><strong>HER2</strong></td>
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<tr>
<td>Jo et al. (2008)</td>
<td>HER-2 (104/105).</td>
<td>-3444 C&gt;T: OR 2.65 (female), 2.26 (non-smokers), 2.19 (non-drinkers); 11985 C&gt;T: OR 2.38 (females), 2.15 (non-smokers), 2.85 (non-drinkers)</td>
<td>62</td>
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<tr>
<td><strong>71</strong></td>
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<tr>
<td>EML4 - ALK fusion gene</td>
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<tr>
<td>Li et al. (2013)</td>
<td>EML4-ALK fusion gene cases (3.37%, 7/208 cases with lung cancer)</td>
<td>Five were female, non-smoking adenocarcinoma</td>
<td>62</td>
</tr>
<tr>
<td><strong>4. Genes related to inflammation and natural Immunity</strong></td>
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<tr>
<td>Kiyohara et al. (2010)</td>
<td>IL1B (462/379, Japanese)</td>
<td>OR 1.51</td>
<td>75</td>
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</tbody>
</table>

GWAS: Genome-wide association studies; TERT: Telomerase reverse transcriptase; CLPTM1L: cleft lip and palate transmembrane 1-like protein; C3orf21: chromosome 3 open reading frame 21; HER2: Human EGFR2; NAT2: N-acetyltransferase 2; NQO1: quinone oxidoreductase 1.
rs8034191) and risk of lung cancer with ORs between 1.30 and 1.32 ($p<9\times10^{-10}$ to $1\times10^{-17}$). The susceptibility region contains three cholinergic nicotine receptor genes, \textit{CHRNA3}, \textit{CHRNA5} and \textit{CHRN4B}. Amos \textit{et al.} (27) and Hung \textit{et al.} (28) showed a direct association between the variants and the risk of lung cancer, irrespective of smoking habit. Thorgeirsson \textit{et al.} demonstrated an association between the same genetic region and smoking habits, as well as an association with risk of lung cancer (29). Therefore, it has been argued whether the association is direct or indirect, that is, whether the risk of lung cancer is in fact associated with any smoking habit. Studies noting an association between 15q variants and nicotine dependence or smoking quantity have so far focused mainly on smokers with lung cancer and chronic obstructive pulmonary disease (29-31). Liu \textit{et al.} demonstrated that the risk of lung cancer was more than five-fold higher among 194 patients with familial lung cancer with either copy of high-risk alleles rs8034191 (OR=7.20) or rs1051730 (OR=5.67), both of which were located in the 15q24-25.1 locus, than among 219 cancer-free controls (32). The HapMap project revealed that the risky alleles of the SNPs at chromosome 15q25, rs8034191 and rs16969968 in Caucasians were found at extremely low frequencies in Asians, suggesting that chromosome 15q25 may have less influence on the risk of lung cancer and smoking behavior in Asians than in Caucasians (31). Ito \textit{et al.} observed no significant association between the variants of rs12914385 and rs931794 on 15q25 and risk of lung cancer in a Japanese population (31).

As noted above, the risky SNPs reported in Caucasians are extremely rare in Asians. Wu \textit{et al.} searched novel SNPs in the \textit{CHRNA3-CHRNB4-CHRNA5} gene cluster on chromosome 15q25 in Chinese and identified four novel SNPs, as shown in Table 1 (33). Moreover, they found that rs6495309T>C SNP located in the \textit{CHRNA3} promoter region alters the ability to bind transcriptional factor organic cation transporter 1, resulting in increased CHRNA3 expression.

An association between nicotine dependence with increased serum levels of nicotine and both \textit{CYP2A6} and \textit{CYP2B6} genes encoding nicotine-metabolizing enzymes as well as both \textit{CHRNB3} and \textit{CHRNA6} genes encoding nicotinic acetylcholine receptor subunits, has been reported (9). Lips \textit{et al.} showed a strong association between 15q gene variants and lung cancer and concluded the existence of an independent association with smoking quantity in a total of 17,300 subjects from five studies (34). The same study showed there to be no association between 15q variant and smoking initiation, cessation, and quitting smoking because the authors observed association between 15q variants and the number of cigarettes smoked per day in former and current smokers, and an association was seen in women, not in men. Although current heavy smoking controls with AA genotype showed there to be a 60%-increased OR for heavy smokers, the lung cancer cases did not show such an association (34). Shiraiishi \textit{et al.} recorded an association in both smokers and non-smokers and in all histological types of lung cancers among Japanese (35).

Wang \textit{et al.} examined the association of 15q25 and smoking, and showed an indirect link between genotype and lung cancer (36, 37). They showed no association between 15q25.1 variation and risk of AC in never smokers (36). They conducted a meta-analysis, pooling their cases and previously published studies on 2,405 never-smoker lung cancer cases and 7,622 controls, and revealed no association between 15q25 variation and lung cancer risk (37). No association between 15q25 and lung cancer in never-smoker women in Asia was noted (38, 39). This finding of no association was widely accepted in an article written by authors from 69 Institutions (40). The article also suggested that the 15q25 variant region may influence the risk of airflow obstruction, that is, lung cancer risk is not mediated solely by the effects of nicotine dependence. This derives from the authors’ observations based on the correlation between 15q25 SNP genotype and \textit{CHRNA5} expression levels in lung and sputum, combined with a finding of increased risk of airflow obstruction in never smokers (40). Moreover, they observed the 15q25.1 region meeting with the iron-responsive element binding protein 2 (\textit{IREB2}) gene by genome-wide association. Fehringer \textit{et al.} reported separately that the minor allele of a variant representing one of the two loci at 15q25 (rs2036534) was associated with increased \textit{IREB2} expression (41). The significance of \textit{IREB2} is that it may play an important role in the repair and remodeling processes that lead to chronic obstructive pulmonary disease in never-smoker patients. \textit{CHRNA3/5} variant was also found to be associated with bronchial hyper-responsiveness in children not exposed to cigarette smoke (42). Thus, a number of articles note that the disease mechanism causing lung cancer is unlikely to involve tobacco addiction. Nicotine acetylcholine receptors could be involved in lung cancer through other mechanisms. It has been suggested that N'-nitrosonornicotine and nitrosamines may facilitate neoplastic transformation by stimulating angiogenesis and tumor growth (43), and cancer cell motility, migration and p63 expression mediated through their interaction with nicotinic acetylcholine receptors (44).

\textbf{5p15. Telomerase reverse transcriptase (\textit{TERT}) and cleft lip and palate transmembrane 1-like protein (\textit{CLPTM1L})}.

GWAS have identified chromosome 5p15.33 as one of the regions that is reproducibly associated with lung cancer risk (27-29). Subsequently, an association of SNPs in the 5p15.33 region with lung cancer risk has been reported (35, 43, 45, 46). Chromosome 5p15.33 comprises of two candidate susceptibility genes, \textit{TERT} and \textit{CLPTM1L}. \textit{TERT} encodes a catalytic subunit of telomerase that maintains telomere ends.
Its overexpression leads to prolongation of the lifespan of the cell and is seen in cancer cells. CLPTM1L is also known as cisplatin resistance-related protein 9 (CRR9p). CLPTM1L confers resistance to apoptosis caused by genotoxic agents in association with up-regulation of the anti-apoptotic protein, B-cell lymphoma-extra large (BCL-XL) (35).

Although the association of rs2361000 SNP in TERT gene with lung cancer risk has been reported in smokers, a strong association was also demonstrated in never smokers (36, 45, 47). Broderick et al. observed a significant increased frequency of rs2736100 (TERT) as the risky allele in cases of AC, but reported no data on never smokers (48). Hsiung et al. observed that the most significant association was rs2736100 at 5p15.33 in lung AC among never smokers (39). Fine mapping analysis of genetic variants in the 5p15.33 region conducted by Pande et al. revealed four SNPs associated with lung cancer risk; rs4975538 is an intronic SNP in TERT, rs451360 and rs370348 are intronic SNPs in CLPTM1L and rs4975615 is in the intergenic region between two genes (49). The rs451360 was significant among never smokers (49).

6q21, BAT3. Recent papers focusing on never-smoker women in Asia (38, 39) showed no evidence of association with lung cancer at 15q25 but found three new susceptible loci at 10q25.2, 6q22.2 and 6q21.32 besides 5q15.33 (CLPTM1L and TERT) and 3q28. The association of 6q21.33 and risk of lung cancer shown by Wang et al. (36, 43) and Broderick et al. (48) could be considered to mediate through a number of transcripts mapping the region of linkage disequilibrium (LD). They wrote that BAT3 represents a strong candidate for lung cancer susceptibility because it is implicated in apoptosis and the protein complexes with EIA-binding protein p300, required for acetylation of p53 in response to DNA damage (48).

The associated risk loci mapping 6p21.33 in which rs3117582 localizes to intron 1 of BAT3 and rs3131379 localizes to intron 10 of MSH5 were revealed by GWAS (43). MSH5 is involved in DNA mismatch repair (MMR) and meiotic recombination. The deficiency of MMR leads to lung cancer. The C allele of rs3117582 is associated with increased risk (OR 1.16), but there is no report of lung cancer in never smokers.

10q25, Vesicle transport through interaction with t-SNAREs homologue 1A (VTI1A). The strongest new association signal, rs7086803 at 10q25.2, located at intron 7 of the VTI1A gene, has been implicated in lung carcinogenesis (38). VTI1A is involved in ACRP30-containing vesicles in adipocytes, and lower amounts of VTI1A in cultured adipocytes can inhibit adiponectin secretion (50). A low level of adiponectin leads to cardiovascular diseases in metabolic syndrome (51). An association between lower adiponectin levels and lung cancer has been reported (52). Petridou et al. showed a relation between adiponectin receptors and the risk of lung cancer but no significant relation with circulating adiponectin levels in smoking patients with lung cancer (53). These alterations may be based on differences in lifestyles, including factors such as obesity and lack of physical activity among others.

Other genes found by GWAS. A GWAS on never smokers conducted by Li et al. revealed a strong correlation between the transcription level of the gene glypican-5 (GPC5) and genotypes of the replicated SNP (rs2352028 at 13q31.3) in 77 non-tumor lung tissue samples, and the expression levels of GPC5 in the matched lung AC tissue were lower by half than in normal tissue (54). Down-regulation of GPC5 may contribute to the development of lung cancer in never smokers. GPC5 is a member of the glypicans gene family. Glypicans are a family of heparin sulphate proteoglycans, and the main function of the membrane-attached glypicans is to regulate the signaling pathway of wingless transformation (WNT), hedgehog, fibroblast growth factors, and bone morphogenetic proteins (54).

Tessema et al. reported that never smokers with primary lung AC had a significantly higher prevalence of methylation of TNFRSF10C (a TNF receptor family member that regulates meiotic G 2/M transition) than current and former smokers (55).

Genotypes of C3orf21, which plays an important role in the formation of NOTCH EGF repeats, as described above in TP63, were nominally-associated with a reduced risk of lung AC among never smokers. C3orf21 is located in 3q29 (26). Chromosomal imbalance in 3q29 has been reported in various types of cancer: NSCLC, prostate cancer, and head and neck squamous cell carcinoma (26). Another novel susceptible locus, 18q11.22 near the adenomatous polyposis coli down-regulated 1 (APCDD1), N-ethylmaleimidedsensitive factor attachment protein gamma (NAPG) and family with sequence similarity 38, member B (FAM38B) genes was also reported in Korean never smokers with NSCLC (56).

Driver Genes: EGFR, KRAS, BRAF, PIK3CA, and EML4-ALK

Epidermal growth factor receptor (EGFR), Kirsten rat sarcoma viral oncogene (KRAS), protooncogene B-Raf (BRAF), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) and EML4-ALK are known as driver genes, and mutations of these genes are seen in patients with AC. Among these the frequency of EGFR mutation was about 50% in non-smoking patients with AC (57).
**EGFR mutation.** EGFR tyrosine kinase inhibitors (EGFR-TKIs) are dramatic anticancer agents, e.g. gefitinib and erlotinib, for lung cancer, especially for AC (58). It was noted that the EGFR mutation in lung cancer associated with sensitivity to EGFR-TKI occurs more frequently in Asian women non-smokers with AC (59-60). EGFR mutation is also considered to be an early event in the pathogenesis of lung AC (60). Wilkerson et al. clearly demonstrated a relation between the subtypes of AC (bronchioid, magnoid, and squamoid) and sequence mutation, copy number, chromosomal instability and methylation (61). Bronchioid was found to occur in female non-smoker patients with a superior survival outcome and who present with well-differentiated, bronchioalveolar morphology, early-stage and EGFR-mutated cancers. On the other hand magnoid and squamoid ACs were found in male patients exposed to smoking, with poor survival outcome and late-stage cancer, and with KRAS, TP53 or serine/threonine kinase 11 (STK11) mutations. STK11 gene regulates cell polarity and functions as a tumor suppressor. The magnoid subtype exhibited increased hypermethylation, a phenomenon similar to the CpG-island-methylator-phenotype observed in other types of cancers (62). EGFR-TKI was used as a potential breakthrough for the treatment of metastatic AC of lung in never smokers (63).

As noted above, AC appears to have a predilection for women, and the association of AC with a smoking habit may be less than that for the other histological subtypes of lung cancer (59). Association of EGFR mutation and estrogen receptor (ER)-α and -β with lung carcinogenesis has been reported (59, 64). Shimizu et al. showed the presence of membranous ERα expression together with EGFR mutation to be an independent factor for survival in patients with lung AC, suggesting a crosstalk between membranous ERα and EGFR mutation (59). Very recently Mazières et al. examined 140 women with AC (63 never smokers and 77 former/current smokers) and found that the never smoker patients with AC were characterized by higher age (67 vs. 58.7 years) and higher frequency of lipidic features (60.3% vs. 37.7%) compared with smokers (12). They also observed increased frequency of EGFR mutation and ERα expression in never smokers with AC (12).

Li et al. clearly demonstrated EGFR mutations in 24.5% (51/208 cases) of patients with lung cancer, and these mutations were identified at high frequencies in females (47.5% vs. 15.0% in males), never smokers (42.3% vs. 13.9% in smokers), and patients with AC (44.2% vs. 8.0% in patients with non-AC) (62).

As the study found patients carrying germline EGFR mutations in cases of familial lung AC, genotyping can be used to predict benefit for treatment with EGFR-TKI, and can also be used as a screening tool to identify patients at-risk of carrying germline EGFR mutations (65). Better response to TKIs was seen in non-smoker (or light smoker) female with lung AC (66). Yamauchi et al. identified a set of 139 gefitinib-sensitive genes which are involved in cancer phenotypes and found that EGF signaling status in cancer cells underlies an aggressive phenotype of cancer cells (67). This is useful for the selection of patients with early-stage lung AC with poor prognosis. Jou et al. revealed polymorphism of EGFR intron 1 in female never smokers with lung cancer (68). Ren et al. observed that 82.7% of lung ACs from non-smoker females were found to harbor oncogenic mutations in the following genes: EGFR, HER2, EML4-ALK, KRAS, BRAF, and PIK3CA (69). A higher human papillomavirus (HPV) may participate in Asian non-smoking lung AC, which responds to EGFR-TKIs (70).

HER2. A proto-oncogene of the receptor tyrosine kinase superfamily, HER2 acts as a binding partner of other members of the EGFR family in the activation of EGFR signaling. Joy et al. revealed that polymorphisms of the HER2 gene are associated with an increased susceptibility to lung cancer in female, non-smoker and non-drinker subgroups in a Korean population (71).

**EML4-ALK.** A fusion protein between EML4 protein and the intracellular signaling portion of the ALK tyrosine kinase receptor has been identified in a small subset of NSCLC patients by Soda et al. (72). Li et al. identified seven patients (3.37% out of 208 patients with lung cancer) who harbored the EML4-ALK fusion gene (62). Six out of the seven positive patients were non-smokers (7.69%, 6/78). The incidence of EML4-ALK translocation in female non-smoking patients with AC was as high as 15.2% (5/33) (62).

**KRAS.** Dogan et al. revealed that the KRAS mutation occurred in 34% of smokers and in 6% of never smokers, but KRAS G>A transition mutations were more likely to be found in never smokers than in smokers (mostly G12D: 58% vs. 20%) while KRAS G12C, the most common G>T transversion mutation, was seen in smokers (73). Dogan et al. suggested this different KRAS mutation could be useful in determining the presence of second-hand smoke in never smokers with lung AC.

**Genes Related to Inflammation/Innate Immunity**

**Interleukin-1 beta (IL1B).** Several SNPs have been examined in the IL1B gene. T>C at position -511 (rs16944), C>T at position -31 (rs1143627) and C>T at position +3954 (rs1143634), of the IL1B gene have been investigated. The rs16944 SNP is known to be correlated with increased intracellular IL1B levels (74). IL1B (rs1143634, 3954C>T) was investigated in a case-control study comprising of 462 lung cancer cases and 379 controls in a Japanese population (75). Association of T allele carriers of the IL1B rs1143634 variant with a loss of 1p and 19q in a subset of NSCLC patients was also observed (76).
polymorphism with risk of lung cancer was seen in never smokers, with an OR 1.11, but higher risk was observed in smokers (OR=5.45) and in heavy alcohol drinkers (OR=2.48). SNPs in \textit{IL2RB} and \textit{BCL2L14} are known to be risky in current smokers and in former smokers (76). There are no reports on genes related to innate immunity in never smokers with AC.

**Summary and Conclusion**

Approximately 25\% of patients with lung cancer worldwide (15\% men, 53\% women) are estimated to be never smokers. The genes associated with susceptibility to lung cancer in smokers are very different from those for susceptibility to AC in never smokers. Several characteristic genes have been found in never smokers with AC: \textit{NAT2}, \textit{TP63}, \textit{C3orf21}, \textit{BAT3}, \textit{VTIIA}, \textit{TNFRSF10C}, and \textit{EGFR}, as shown in Table II.

Among them, the \textit{EGFR} gene is the most notable and frequently reported, regardless of differences in ethnicity. It is seen in the early stage of AC in never smokers. Saxena \textit{et al.} reported a bi-directional crosstalk between leptin and insulin-like growth factor-I signaling promotes invasion and migration of breast cancer cells, \textit{via} transactivation of EGFR, \textit{i.e.}, carcinogenesis in never-smoker women with lung AC is very similar to that in breast cancer (81). \textit{VTIIA} is also related to the mechanism of cancer occurrence in metabolic syndrome \textit{via} association with lower adiponectin (38). Zhang \textit{et al.} reported strong evidence of linkage of 5p14 with metabolic syndrome and risk of cancer (11). Obesity, metabolic syndrome and type II diabetes mellitus indicate a higher risk for lung cancer (52, 53).

In conclusion, prevention is the most important and most valuable factor. Cessation of smoking is, of course, necessary, but in and of itself is not sufficient to prevent lung cancer altogether because a quarter of patients with lung cancer are never smokers. It is more important to provide effective education to improve lifestyle with emphasis on prevention of obesity, metabolic syndrome, diabetes mellitus, and heavy drinking, and promoting daily physical activity, especially in the so-called developed countries.

**Conflicts of Interest**

The Authors confirm that there are no conflicts of interest regarding the contents of this article.

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References


12493-1500, 2010.


18493-1500, 2010.


22493-1500, 2010.


28493-1500, 2010.


34493-1500, 2010.


