Nicotine dependence may link the 15q25 locus to lung cancer risk

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The nicotinic 15q25 locus has been implicated in lung cancer risk, with an odds ratio of ~1.3. The same locus is associated with nicotine dependence due to cigarette smoking and with smoking-associated chronic obstructive pulmonary disease, which is a risk factor for lung cancer. Our meta-analysis of reported studies shows that this locus was not associated with lung cancer risk in >1000 never-smoker cases and >1800 controls. Review of exposure-response data for lung cancer risk showed that less than a half-cigarette per day may confer the same risk of lung cancer as that conferred by the 15q25 locus. Given the lack of effect in never-smokers and the known common and variable underreporting of smoking habit in studies on smoking-associated diseases, we cannot exclude that the association between the 15q25 locus and lung cancer risk is indirect, deriving from association of the same locus with smoking habit. Since nicotine is not carcinogenic, available data do not provide plausibility of the association between the nicotinic 15q25 locus and lung cancer pathogenesis. Thus, a direct link between the 15q25 locus and lung cancer risk has yet to be established.

Introduction

Three genome-wide association studies (GWAS) have reported an association between a chromosome 15 region (15q25) and lung cancer risk (1–3). Of six genes mapping in the locus that show extensive linkage disequilibrium, three (CHRNA5, CHRNA3 and CHRN4) encode nicotinic acetylcholine receptor (nAChR) subunits, whose biological function may underlie the association with smoking habit. Indeed, the 15q25 locus has also been associated with nicotine dependence due to cigarette smoking (3), which represents the main environmental risk factor for lung cancer (4). Herein, we explored the possibility that the nicotinic 15q25 locus is associated with lung cancer risk because of an individual’s genetic predisposition to smoking habit, which in turn causes an increased risk of lung cancer.

The nicotinic 15q25 locus is associated with nicotine dependence

Association between the nicotinic 15q25 locus and smoking habit has been documented and confirmed by a number of studies. A large case–control study targeting 16 nAChR subunit genes for nicotine dependence due to cigarette smoking showed significant associations with the CHRNA5–CHRNA3–CHRN4 gene cluster on chromosome 15q25, with the CHRNA3–CHRNA5 gene cluster on chromosome 8 and with the CHRNA4–CHRNA6 gene cluster on chromosome 2. The association with the α5-nicotinic receptor subunit (CHRNA5) gene exhibited a 2-fold increase in risk of developing nicotine dependence (5).

A variant of CHRNA3 (rs1051730) showed a statistically significant association with the number of cigarettes smoked per day in >10 000 smokers (3). A study involving three independent populations of European origin, with ~15 000 individuals, showed significant association of a common haplotype in the CHRNA3–CHRNA5 gene cluster with the ‘cigarettes per day’ quantitative trait (6). A study in 219 pedigrees of European descent (~2000 subjects) found a statistically significant association of a non-synonymous coding single-nucleotide polymorphism (SNP) of the CHRNA5 gene (rs16969968) with smoking habit (7).

The nicotinic 15q25 locus is associated with the smoking-related chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide (8). It is characterized by the presence of airflow limitation due to chronic bronchitis or emphysema. Airflow obstruction is generally progressive, may be accompanied by airway hyper-reactivity, and may be partially reversible.

By GWAS, SNPs mapping in the nAChR locus on chromosome 15q25 (CHRNA3–CHRNA5) showed highly statistically significant associations with COPD, with the strongest association observed for SNP rs8034191, which maps to the amidoglucoside phosphotransferase domain containing 1 (AGPHD1) gene located close to CHRNA5 (9).

Besides the genetic association with the 15q25 locus, COPD risk is associated with cigarette smoking, although quantitation of such risk is still uncertain, in part because of imprecise and variable definitions of COPD in the past. Estimations from large mortality studies involving up to ~1 million USA adults indicate hazards ratios (HRs) ranging from 3.1 to 12.3 for COPD associated with smoking (10–12). In an incidence study carried out in the prospective population-based Rotterdam Study cohort of 7983 subjects aged >55 years, an HR of 6.3 in current smokers for definite COPD was reported (13).

Once diagnosed, COPD increases the risk of developing lung cancer by ~2-fold (14,15), although the precise mechanism linking COPD to lung tumorigenesis is not fully understood. Chronic inflammation and immune dysfunction, characteristics of COPD, may constitute tumor promoter conditions underlying the increased risk of lung cancer in COPD patients.

The nicotinic 15q25 locus is associated with lung cancer in smokers but not in never-smokers

In all three GWAS, quantitative estimates of the association between SNPs mapping in the 15q25 locus and lung cancer risk consisted in odds ratios (ORs) ~1.3 (1–3).

Four studies in Caucasian and in Japanese subjects have analyzed the association of lung cancer risk with the nicotinic 15q25 locus in never-smokers; in three of these studies, the synonymous SNP rs1051730, mapping to CHRNA3, was genotyped (1,16,17), whereas one study genotyped the missense SNP rs16969968 mapping to CHRNA5 (18). Although the frequency of the rare alleles for both rs1051730 and rs16969968 was much lower in the Japanese population (0.013) than in Caucasians (~0.35), the haplotype defined by these SNPs was significantly associated with lung cancer risk also in Japanese subjects (16). Analysis of the HapMap genotyping data of the two SNPs in both the Caucasian population (CEU) and the Japanese population (JPT) (www.hapmap.org; accessed 31 July 2009) showed that they are in complete linkage disequilibrium, with $D^\prime = 1.0$ and $r^2 = 1.0$ (CEU; $n = 165$; JPT; $n = 86$; JLIN analysis (19)). Therefore, we considered rs1051730 and rs16969968 as a single marker and conducted a meta-analysis of the results of the four studies to obtain an overall estimate of the association between

Abbreviations: CI, confidence interval; COPD, chronic obstructive pulmonary disease; GWAS, genome-wide association studies; HR, hazards ratio; nAChR, nicotinic acetylcholine receptor; OR, odds ratio; SNP, single-nucleotide polymorphism.
nicotinic 15q25 locus and lung cancer risk in non-smokers. The four studies included a total of 1017 non-smoker lung cancer cases and 1872 controls.

Meta-analysis of the association of the rare allele carrier status (risk allele) with lung cancer risk revealed no statistically significant association in never-smokers, with OR = 1.20 (95% confidence interval (CI) 0.77–1.86; P = 0.4219) (Figure 1), and indicated significant heterogeneity among the four studies (P = 0.0168, Q-statistic; $\tau^2 = 0.14$), probably due to the different ethnicity of the series. Similarly, comparison of the homozygous individuals with the homozygous individuals carrying the common allele indicated no statistically significant association, with OR = 1.15 (95% CI 0.73–1.82), and significant heterogeneity among the studies (P = 0.0141, Q-statistic; $\tau^2 = 0.15$).

Analysis of the effects of rare allele homozygosity excluding the Japanese study (16), which reported only one homozygous subject, again revealed no significant association with lung cancer risk, with OR = 1.15 (95% CI 0.83–1.59); in this case, no significant heterogeneity among studies was detected (P = 0.7907, Q-statistic; $\tau^2 = 0$).

Note that a fifth study (2) also analyzed the association between the 15q25 locus and lung cancer risk in non-smokers but did not report the genotypes of cases and controls and thus could not be included in our meta-analysis. That study, which reported a significant association between the intronic SNP rs8034191 mapping in AGPHD1 and lung cancer risk in former and current smokers (OR ~1.3), also showed a weak association in never-smokers (352 cases and 2057 controls; OR = 1.25, 95% CI 1.05–1.49, P = 0.013, codominant model). However, a significant association in almost-never smokers (cumulative consumption of <20 pack-years), which would be expected when smoking status is defined by cumulative tobacco consumption, was not found (P = 0.107). These results are inexplicably contradictory regarding the association of the 15q25 locus with lung cancer risk, considering the close similarity of non-smokers and very light smokers. SNP rs8034191 genotyped in that study showed a tight linkage disequilibrium ($D^* = 1.0, r^2 = 0.89$) with either rs1051730 or rs16969968 in the CEU population (JLIN analysis).

Less than a half-cigarette per day confers a higher lung cancer risk than does the nicotinic 15q25 locus

Data from the accurate exposure-response study in British doctors (4) and from a more recent prospective cohort study (20) can be used to estimate the increase in HR associated with increasing smoking by one cigarette per day. Indeed, in the exposure categories, the HR value in each category divided by the mean number of cigarettes per day followed by calculation of the overall mean value predicts that an increase of one cigarette per day confers an increased mean HR of ~1.025 in the British doctor study and a mean HR of 1.912 and 1.1414 in men and women, respectively, in current smokers enrolled in the recent prospective study. In the latter cohort, HRs of 1.176 and 1.025 in the British doctor study and a mean HR of 1.912 and 1.1414 in men and women, respectively, in current smokers enrolled in the recent prospective study. In the latter cohort, HRs of 1.176 and 1.025 in men and women, respectively, were calculated for subjects who stopped smoking for 1–5 years (ex-smokers) (20).

Thus, the OR ~1.3 observed in the three GWAS for association between the chromosome 15q25 locus and lung cancer risk, i.e. an increase of 0.3 over that of controls (OR = 1.0), corresponds to an increased risk provided by ~0.3 cigarettes per day based on the data of Doll et al. (4) and to that provided by 0.16–0.35 cigarettes per day based on the data of the study by Freedman (20). These calculations underline the crucial importance of reporting accurate cigarette consumption in association studies addressing smoking-related diseases.

Of the three GWAS, lung cancer cases show a higher prevalence of smokers than controls in two series (1,2), whereas in the third series, smoking habits of lung cancer cases and controls were not reported (3). Thus, besides the statistical adjustments for smoking, none of the series has considered a possible bias due to underreporting smoking habit or cigarette consumption, especially if the frequencies of such eventual underreporting differed in cases and controls. Indeed, since the nicotinic 15q25 locus is associated with smoking habit, a modest underreporting of smoking in cases might explain the association of this locus with lung cancer risk.

<table>
<thead>
<tr>
<th>Study</th>
<th>OR (95% CI)</th>
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<tbody>
<tr>
<td>Shiraiishi et al., 2009</td>
<td>2.56 [1.26, 5.21]</td>
</tr>
<tr>
<td>Amos et al., 2008</td>
<td>1.05 [0.71, 1.55]</td>
</tr>
<tr>
<td>Falvella et al., 2009</td>
<td>1.32 [0.69, 2.54]</td>
</tr>
<tr>
<td>Spitz et al., 2008</td>
<td>0.82 [0.65, 1.03]</td>
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**Fig. 1.** Forest plot representing lung cancer risk and nicotinic 15q25 locus risk-allele carrier status in never-smokers. Weighted average of table ORs and 95% CI for lung cancer in non-smokers compares carriers of the rare allele (heterozygous (G/A) and homozygous (A/A) genotypes of the matching rs1051730 or rs16969968) to the homozygous (G/G) common genotype. Meta-analysis was carried out using the random-effects (RE) model and the Meta-Analysis Package ‘metafor’ for R, written by Wolfgang Viechtbauer (wvb@wvbauer.com).

**Fig. 2.** Schematic representation of our hypothesis on the link between the 15q25 locus and lung cancer. Several studies have identified the association of the 15q25 locus with nicotine dependence derived by cigarette consumption. In turn, smoking habit is well known to be the major risk factor of lung cancer and of COPD. The latter disease, whose risk is also associated to the 15q25 locus, can be crucial in the development of lung cancer through promoting inflammatory processes. Thus, a direct role for the 15q25 locus in the pathogenesis of lung cancer remains to be demonstrated.

Unfortunately, a tendency to decrease the self-reported cigarette use is common in smokers, as demonstrated by a meta-analysis of the studies comparing the amount of self-reported cigarette use and the cotinine levels (21). Indeed, self-reports underestimated true smoking prevalence in most of the studies reviewed, with large variations among them (21). Such bias may be particularly relevant in females (22,23), in certain ethnic populations (24) and in certain cultural or socio-economic groups (25). Thus, unpredictable variations in underreporting may be particularly relevant in consortium studies, such as the GWAS, where heterogeneous series are mixed.
together to obtain large numbers of subjects in order to improve the statistical power of the studies.

No evidence that nicotine is ‘the estrogen’ for lung cancer cells

The association between the nicotinic 15q25 locus and lung cancer risk appears to be supported by reports on stimulation of cancer cell growth by nicotine, an exogenous ligand of nAChR. Indeed, it has been proposed that nicotine is for lung cancer what estrogen is for breast cancer, i.e. a driver of cancer growth and development of lung cancer (26).

However, evidence for stimulation of cancer cell growth by nicotine derives from sparse studies in vitro or in xenografts of non-lung cancer cells (27,28) results that contrast with a study showing no effect of in vivo nicotine treatment on growth of a grafted human small-cell lung cancer cell line in nude mice (29). In addition, the ability of nicotine to bind to several nAChR subunits makes it difficult to attribute any effect on proliferation of lung cancer cells in vitro or in vivo specifically to the 15q25 locus since any of several nicotinic loci might mediate such effects. Indeed, studies on alpha7-nAChR antagonists in A549 cells point to a possible involvement of the CHRNA7 rather than the CHRNA5 locus (30), whereas another study points to the stimulation of beta (2)-adrenoceptors by nicotine (27). Finally, there is no evidence of nicotine carcinogenicity in vivo; indeed, a lifelong (2 years) treatment of rats with nicotine by inhalation at a concentration producing twice the plasma concentration found in heavy smokers did not increase the incidence of any tumor type, including lung tumors, over control rates (31). No lung tumor promotion studies on nicotine are available.

Conclusions

Several studies showed that the 15q25 locus is statistically associated with smoking habit as well as with smoking-related COPD and lung cancer (Figure 2). The relatively low quantitative estimates of the association of the 15q25 locus and lung cancer (OR ~1.3), corresponding to a risk provided by less than a half-cigarette per day, may suggest an indirect association of the 15q25 locus with lung cancer risk (Figure 2). Also, we cannot exclude that a possible bias due to the underreporting of smoking habit in cases plays a role in the putative association of the 15q25 locus with lung cancer risk.

Our meta-analysis of four studies in never-smokers, comprising a total of >1000 cases and >1800 controls, revealed no statistically significant association between the chromosome 15q25 locus and lung cancer risk, consistent with the hypothesis that the association between the 15q25 locus and lung cancer is due mainly to the association of the same locus with smoking habit, which in turn modulates lung cancer risk (Figure 2). Overall, there is no evidence to date that nicotine is carcinogenic or co-carcinogenic or that it can act as a tumor promoter in vivo to support the plausibility of a link between the nicotinic 15q25 locus and lung cancer pathogenesis. Proof of a direct effect of the 15q25 locus on lung cancer risk awaits demonstration by additional studies and by biological assays of variations in candidate genes.

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References


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