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Tobacco Smoking, *NBS1* Polymorphisms, and Survival in Lung and Upper Aerodigestive Tract Cancers with Semi-Bayes Adjustment for Hazard-ratio Variation

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Abstract

Purpose—Although single nucleotide polymorphisms (SNPs) of *NBS1* have been associated with susceptibility to lung and upper aerodigestive tract (UADT) cancers, their relations to cancer survival and measures of effect are largely unknown.

Methods—Using follow-up data from 611 lung-cancer cases and 601 UADT-cancer cases from a population-based case-control study in Los Angeles, we prospectively evaluated associations of tobacco smoking and 5 *NBS1* SNPs with all-cause mortality. Mortality data were obtained from the Social Security Death Index. We used Cox regression to estimate adjusted hazard ratios (HR) for main effects and ratios of hazard ratios (RHR) derived from product terms to assess hazard-ratio variations by each SNP. Bayesian methods were used to account for multiple comparisons.

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Results—We observed 406 (66%) deaths in lung-cancer cases and 247 (41%) deaths in UADT-cancer cases with median survival of 1.43 and 1.72 years, respectively. Ever tobacco smoking was positively associated with mortality for both cancers. We observed an upward dose-response association between smoking pack-years and mortality in UADT squamous cell carcinoma. The adjusted HR relating smoking to mortality in non-small cell lung cancer (NSCLC) was greater for cases with the GG genotype of *NBS1* rs1061302 than for cases with AA/AG genotypes (semi-Bayes adjusted RHR = 1.97; 95% limits = 1.14, 3.41).

Conclusions—A history of tobacco smoking at cancer diagnosis was associated with mortality among patients with lung cancer or UADT squamous cell carcinoma. The HR relating smoking to mortality appeared to vary with the *NBS1* rs1061302 genotype among NSCLC cases.

Keywords

Survival; *NBS1*; Lung cancer; Upper aerodigestive tract (UADT) cancers; Tobacco; Bayesian methods

Introduction

Cancer is the second leading cause of death both worldwide and in the United States [1–3]. Although cancer diagnosis and treatment have largely improved over the past 30 years in the U.S., the five-year survival rate of lung cancer is only 16% and has improved little over the same period [2]. While a similar pattern of cancer survival also applies to esophageal cancer in the U.S. (5-year survival rate of 19%) [2], cancers of other upper aerodigestive tract (UADT) sites have shown improved treatment outcomes and survival rates in recent decades [4, 5]. Furthermore, tobacco smoking is a well-known risk factor for lung and UADT cancers [6] and is inversely associated with lung cancer survival [7, 8]. However, few studies with relatively large sample sizes have examined pre-diagnostic tobacco smoking and alcohol drinking in relation to UADT cancer survival.

The associations between genetic variations such as single nucleotide polymorphisms (SNPs) on *NBS1* and cancer susceptibility have been reported in several studies of lung [9–11] and UADT cancers [10, 12]. In lung and UADT tumor tissues, researchers have found elevated expression of the *NBS1* product, nibrin, which plays an important role in DNA damage repair by forming the hMRE11–hRAD50–NBS1 (MRN) nuclease complex [11–14]. The association between *NBS1* SNPs and cancer susceptibility is postulated through the functional change of the MRN nuclease complex, resulting in diminished DNA repair ability [11]. While studies suggest that *NBS1* polymorphisms may be involved in carcinogenesis at several cancer sites [15, 16], there is limited information on their relationship to lung or UADT cancer survival. Moreover, it is not clear whether such relationships differ across environmental risk factors, such as tobacco smoking or alcohol drinking.

Our study aims to explore potential prognostic roles of *NBS1* SNPs for lung and UADT cancers, given their high association with cancer susceptibility. We hypothesized that five *NBS1* polymorphisms might be positively associated with overall mortality among patients with lung or UADT cancer. These polymorphisms might also modify the associations between tobacco smoking or alcohol drinking and mortality, leading to variation of the hazard ratios between exposure strata. Our study population consists of cancer patients recruited in a population-based case-control study in Los Angeles County.

Materials and Methods

Study design and participants

A population-based case-control study of lung and UADT cancers was conducted in Los Angeles County from 1999 to 2004 and approved by the Institutional Review Board of the University of California at Los Angeles and the University of Southern California; all participants provided signed informed consent. Detailed information has been described elsewhere [10, 17, 18]. Briefly, newly diagnosed cancer patients were recruited through the rapid ascertainment system of the USC Cancer Surveillance Program for Los Angeles County. Participants met the following inclusion criteria: (i) a resident of Los Angeles County at the time of diagnosis; (ii) diagnosed at age 18 to 65 years old during the study period; (iii) spoke either English or Spanish or were accompanied by translators during interview. Among eligible patients, the recruitment rates were 39% (611 of 1,556) for lung and 46% (601 of 1,301) for UADT cancer patients. Cancer diagnosis was verified by histological reports (in over 95% of patients), magnetic resonance imaging, computed tomography scan, or other diagnostic methods. There were 611 lung cancer patients and 601 UADT cancer patients. Among the latter there were 527 patients with squamous cell carcinoma, with the majority located at the oropharynx (n=338), larynx (n=90), nasopharynx (n=48), and esophagus (n=34), and 74 patients with adenocarcinomas, all confined to the esophagus.

Each participant was interviewed in-person with a standardized questionnaire. The questionnaire included demographic factors, detailed information of tobacco smoking and alcohol consumption (one year before diagnosis of cases), medical history, history of occupational and environmental exposures, and family history of cancer. We calculated smoking pack-years and alcohol drink-years which represent cumulative exposure history [17]. Participants provided buccal cell samples for DNA extraction and SNP genotyping at rates of 89%, 68%, 88% and 90% for lung, oro-/nasopharyngeal, laryngeal and esophageal cancer patients, respectively. Tumor tissue specimens were available from 190 lung cancer patients who underwent surgery.

Genotyping

SNPs on *NBS1* were selected based on the following criteria: (i) genomic context suggesting a possibility of functional change, such as SNPs located in an exon or 3' untranslated region (3'-UTR); (ii) SNPs previously reported with minor allele frequency of at least 5% in the SNP database of the National Center for Biotechnology Information [19]; (iii) SNPs associated with disease outcomes including smoking-related cancers. Five SNPs (rs709816, rs1061302, rs1063053, rs1063054 and rs2735383) were selected and genotyped by TaqMan and SNPlex platforms (Applied Biosystems, Foster City, CA). All five SNPs were genotyped by the SNPlex platform and four SNPs (rs1061302, rs1063053, rs1063054 and rs2735383) were validated by TaqMan. An average Cohen's kappa coefficient of 0.919 suggested high agreement of genotyping results between two platforms [10]. For SNPs assayed by both methods, we used results from TaqMan because the TaqMan assays have higher and more reliable conversion rates compared to the SNPlex assays during low-throughput genotyping [20]. For quality control, 5% of random samples were re-analyzed to evaluate reproducibility with a concordance above 99%. Preliminary analysis indicated that three SNPs (rs1063054, rs1063053 and rs2735383) were in high linkage disequilibrium (LD) ($r^2 > 0.99$) [10]. We selected rs1063054 for analysis to be consistent with our previous research [10]. The *NBS1* rs1063054 also had the highest call rate. Finally, three *NBS1* SNPs--rs709816 (exon 10, D399D), rs1061302 (exon 13, P672P), and rs1063054 (3'UTR)--were analyzed on survival of lung and UADT cancer patients.

Follow-Up Survival Data

We used the Social Security Death Index (SSDI) to acquire participants' death information. The SSDI is generated from the public Death Master File of the U.S. Social Security Administration and provides death records of qualified social security recipients. The SSDI is accessible through several commercial providers; we used the Social Security Death Index Interactive Search. A death record contains information of a decedent's first and last name, social security number (SSN), last benefit, birth date, death date, last residence, and state issued. We first used the nine-digit SSN with information of name and birth date to retrieve the participant's record. If a record was unavailable, we used the first three or last four digits of the participant's SSN, birth date, and first/last name. These records were last retrieved on October 31, 2011. We noticed a variable lag between cancer diagnosis and enrollment to the original case-control study. The median (interquartile range, IQR) lag time in months was 3.7 (2.9–5.1) and 4.1 (3.1–5.6) for patients with lung and UADT cancer, respectively. We defined two follow-up durations (survival times): one started from patient enrollment date, and the other started from the date of diagnosis of cancers under study, until death date or October 31, 2011. Since very similar results were observed using both survival time periods, we presented results using the follow-up period starting from patient enrollment date. Participants whose death records could not be acquired from the SSDI (205 lung cancer patients and 354 UADT cancer patients) were treated as alive (right-censored) on October 31, 2011.

Statistical Methods

Kaplan-Meier survival analysis and log-rank tests were used for the initial survival analysis. Time to death in days was measured from the date of patient enrollment to the date of death reported in the SSDI. We used Cox proportional-hazards models fitted by partial likelihood to estimate crude and adjusted hazard ratio (HR) and 95% confidence intervals (95% CI). The proportionality assumption of each model was assessed by including product terms of regressors and log-transformed time (measured in days), which led us to stratify on sex ($P = 0.003$ for product with log-transformed time).

The models included age, sex, ethnicity (non-Hispanic White, Hispanic, African American, Asian/Pacific Islander, and others), education (less than 12, 13 to 16, and more than 16 years), tumor histologic grade, tobacco smoking (in pack-years), and alcohol consumption (in drink-years). The tumor histologic grade was originally categorized into grade 1 (well-differentiated) to grade 4 (undifferentiated) or grade X (undetermined grade) according to the American Joint Committee on Cancer (AJCC) recommendations [21]. We then re-categorized the histologic grade into high grade (grade 3 to 4), non-high grade (grade 1 to 2), and undetermined (grade X) for adjustment. The associations between variant alleles of three SNPs and survival were examined in additive genetic models, followed by dominant (at least one variant allele vs. wild type allele) or recessive (two variant alleles vs. at least one wild type allele) models. We also calculated adjusted HR and 95% CI by histologic subtypes and cancer sites. Since squamous cell carcinoma and adenocarcinomas at the UADT sites are different diseases in terms of causes and risk factors [22–24], we presented results for all UADT cancer patients and histological subtypes, with an emphasis on UADT squamous cell carcinoma.

Stratified analyses were performed across “ever/never” tobacco smoking or alcohol drinking, assuming dominant or recessive genetic models. To examine variation in the hazard ratios, we added product terms (SNP*smoking, SNP*drinking, and smoking*drinking) to the models and reported ratios of hazard ratios ($RHR = e^b$, where b = estimated coefficient for the product term).

To address multiple-comparison issues, we computed the Bayesian false-discovery probability (BFDP) for main-effect associations and stratified analyses [25], and used semi-Bayes adjustment for product-term coefficients [26–28]. The BFDP is based on the false-positive report probability [29] and provides the probability of a false discovery given the estimated association, prior probability for alternative hypothesis, and a *priori* ratio of costs between a false non-discovery to a false discovery. We set the ratio of costs (threshold for noteworthiness) to 0.75 as suggested in the original papers [25, 29]. It is also a commonly accepted threshold for cancer molecular epidemiology studies with medium sample size [30–32]. We calculated BFDP in two scenarios where prior probabilities for a non-null association were 0.20 and 0.10, respectively, with prior variance 0.05.

In semi-Bayes adjustment for product-term coefficients [26–28], we assigned independent normal priors with mean zero, variance 0.5 (95% prior limits of $\frac{1}{4}$ and 4 after exponentiation) to three continuous product terms of SNP*smoking pack-years, SNP*alcohol drink-years, and smoking pack-years*alcohol drink-years. The pack-years and drink-years were rescaled to units of 40 pack-years and 80 drink-years, respectively [26, 33]. The same priors were also used for three product terms of SNP*smoking, SNP*drinking, and smoking*drinking with smoking and drinking coded as binary “ever/never” variables. Posterior estimates were calculated using data augmentation [26, 34]. Analyses were performed by the SAS version 9.1 (SAS Institute, Cary, NC) and Stata/IC 10.1 (Stata Corporation, College Station, Texas).

Results

Tables 1(a) and 1(b) show the baseline characteristics of lung and UADT cancer patients, respectively. During follow-up, 406 (66%) deaths among lung cancer patients and 247 (41%) deaths among UADT cancer patients were identified. The median follow-up was 26.3 (IQR: 9.5–118.5) and 102.2 (22.6–121.7) months for lung and UADT cancer patients. Shown in Table 1(a), female lung cancer patients had a longer median follow-up than male (41.3 [12.0–123.1] vs. 18.5 [7.0–113.8] months) with an adjusted HR of 0.73 (95% CI: 0.59–0.91). There was no consistent relation of ethnicity to survival among lung-cancer patients, although Hispanic patients with small cell lung cancer had worse survival than Whites (adjusted HR = 2.55 [1.04–6.27], Supplementary Table S1). Lung cancer patients with high (36%) or undetermined (36%) grade tumors showed increased hazards of death consistently across all histologic subtypes compared to those with low grade tumors. Among lung cancer patients, 82% were ever smokers and 33% smoked more than 40 pack-years. Tobacco smoking was positively associated with mortality among lung-cancer patients. The Kaplan-Meier plot in Fig. 1(a) shows that both survival curves for lung cancer patients who had ever smoked differed from that of never smokers since the second year of follow-up ($P = 0.11$ and 0.002).

Table 1(b) shows that among UADT cancer patients, 74 patients (12%) had adenocarcinoma of the esophagus with a median follow-up of 41.9 (IQR: 12.4–112.7) months. Among 527 squamous cell carcinoma patients, the median follow-up was 103.5 (IQR: 26.0–122.4) months. Tobacco smoking was strongly related to the overall mortality in UADT squamous cell carcinoma with a clear upward dose-response pattern. We did not observe such association in esophageal adenocarcinoma. A monotonic trend was observed between smoking pack-years and overall survival of squamous cell carcinoma at the UADT sites (Table 1(b), P -value for trend < 0.001). The Kaplan-Meier plot in Fig. 1(b) showed three clearly separate survival curves: UADT squamous cell carcinoma patients who smoked at least 40 pack-years prior to diagnosis had the lowest survival, followed by patients who smoked less than 40 pack-years. As shown in Supplementary Table S2, squamous cell carcinoma patients had higher risk of all-cause mortality in current (adjusted HR = 2.50

[95% CI: 1.42–4.39]) and possibly in former smokers (adjusted HR = 1.37 [0.88–2.13]), compared with never smokers. Little or no association was observed between age, sex, education, or alcohol consumption and survival. However, African Americans with squamous cell carcinoma experienced a lower survival than did non-Hispanic whites (adjusted HR = 1.73 [1.17–2.54]).

Fig. 2(a) shows associations between *NBS1* SNPs and all-cause mortality. Large cell lung carcinoma patients who carry variant C allele of *NBS1* rs709816 showed shorter survival (adjusted HR = 1.63 [1.04–2.55]). Small cell lung carcinoma patients with variant G allele of *NBS1* rs1061302 showed higher death hazards (adjusted HR = 1.96 [1.22–3.15]). Supplementary Table S3(a) provides the Bayesian false-discovery probability. Given a prior probability of 0.20, both associations described had BFDP < 0.75, indicating noteworthy observations.

Fig. 2(b), 2(c), and Supplementary Table S4 give results of stratified analysis on tobacco smoking and alcohol drinking. Among lung cancer patients who ever smoked, carrying a GG genotype of *NBS1* rs1061302 was associated with lower survival in non-small cell lung cancer (NSCLC, adjusted HR = 1.41 [0.97–2.04]), squamous cell carcinoma (adjusted HR = 2.51 [1.00–6.26]), and small cell carcinoma (adjusted HR = 2.54 [1.00–6.44]). Among non-drinking patients with UADT squamous cell carcinoma, we observed inverse associations between *NBS1* rs1063054 genotype and overall death (AC/CC vs. AA, adjusted HR = 0.32 [0.13–0.79]). As shown in Supplementary Table S3(b), the BFDP values for these analyses indicated noteworthy findings except possibly for small cell lung carcinoma (BFDP = 0.76) and squamous cell lung carcinoma (BFDP = 0.75).

We explored variations of the hazard ratios relating tobacco smoking to survival among NSCLC patients by *NBS1* rs1061302, and relating alcohol drinking to survival among patients with UADT squamous cell carcinoma by *NBS1* rs1063054. Results are shown in Table 2. Compared to NSCLC patients who never smoked and carry AA or AG genotype of *NBS1* rs1061302, never smokers who carry GG genotype appeared to have had improved overall survival (adjusted HR = 0.47 [0.21–1.08]) while ever-smokers who carry AA or AG genotype had similar survival (adjusted HR = 1.07 [0.74–1.57]). The adjusted ratio of hazard ratios (RHR) relating smoking and mortality among NSCLC patients across *NBS1* rs1061302 genotypes was 3.00 (1.22–7.38). In patients with squamous cell carcinoma at the UADT sites, hazard ratios relating alcohol drinking and survival differed across strata of *NBS1* rs1063054 (adjusted RHR = 2.90 [1.14–7.33]). Both hazard-ratio variations were noteworthy only under the setting of prior probability 0.20 for non-null association in the BFDP adjustment.

The Kaplan-Meier plots for survival, stratified by SNP genotypes and smoking (NSCLC) or drinking (UADT squamous cell carcinoma), are shown in Fig. 3(a) and 3(b). Among NSCLC patients who ever smoked, the *NBS1* rs1061302 GG carriers had lower survival than AA/AG carriers ($P = 0.048$). For non-smokers, two NSCLC survival curves across *NBS1* rs1061302 genotypes were not separate ($P = 0.18$, data not shown). Among NSCLC patients with GG genotype of *NBS1* rs1061302, smokers had lower survival than non-smokers ($P = 0.011$). For UADT squamous cell carcinoma patients who never drank, we observed differences in survival curves across *NBS1* 1063054 genotypes ($P = 0.001$) and between drinkers and non-drinkers with AC or CC genotype of *NBS1* rs1063054 ($P = 0.031$).

Table 3 shows the semi-Bayes results. Among NSCLC patients, the exponentiated estimate of the continuous rs1061302*smoking coefficient was 1.90 (95% posterior limits: 1.11, 3.26). The model contained two additional continuous products of rs1061302*drink-year

and pack-year*drink-year for confounding control. Smoking pack-year and alcohol drink-year were rescaled to 40 pack-years and 80 drink-years, centered at their mean values. The binary rs1061302*smoking product had an exponentiated estimate of 2.26 (95% posterior limits: 1.06, 4.81) after semi-Bayes adjustment.

Discussion

Associations between three *NBS1* SNPs and all-cause mortality among lung and UADT cancer patients were examined in this study. Tobacco smoking (either pack-years or current/former smokers) was found to be associated with all-cause mortality for patients with UADT squamous cell carcinoma. Although our study did not observe clear associations between *NBS1* polymorphisms and survival in lung or UADT cancer patients, stratified analyses by cancer subtypes and cancer risk factors suggested (1) Borderline yet consistent associations between *NBS1* rs1061302 GG genotype and lower survival among smokers with NSCLC, squamous cell lung carcinoma, or small cell lung carcinoma; (2) Improved survival among non-drinking UADT squamous cell carcinoma patients who carry the AC or CC genotype of *NBS1* rs1063054. We applied two Bayesian approaches to account for multiple comparisons. After semi-Bayes shrinkage, it still appeared that *NBS1* rs1061302 may modify hazard-ratio relating tobacco smoking to survival among NSCLC patients.

Tobacco smoking is known to have immunosuppressive effects on local tissues via induction of pro-inflammatory cytokines and chemokines and suppression of antigen recognition and response [35, 36]. The immunosuppression results in increased susceptibility to infections targeting the lungs, a common cause of death among lung cancer patients [37]. Thus, as expected, we observed an association between tobacco smoking and shorter survival among lung cancer patients [7, 38]. We also noticed higher risk of overall mortality in patients with squamous cell carcinoma at the UADT sites who were current (adjusted HR = 2.50 [1.42–4.39]), and possibly former smokers (adjusted HR = 1.37 [0.88–2.13]), than never smokers. Several studies have observed that head and neck cancer patients who were non-smokers or who had quit smoking before cancer diagnosis, had improved disease prognosis and survival [39–42]. Additionally, we observed a positive monotonic association between smoking pack-years prior to cancer diagnosis and all-cause mortality in UADT squamous cell carcinoma; heavy smokers who smoked more than 40 pack-years had the lowest survival. Both Kaplan-Meier survival curves (Fig. 1(b)) and multivariable proportional-hazards models (Table 1(b)) conformed to a simple dose-response relationship.

The *NBS1* gene codes 754 amino acids (a.a.) and is involved in DNA double-strand break (DSB) repair [43]. Its gene-product, nibrin, has three known functional regions: N-terminus (a.a. position 1–196), central region (a.a. position 278–343) and C-terminus (a.a. position 665–693) [43]. The C-terminus is the binding site for the MRN complex, which participates in DSB repair and activates a damage checkpoint kinase ATM [44–46]. The *NBS1* gene has more than 600 polymorphisms [11]. Associations between *NBS1* polymorphisms with cancer susceptibility have been widely studied, in particular *NBS1* rs1805794 (E184Q) -- a 3'-UTR polymorphism in a complete LD ($r^2 = 1$) with rs1061302 (exon 13, P672P) in our study. The susceptibility of lung [9–11], head and neck [10, 12], prostate [47], breast [48–50], bladder [10, 51, 52], leukemia [53], and liver cancers [10, 54] has been shown to be associated with *NBS1* rs1805794 or rs1061302. Our study further elucidates the relationship between *NBS1* SNPs and mortality. One recent pilot study reported an improved progression-free survival with minor C allele of *NBS1* rs1805794 (SNP in a complete LD with *NBS1* rs1061302 in our study) among 147 Chinese patients with inoperable NSCLC who received platinum-based chemotherapy [55]. Our study suggests an increased hazard of overall death among NSCLC patients carrying the minor G allele of rs1061302. Results from these two studies however require cautious interpretation since two study groups have

distinct inclusion/exclusion criteria and ethnic groups. While the pilot study focused specifically on Chinese patients with inoperable NSCLC, our study population consisted of several ethnic groups with unreported cancer stage.

Several studies also reported associations between *NBS1* overexpression and head and neck cancer progression, including larger lymph node involvement, more advanced cancer stage, higher metastasis rate, and poorer cancer prognosis [12–14, 56]. Our findings, along with these emerging clinical observations, further raise awareness of a potential prognostic role for *NBS1* polymorphisms in cancer progression and outcome. Since the *NBS1* rs1061302 is located at the binding domain of MRN complex, it might hinder MRN binding ability and DSB repair function and thus be related to carcinogenesis and cancer progression [10]. Functional studies are needed to explore associations between *NBS1* SNPs and *NBS1* protein expression.

We observed variation in hazard-ratio relating tobacco smoking to mortality among NSCLC patients across *NBS1* rs1061302 genotypes, shown in Table 3 and Fig. 3(a). The Kaplan-Meier plot in Fig. 3(a) indicates the variation – survival curves by smoking status highly overlapped in NSCLC patients with AA/AG genotype (curve (1) and (2)) and were separate among patients with GG genotype (curve (3) vs. (4), $P = 0.011$). Similarly, the proportional-hazards model also suggested hazard-ratio variation: the adjusted HR relating *NBS1* rs1061302 GG genotype to all-cause mortality in NSCLC patients was 1.41 (0.97–2.04) among smokers and 0.55 (0.22–1.39) among non-smokers. Unfortunately, the latter was imprecise. These findings need further replication. The hazard-ratio variation was still clear (semi-Bayes adjusted RHR = 1.90; 95% limits = 1.11, 3.26, Table 3) after semi-Bayes shrinkage with independent normal priors (mean 0, variance 0.5) for three product terms. The prior assumes that our bets for the SNP*smoking RHR were centered on the null and includes a 16-fold range (adjusted RHR were $\frac{1}{4}$ to 4) with 95 percent certainty [26, 57]. After shrinkage and adjustment for two potential confounders of SNP*drinking and smoking*drinking, we were more confident that there exists variation in the adjusted HR relating smoking to mortality among NSCLC patients by the *NBS1* rs1061302. Even when we assigned strong, conservative product-term coefficient priors with mean zero, variance 0.125 (95% prior limits: 0.5, 2.0 after exponentiation), the hazard-ratio variation remained apparent (semi-Bayes adjusted RHR: 1.55; 95% limits: 0.99, 2.44; data not shown).

Consistent with previous studies [7, 8, 58], we found higher survival among never and former smokers than current smokers who developed lung cancer. Additionally, it appeared that smoking NSCLC patients with *NBS1* rs1061302 GG genotype showed the highest hazard of mortality, compared to non-smoking patients with AA/AG genotype. These findings underscore the importance of reducing smoking for improved cancer prognosis, especially for high risk groups with genetic variants such as *NBS1* rs1061302 GG genotype carriers.

Among study limitations, cancer stage and treatment information were unavailable. These two factors influence cancer outcomes and survival and might confound associations between SNP and survival through the selection process. We included tumor histologic grade in analyses to improve accuracy because Sun et al (2006) reported that tumor grade was associated with NSCLC mortality, controlling for clinical stage and treatment [59]. We also conducted sensitivity analyses, assuming that 190 (31%) patients who provided lung tumor tissue had early-stage lung cancer and the other 421 patients without tissue specimens had late-stage lung cancer. Using the availability of tissue specimen as a proxy for stage, sensitivity analyses yielded similar associations.

Lag time between death date and event reporting date to the SSDI presented an additional challenge to our analysis. Patients who died during study follow-up may not have been reported to the SSDI. This measurement error would likely be non-differential between comparison groups and either had limited influence on observed associations or biased the associations toward the null. For the purpose of another study, we updated death records in our dataset from the SSDI to July 15, 2012. We identified one more death record for a UADT patient and re-ran the analyses. Results from the updated dataset were very similar to those from the original analyses. For example, adjusted HR relating *NBS1* rs1063054 to survival among UADT cancer patients changed from 0.75 (0.56–1.00) to 0.76 (0.56–1.02). Among non-drinking patients with UADT squamous cell carcinoma, the adjusted HR only changed slightly from 0.32 (95% CI: 0.13–0.79) to 0.31 (95% CI: 0.12–0.76).

Selection bias may also be an issue due to non-participation of eligible cancer cases. We captured patients capable of participating in an interview, which might have included an excess proportion of patients with an early cancer stage. Some eligible patients did not participate in our study due to death before interview, incorrect contact information, being too sick to be interviewed, and refusal [17]. Among UADT cancer patients, the collection rate of buccal cells was relatively low (68%) in oro-/nasopharyngeal cancer patients [10]. This may also have contributed to selection bias. The direction of biased associations, however, is difficult to determine as the distribution of *NBS1* polymorphisms and risk factors for mortality is unknown among non-participants. The extent of bias would be limited for UADT cancer patients since less than 15% of eligible patients were excluded due to death or sickness before enrollment [17].

In summary, we observed an upward dose-response association between smoking pack-years and mortality of patients with UADT squamous cell carcinoma and associations between tobacco smoking and mortality in lung cancer patients. Our study further suggested several associations between *NBS1* polymorphisms and all-cause mortality in smoking lung cancer patients and non-drinking patients with squamous cell carcinoma at the UADT sites. Our results indicated that *NBS1* rs1061302 may modify the hazard-ratio relating tobacco smoking (both ever/never status and pack-years) to mortality of NSCLC patients. These findings underscore the importance of smoking cessation for improving survival of cancer patients. Further studies are needed to explore *NBS1* polymorphisms associations with *NBS1* protein expression, as well as with cancer outcomes, among better-defined subgroups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Kaplan-Meier analysis and log-rank P -values for tobacco smoking pack-years (PYs) in patients with (a) lung cancer; (b) UADT squamous cell carcinoma

Fig. 2.

(a) Selected associations of *NBS1* SNPs with mortality. (b) Associations of *NBS1* rs1061302 (recessive model) with mortality of lung cancer patients by smoking status. (c) Associations of *NBS1* rs1063054 (dominant model) and mortality of UADT cancer patients by drinking status

aHR adjusted hazard ratio, *CI* confidence interval, *NSCLC* non-small cell lung cancer, *UADT* upper aerodigestive tract

^aModels included age, gender, ethnicity, education, drink-year and pack-year. Among non-drinkers, the drink-year was zero.

^bModel stratified on sex

^cAssociations among non-smokers were not presented due to small sample sizes

^dValues of BFPD were less than 0.75 given a prior probability of 0.20, shown in Supplementary Table S4, indicating noteworthy observations

Fig. 3. Kaplan-Meier analysis and log-rank P -values for (a) *NBS1* rs1061302 by tobacco smoking in NSCLC patients; (b) *NBS1* rs1063054 by alcohol drinking in patients with UADT squamous cell carcinoma

Table 1(a)

Distribution and adjusted hazard ratios (aHR) of lung cancer patients by selected covariates

	Overall lung cancer (n = 611)				NSCLC (n = 507)				Small cell lung cancer (n = 75)			
	Death/All	Median follow-up ^a	aHR (95 % CI)	Death/All	Median follow-up ^a	aHR (95 % CI)	Death/All	Median follow-up ^a	aHR (95 % CI)	Death/All	Median follow-up ^a	aHR (95 % CI)
All	406/611	26.3		324/507	33.5		60/75	12.9				
Sex												
Male	215/303	18.5	1	180/259	23.9	1	25/32	10.0	1			
Female	191/308	41.3	0.73 (0.59–0.91)	144/248	57.6	0.68 (0.53–0.87)	35/43	17.1	1.04 (0.52–2.05)			
Tumor grade												
Low - intermediate	90/169	83.4	1	88/167	85.3	1	1/1	45.4	1			
High	154/222	18.7	1.60 (1.23, 2.09)	107/163	25.6	1.38 (1.04, 1.84)	30/38	10.2	3.91 (0.45, 34.40)			
Undetermined	161/219	20.4	1.87 (1.43, 2.44)	128/176	20.5	1.89 (1.43, 2.49)	29/36	15.7	2.61 (0.30, 22.51)			
Missing	1	4.4		1	4.4							
Tobacco smoking												
Never	61/110	64.1	1	55/100	64.1	1	2/4	107.8	1			
<40 (pack-years)	202/299	25.5	1.53 (1.11–2.12)	159/246	32.4	1.43 (1.00, 2.01)	32/39	12.9	6.08 (1.09, 34.04)			
40 or more (pack-years)	143/202	18.0	1.53 (1.06–2.22)	110/161	22.3	1.50 (1.00, 2.24)	26/32	9.5	5.72 (0.90, 36.25)			
Test for trend			P = 0.37			P = 0.42			P = 0.94			

aHR adjusted hazard ratio, CI confidence interval

^aMedian follow-up in months.

Table 1(b)
Distribution and adjusted hazard ratios (aHR) of UADT cancer patients by selected covariates

	UADT cancer (n = 601)			UADT, Squamous cell (n = 527)			Esophageal adenocarcinoma (n = 74)		
	Death/All	Median follow-up ^a	aHR (95 % CI)	Death/All	Median follow-up ^a	aHR (95 % CI)	Death/All	Median follow-up ^a	aHR (95 % CI)
All	247/601	102.2		205/527	103.5		42/74	41.9	
Sex									
Male	190/454	101.4	1	154/391	103.1	1	36/63	43.0	1
Female	57/147	104.0	1.02 (0.74–1.41)	51/136	106.0	1.07 (0.76–1.51)	6/11	40.7	0.88 (0.26–3.05)
Tumor grade									
Low - intermediate	172/399	101.0	1	151/369	101.8	1	21/40	79.1	1
High	41/121	107.4	0.87 (0.61–1.23)	29/100	108.8	0.76 (0.50–1.16)	12/21	40.7	0.89 (0.41–1.95)
Undetermined	34/81	101.8	0.99 (0.68–1.43)	25/68	104.5	0.84 (0.55–1.30)	3/13	28.9	1.67 (0.70–3.99)
Tobacco smoking									
Never	52/182	111.6	1	41/164	113.3	1	11/18	57.8	1
<40 (pack-years)	122/291	101.2	1.59 (1.10–2.29)	100/252	102.1	1.71 (1.13–2.59)	22/39	31.2	0.94 (0.36–2.45)
40 or more (pack-years)	73/128	76.7	2.17 (1.41–3.36)	64/111	78.9	2.43 (1.49–3.96)	9/17	20.5	1.10 (0.33–3.73)
Test for trend			P = 0.001			P < 0.001			P = 0.659

aHR adjusted hazard ratio, CI confidence interval

^aMedian follow-up in months.

Table 2
Variation in hazard ratios relating cancer risk factors to cancer survival by *NBS1* polymorphisms

<i>NBS1</i> SNP/genotypes	Cancer risk factors	Cancer type ^a , death/all (%)	<i>aHR</i> ^b (95 % CI)	<i>BFDPC</i> ^c prior probability	
				0.20	0.10
<i>NBS1</i> rs1061302					
	Smoking	NSCLC			
AA/AG	Never	36/66 (55)	1		
AA/AG	Ever	183/315 (58)	1.07 (0.74–1.57)	0.85	0.93
GG	Never	7/17 (41)	0.47 (0.21–1.08)	0.76	0.88
GG	Ever	34/45 (76)	1.53 (0.95–2.47)	0.73	0.86
Adjusted Ratio of Hazard Ratios (<i>aHR</i>) ^b			3.00 (1.22–7.38)	0.72	0.85
<i>NBS1</i> rs1063054					
	Drinking	UADT, Squamous cell carcinoma			
AA	Never	18/34 (53)	1		
AA	Ever	53/135 (39)	0.55 (0.31–0.97)	0.70	0.84
AC/CC	Never	8/41 (20)	0.34 (0.15–0.78)	0.69	0.83
AC/CC	Ever	71/192 (37)	0.54 (0.31–0.93)	0.66	0.81
Adjusted Ratio of Hazard Ratios (<i>aHR</i>) ^b			2.90 (1.14–7.33)	0.74	0.86

aHR adjusted hazard ratio, *CI* confidence interval, *BFDPC* Bayesian false-discovery probability, *aHR* adjusted ratio of hazard ratios

^aCancer types are non-small cell lung cancer (NSCLC) and squamous cell carcinoma at the UADT sites, respectively.

^bModels adjusted for age, gender, ethnicity, education, tumor grade, alcohol drink-years (NSCLC) and smoking pack-years (UADT).

^cThe *BFDPC* threshold (ratio of costs between false negative and false positive findings) was 0.75 as suggested in original papers. Prior variance for alternative hypothesis was 0.05.

Table 3

Maximum Partial-Likelihood (ML) and semi-Bayes (SB) estimates of exponentiated product-term coefficients (ratios of hazard ratios, RHR) using independent mean zero, variance $\frac{1}{2}$ coefficient priors (95% prior limits $\frac{1}{4}$, 4 after exponentiation)

Product term	ML estimates ^a (95 % confidence limits)	SB estimates ^a (95 % posterior limits)	ML estimates ^b (95 % confidence limits)	SB estimates ^b (95 % posterior limits)
Non-small cell lung cancer				
<i>NBSI</i> rs709816 (CC/TC vs. TT)				
SNP*smoking	1.35 (0.85, 2.14)	1.30 (0.84, 2.01)	1.91 (0.83, 4.38)	1.58 (0.77, 3.23)
SNP*drinking	0.81 (0.62, 1.06)	0.82 (0.63, 1.06)	0.78 (0.40, 1.52)	0.84 (0.46, 1.51)
Smoking*drinking	0.95 (0.78, 1.16)	0.96 (0.79, 1.16)	1.67 (0.80, 3.49)	1.48 (0.78, 2.82)
<i>NBSI</i> rs1061302 (GG vs. AA/AG)				
SNP*smoking	2.13 (1.18, 3.83)	1.90 (1.11, 3.26)	3.42 (1.28, 9.12)	2.26 (1.06, 4.81)
SNP*drinking	1.01 (0.62, 1.65)	1.02 (0.65, 1.62)	0.71 (0.31, 1.59)	0.86 (0.43, 1.70)
Smoking*drinking	1.06 (0.90, 1.24)	1.06 (0.90, 1.24)	1.59 (0.79, 3.18)	1.44 (0.77, 2.66)
<i>NBSI</i> rs1063054 (AC/CC vs. AA)				
SNP*smoking	1.26 (0.86, 1.85)	1.24 (0.86, 1.78)	1.45 (0.73, 2.89)	1.25 (0.68, 2.29)
SNP*drinking	0.86 (0.67, 1.09)	0.86 (0.68, 1.09)	0.71 (0.39, 1.30)	0.79 (0.46, 1.36)
Smoking*drinking	1.03 (0.88, 1.21)	1.03 (0.88, 1.20)	1.77 (0.88, 3.55)	1.53 (0.83, 2.83)
UADT, squamous cell carcinoma				
<i>NBSI</i> rs709816 (CC/TC vs. TT)				
SNP*smoking	1.53 (0.81, 2.93)	1.43 (0.80, 2.54)	1.31 (0.50, 3.43)	1.19 (0.54, 2.62)
SNP*drinking	1.07 (0.92, 1.23)	1.07 (0.93, 1.24)	0.90 (0.33, 2.48)	0.95 (0.42, 2.13)
Smoking*drinking	0.94 (0.87, 1.02)	0.94 (0.87, 1.02)	1.06 (0.40, 2.80)	1.04 (0.47, 2.30)
<i>NBSI</i> rs1061302 (GG/AG vs. AA)				
SNP*smoking	1.59 (0.90, 2.82)	1.49 (0.88, 2.52)	1.48 (0.62, 3.52)	1.37 (0.66, 2.83)
SNP*drinking	0.94 (0.78, 1.13)	0.95 (0.79, 1.14)	1.56 (0.63, 3.86)	1.41 (0.66, 2.97)
Smoking*drinking	0.89 (0.80, 1.00)	0.89 (0.80, 1.00)	0.94 (0.36, 2.45)	0.96 (0.43, 2.11)
<i>NBSI</i> rs1063054 (AC/CC vs. AA)				
SNP*smoking	1.73 (0.97, 3.11)	1.59 (0.93, 2.73)	1.00 (0.41, 2.43)	1.07 (0.52, 2.23)
SNP*drinking	1.01 (0.87, 1.18)	1.02 (0.88, 1.19)	2.83 (1.05, 7.67)	2.02 (0.93, 4.40)
Smoking*drinking	0.93 (0.85, 1.01)	0.93 (0.85, 1.01)	1.06 (0.39, 2.84)	1.01 (0.46, 2.23)

^a continuous main effects and product-terms in models: tobacco smoking (40 pack-years) and alcohol drinking (80 drink-years)

^b binary main effects and product-terms in models: ever/never tobacco smoking and alcohol drinking