ORIGINAL RESEARCH

Genetic variants of SP-D confer susceptibility to radiation pneumonitis in lung cancer patients undergoing thoracic radiation therapy

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Abstract

Background: Surfactant protein D (SP-D) is an innate immunity molecule in the alveoli. However, the associations between genetic variants of SP-D and radiation pneumonitis (RP) have never been investigated.

Methods: The Linkage disequilibrium of SP-D and tagSNPs were analyzed by using Haploview 4.1. Eight tagSNPs were genotyped among 396 lung cancer patients who received thoracic radiation therapy with follow-up time (median [P25, P75]: 11[6, 18]) using improved multiplex ligation detection reaction (iMLDR). The associations between clinical characteristics, tagSNP alleles, genotypes, haplotypes and onset time of grade ≥2 or ≥3 RP were evaluated by using univariate and multivariate Cox proportional hazard regression model.

Results: Three tagSNPs of SP-D (rs1998374, rs911887 and rs2255326) were significantly associated with grade ≥2 RP in multivariate analysis with multiple testing (Q test). The rs199874 had a protective effect for grade ≥2 RP in the dominant model (Hazard ratio (HR), 0.575; 95% confidence interval (CI), 0.378‐0.875). The homozygous mutant genotype for rs911887 had risk effect for grade ≥2 RP (HR, 2.209; 95% CI, 1.251‐3.902). The A mutant allele of rs2255326 also showed an elevated risk for grade ≥2 RP (HR, 1.777; 95% CI, 1.283‐2.461) and this risk effect was still significant in the recessive genetic model (HR, 3.320; 95% CI, 1.659‐6.644) and dominant
1 | INTRODUCTION

With the increasing lung cancer patients diagnosed, more and more lung cancer patients receive thoracic radiotherapy as a part of standard treatment regimen. However, approximately 16%-30% of lung cancer patients experience moderate to severe radiation-induced pneumonitis (RP) within the first 3 months of thoracic radiation therapy, even for those who were treated with intensity–modulated radiation therapy (IMRT). RP limits the escalation of radiation dose necessary to achieve curative effects while reducing the quality of life for patients. Therefore, establishing reliable predictors of RP is a critical step towards maximizing efficacy of treatment for lung cancer patients while minimizing adverse effects associated with thoracic radiation.

Multiple therapeutic and patient–related RP risk factors include performance status (PS), smoking status, chemotherapy, and dosimetric parameters. However, they are not sufficient to fully explain why under similar radiotherapy doses fraction some patients develop RP while the rest never do, which suggests a genetic basis for RP. RP genetic association studies had identified some RP susceptibility genes, further supporting a genetic basis in RP development. These RP susceptibility genes identified thus far were involved in the DNA repair pathways, oxidative stress pathways, cellular signaling pathways, and inflammatory response to ionizing radiation.

Surfactant protein D (SP-D), a component of lung surfactants, which reduces surface tension at the pulmonary air–liquid interface and enhances defense against pathogens as the first line of innate pulmonary immunity, has never been investigated with regard to the association between its genetic variants and the risk of RP. Previous study showed that serum levels of SP-D in RP patients were elevated. More specifically, the genetic polymorphisms in SP-D were associated with some lung injuries such as COPD, interstitial pneumonia, asthma, lung cancer, and infectious lung diseases. Therefore, we suspected that single–nucleotide polymorphisms (SNPs) of SP-D would also be associated with RP.

In this study, two endpoints of grade ≥2 or grade ≥3 RP were observed and the potential associations of SP-D were explored in lung cancer patients treated with thoracic radiotherapy. To comprehensively investigate this association, the alleles, genotypes and haplotypes of tagSNPs in SP-D were analyzed after clinical–risk RP factors were evaluated.

2 | MATERIALS AND METHODS

2.1 | Patients

Lung cancer patients receiving thoracic radiotherapy were recruited at the Department of Radiation Oncology at Daping Hospital (Chongqing, China) between February 2006 and March 2011, and were continually recruited at the Cancer Center of Chengdu Military General Hospital (Chengdu, China) between April 2011 and April 2017. The inclusion criteria were (a) histological and cytological confirmation of lung cancer; (b) genomic DNA samples and clinical data availability; (c) patients who did not develop RP during the follow–up period must have received a radiation dose ≥40 Gy; (d) patient records with at least a 6-month follow–up period. The exclusion criteria included (a) patients who had previous thoracic irradiation; (b) patients who had severe cardiopulmonary diseases; (c) patients who had blood relatives already enrolled in the study. Written informed consent was obtained from every patient before radiotherapy treatment. Key patient characteristics, including PS, smoking history, lung cancer stage, and chemotherapy, were all recorded. A 1-ml peripheral blood sample was collected from each patient before radiotherapy, and genomic DNA was extracted from the blood samples using Wizard Genomic DNA Purification Kit (Promega, USA). The isolated DNA samples’ purity and concentration were determined by spectrophotometric measurement of absorbances at 260 and 280 nm.

2.2 | Treatment and follow-up

All patients received radiotherapy with 6-MV X-rays from a linear accelerator (Varian, USA). The dosimetric parameters
were obtained from the treatment planning system. The median total radiation dose was 55.1 Gy (range: 22 to 72 Gy), with a mean of 2.2 Gy (range: 1.5 to 3 Gy) administered per radiation treatment. 96.2% of the patients (n = 381) received IMRT (intensity–modulated radiation therapy) and others received three–dimensional conformal radiotherapy with total radiation dose ranging from 40 Gy to 70 Gy. The dosimetric parameters for the risk of RP such as the percentage of lung volume receiving greater than 5, 10, 20, 30 Gy (V5, V10, V20, V30) were obtained from the dose–volume histograms (DVH).

During radiation therapy, patients were monitored weekly. They were checked every month from 1 to 3 months after radiotherapy and then every 3 months afterwards during follow‐up observations. Follow‐up evaluations included interval history, physical examination, chest CT or PET/CT, pulmonary functional tests, and routine blood tests. RP was assessed firstly at each follow–up visit and diagnosed by clinical presentation and radiographic abnormalities, including ground–glass opacity, attenuation, or consolidation changes within the radiation field. Then, RP was graded by two radiation oncologists independently who were blinded from the genotyping results according to the Common Terminology Criteria for Adverse Events version 3.0. Patients with clinical symptoms such as shortness of breath, dry cough, low–grade fever, chest tightness and/or pain were graded >2 RP. The RP patients with oxygen therapy in medical history were graded >3 RP. The time to end–point development was calculated from the beginning of the radiation therapy for each patient to the final follow–up date October, 2017.

TABLE 1 (Continued)

| Characteristic                                      | Descriptive statistics       |
|-----------------------------------------------------|------------------------------|
| Gender, n (%)                                       |                             |
| Male                                                | 309 (78.03)                 |
| Female                                              | 87 (21.97)                  |
| Age (y), mean ± SD                                  | 59.15 ± 9.72                |
| Age group (y), n (%)                                |                             |
| ≤60                                                 | 205 (51.77)                 |
| >60                                                 | 191 (48.23)                 |
| Histology, n (%)                                    |                             |
| SCLC                                                | 97 (24.49)                  |
| NSCLC                                               | 299 (75.51)                 |
| Adenocarcinoma                                      | 116 (38.80)                 |
| Squamous cell carcinoma                             | 173 (57.86)                 |
| Adenosquamous carcinoma                             | 10 (3.34)                   |
| Tumor location in the lung, n (%)                   |                             |
| Upper lobe                                          | 176 (44.44)                 |
| Middle lower lobes                                  | 220 (55.56)                 |
| Stage, n (%)                                        |                             |
| I, II                                               | 47 (11.87)                  |
| IIIA                                                | 99 (25.00)                  |
| IIIB                                                | 102 (25.76)                 |
| IV                                                  | 148 (37.37)                 |
| PS, n (%)                                           |                             |
| ≤2                                                  | 383 (96.72)                 |
| >2                                                  | 13 (3.28)                   |
| Smoking history, n (%)                              |                             |
| Never                                               | 155 (39.14)                 |
| Ever                                                | 241 (60.86)                 |
| Pulmonary lobectomy, n (%)                          |                             |
| No                                                  | 332 (83.84)                 |
| Yes                                                 | 64 (16.16)                  |
| Chemotherapy, n (%)                                 |                             |
| No                                                  | 10 (2.53)                   |
| Irinotecan and platinum                             | 97 (24.49)                  |
| Pemetrexed and cisplatin                            | 111 (28.03)                 |
| Paclitaxel and cisplatin                            | 178 (44.95)                 |
| Radiation dose fractionation (Gy)                   |                             |
| Mean ± SD                                           | 2.19 ± 0.58                 |
| Median (P25, P75)                                   | 2 (2.2)                     |
| Radiation dose fractionation group (Gy), n (%)       |                             |
| ≤2 (conventional radiotherapy)                      | 276 (69.70)                 |
| >2 (hypofractionation)                              | 120 (30.30)                 |
| Radiation dose (Gy)                                 |                             |
| Mean ± SD                                          | 55.09 ± 7.12                |

NSCLC, nonsmall cell carcinoma; PS, performance status of ECOG score standard; SCLC, small cell carcinoma; V10 (%), percentage of the lung volume that received more than 10 Gy; V20 (%), percentage of the lung volume that received more than 20 Gy; V30 (%), percentage of the lung volume that received more than 30 Gy; V5 (%), percentage of the lung volume that received more than 5 Gy.
|                                | Grade ≥2 RP |                           | Grade ≥3 RP |                           |
|--------------------------------|-------------|---------------------------|-------------|---------------------------|
|                                | HR 95% CI   | P                         | HR 95% CI   | P                         |
| Gender                         |             |                           |             |                           |
| Male                           | 1           |                           | 1           |                           |
| Female                         | 0.706       | 0.405-1.230               | 0.791       | 0.348-1.797               |
| Age group (y)                  |             |                           |             |                           |
| ≤60                            | 1           |                           | 1           |                           |
| >60                            | 1.277       | 0.840-1.942               | 2.351       | 1.186-4.659               |
| Histology                      |             |                           |             |                           |
| SCLC                           | 1           |                           | 1           |                           |
| Adenocarcinoma                 | 0.900       | 0.508-1.595               | 0.760       | 0.323-1.791               |
| Squamous cell carcinoma        | 0.930       | 0.554-1.561               | 0.764       | 0.351-1.663               |
| Adenocarcinoma                 | 1.440       | 0.432-4.798               | 1.968       | 0.436-8.880               |
| Tumor location in the lung     |             |                           |             |                           |
| Upper lobe                     | 1           |                           | 1           |                           |
| Middle lower lobes             | 0.936       | 0.615-1.425               | 0.642       | 0.339-1.217               |
| Stage                          |             |                           |             |                           |
| I,II                           | 1           |                           | 1           |                           |
| IIIA                           | 1.581       | 0.716-3.492               | 1.435       | 0.290-7.109               |
| IIIB                           | 1.452       | 0.652-3.231               | 3.590       | 0.821-15.698              |
| IV                             | 1.227       | 0.562-2.676               | 2.475       | 0.566-10.824              |
| PS                             |             |                           |             |                           |
| ≤2                             | 1           |                           | 1           |                           |
| >2                             | 2.370       | 1.034-5.431               | 3.584       | 1.271-10.106              |
| Smoking history                |             |                           |             |                           |
| Never                          | 1           |                           | 1           |                           |
| Ever                           | 1.617       | 1.023-2.556               | 1.422       | 0.717-2.818               |
| Pulmonary lobectomy            |             |                           |             |                           |
| No                             | 1           |                           | 1           |                           |
| Yes                            | 0.805       | 0.438-1.480               | 0.979       | 0.409-2.340               |
| Chemotherapy                   |             |                           |             |                           |
| No                             | 1           |                           | 1           |                           |
| Irinotecan and platinum        | 1.097       | 0.259-4.654               | 0.516       | 0.114-2.326               |
| Pemetrexed and cisplatin       | 0.987       | 0.233-3.188               | 0.367       | 0.079-1.698               |
| Paclitaxel and cisplatin       | 1.049       | 0.253-4.341               | 0.410       | 0.094-1.783               |
| Radiation dose fractionation group (Gy) | | | | |
| ≤2 (conventional radiotherapy) | 1           |                           | 1           |                           |
| >2 (hypofractionation)         | 0.847       | 0.530-1.355               | 0.509       | 0.224-1.156               |
| Radiation dose (Gy)            | 0.971       | 0.945-0.997               | 0.951       | 0.916-0.987               |
| V5 (%)                         | 1.017       | 1.003-1.030               | 1.024       | 1.003-1.045               |
| V10 (%)                        | 1.028       | 1.011-1.046               | 1.042       | 1.015-1.069               |
| V15 (%)                        | 1.038       | 1.012-1.065               | 1.058       | 1.018-1.100               |
| V30 (%)                        | 1.063       | 1.026-1.101               | <0.001      | 1.063                    |

*P* were calculated by univariate Cox proportional hazard regression model.

95% CI, 95% confidence interval; HR, hazard ratio; PS, performance status of ECOG score standard; RP, Radiation Pneumonitis; SCLC, small cell carcinoma; V10 (%), percentage of the lung volume that received more than 10 Gy; V20 (%), percentage of the lung volume that received more than 20 Gy; V30 (%), percentage of the lung volume that received more than 30 Gy; V5 (%), percentage of the lung volume that received more than 5 Gy.
2.3 | TagSNPs selection and genotyping

Linkage disequilibrium of SP-D and tagSNPs in SP-D were analyzed by using HaploView 4.0 software (Supplementary Figure S1). A total of eight tagSNPs (rs721917, rs2243639, rs726288, rs1923536, rs1998374, rs911887, rs2255326 and rs75074551), which captured 100% of 36 alleles with MAF >0.1 with a mean r² of 0.959, were genotyped by experts who were blinded to the clinical information using improved multiplex ligation detection reaction (iMLDR) method (Genesky Biotechnologies Inc, Shanghai, China). A 5% blind, random sample of study subjects was genotyped twice and the genotype concordance rate was 100% and the call rate was 100%.

2.4 | Statistical methods

The Hardy-Weinberg equilibrium (HWE) test was conducted via Pearson χ² goodness-of-fit test. We observed two endpoints: development of grade ≥2 RP and development of grade ≥3 RP. The time to the end-point was calculated from the start of radiotherapy for each patient. The SPSS 16.0 statistical package (SPSS, Chicago, IL) was used for the statistical analyses. The associations between clinical characteristics, tagSNP alleles, genotypes, haplotypes and onset time of grade ≥2 or ≥3 RP were evaluated by using univariate and multivariate Cox proportional hazard regression model. Because eight SNPs and many tests were performed, the Q value that represents a measure of significance in terms of the false discovery rate was used to adjust the significance level for individual SNPs. Stepwise multivariate Cox proportional hazard regression model was used to select independent risk factors and the probabilities of entry and removal were 0.05 and 0.10. 95% CI, 95% confidence interval; HR, hazard ratio; RP, Radiation Pneumonitis; V10 (%), percentage of the lung volume that received more than 10 Gy; V30 (%), percentage of the lung volume that received more than 30 Gy.

### Table 3

| Grade ≥2 RP | Grade ≥3 RP |
|-------------|-------------|
| Age group (y) | | |
| ≤60 | 1 | |
| >60 | 2.373 | 1.197-4.703 | 0.013 |
| V10 (%) | 1.042 | 1.016-1.070 | 0.002 |
| V30 (%) | 1.063 | 1.026-1.101 | <0.001 |

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### TABLE 3

Multivariate analysis between clinical characteristics and grade ≥2 RP or grade ≥3 RP (n = 396)

3 | RESULTS

3.1 | Patients, treatment, and radiation dosimetric characteristics

A total of 396 patients with a mean age of 59.15 years (range, 23 to 80 years) were analyzed in this study (Table 1). Most of them (88.13%, n = 349) had stage III/IV lung cancer (according to the 7th lung cancer TNM classification and staging system) and 97.47% (n = 386) were treated with a combination of chemotherapy and radiotherapy. The chemotherapies of these patients were irinotecan and platinum for SCLC. Pemetrexed and cisplatin were adopted as first-line chemotherapy for adenocarcinoma in NSCLC and paclitaxel and cisplatin were used for squamous cell carcinoma and adenocarcinoma of the lung (ASC). Dosimetric parameters for each subvolume V5, V10, V20 and V30 were shown in Table 1. The median follow-up periods were 11.4 months (range: 6-58).

### Table 2

| Grade ≥2 RP | |
|-------------|-------------|
| Age group (y) | |
| ≤60 | 1 | |
| >60 | 2.373 | 1.197-4.703 | 0.013 |
| V10 (%) | 1.042 | 1.016-1.070 | 0.002 |
| V30 (%) | 1.063 | 1.026-1.101 | <0.001 |

### Table 3

Multivariate analysis between clinical characteristics and grade ≥2 RP or grade ≥3 RP (n = 396)

3.2 | Clinical and dosimetric variables associated with grade ≥2 or ≥3 RP

After radiotherapy, 88 (22.22%) patients experienced grade ≥2 RP and 38 (9.60%) patients developed grade ≥3 RP. The mean occurrence time of grade ≥2 RP or grade ≥3 RP were 2.8 months and 2.7 months, respectively. To determine whether any confounding factors were influencing the risk of RP, the association between RP and clinical-dosimetric characteristics was investigated first. The clinical variables associated with grade ≥2 RP were PS, smoking history, radiation dose and V5-V30 (Table 2). The clinical variables associated with grade ≥3 RP were age, PS, radiation dose and V5-V30 in univariate analysis (Table 2). In the stepwise multivariate Cox proportional hazard regression model, V30 was associated with grade ≥2 RP (HR, 1.063; 95% CI, 1.026-1.101) and both age group (HR, 2.373; 95% CI, 1.197-4.703) and V10 (HR, 1.042; 95% CI, 1.016-1.070) were associated with grade ≥3 RP (Table 3).
| tagSNP allele/ genotypes | All Radio-therapy patients | Grade ≥2 RP | Univariate analysis<sup>a</sup> | Multivariate analysis<sup>b</sup> |
|--------------------------|---------------------------|----------------|-----------------|-----------------|
|                          | case No. | HR  | 95% CI | P | Q<sup>d</sup> | HR  | 95% CI | P | Q<sup>d</sup> |
| rs721917 | G | 477 | 110 | 1 | | 1 | | |
| | A | 315 | 66 | 0.909 | 0.670-1.234 | 0.542 | 0.587 | 0.924 | 0.681-1.253 | 0.610 | 0.670 |
| | GG | 143 | 36 | 1 | | 1 | | |
| | GA | 191 | 38 | 0.781 | 0.495-1.232 | 0.288 | 0.416 | 0.813 | 0.515-1.282 | 0.372 | 0.484 |
| | AA | 62 | 14 | 0.905 | 0.488-1.677 | 0.750 | 0.750 | 0.921 | 0.497-1.709 | 0.795 | 0.827 |
| rs2243639 | C | 612 | 140 | 1 | | 1 | | |
| | T | 180 | 36 | 0.874 | 0.517-1.441 | 0.471 | 0.517 | 0.911 | 0.631-1.315 | 0.618 | 0.670 |
| | CC | 227 | 54 | 1 | | 1 | | |
| | CT | 158 | 32 | 0.848 | 0.498-1.453 | 0.463 | 0.517 | 0.866 | 0.521-1.387 | 0.588 | 0.670 |
| | TT | 11 | 2 | 0.775 | 0.189-3.181 | 0.724 | 0.724 | 0.875 | 0.213-3.602 | 0.854 | 0.854 |
| rs726288 | C | 616 | 144 | 1 | | 1 | | |
| | T | 176 | 32 | 0.758 | 0.517-1.111 | 0.156 | 0.267 | 0.757 | 0.413-1.387 | 0.154 | 0.287 |
| | CC | 237 | 58 | 1 | | 1 | | |
| | CT | 142 | 28 | 0.771 | 0.491-1.211 | 0.259 | 0.397 | 0.791 | 0.434-1.422 | 0.308 | 0.435 |
| | TT | 17 | 2 | 0.478 | 0.117-1.965 | 0.724 | 0.724 | 0.443 | 0.108-1.814 | 0.258 | 0.394 |
| rs1923536 | C | 753 | 163 | 1 | | 1 | | |
| | T | 39 | 13 | 1.548 | 0.880-2.724 | 0.129 | 0.254 | 1.540 | 0.875-2.709 | 0.134 | 0.269 |
| | CC | 358 | 76 | 1 | | 1 | | |
| | CT | 37 | 11 | 1.376 | 0.731-2.590 | 0.322 | 0.419 | 1.380 | 0.733-2.597 | 0.318 | 0.435 |
| | TT | 1 | 1 | 8.091 | 1.115-58.687 | 0.039 | 0.112 | 6.388 | 0.878-46.459 | 0.067 | 0.161 |
| rs1998374 | T | 460 | 116 | 1 | | 1 | | |
| | C | 332 | 60 | 0.696 | 0.51-0.951 | 0.023 | 0.077 | 0.695 | 0.509-0.951 | 0.022 | 0.072 |
| | TT | 133 | 40 | 1 | | 1 | | |
| | CT | 194 | 36 | 0.582 | 0.371-0.913 | 0.013 | 0.068 | 0.584 | 0.372-0.916 | 0.019 | 0.072 |
| | CC | 69 | 12 | 0.551 | 0.289-1.051 | 0.070 | 0.183 | 0.549 | 0.288-1.046 | 0.068 | 0.161 |
| | CT+CC | 263 | 48 | 0.574 | 0.377-0.873 | 0.009 | 0.041 | 0.575 | 0.378-0.875 | 0.008 | 0.040 |
| rs911887 | T | 514 | 98 | 1 | | 1 | | |
| | C | 278 | 78 | 1.518 | 1.127-2.044 | 0.006 | 0.039 | 1.457 | 1.082-1.963 | 0.013 | 0.057 |
| | TT | 168 | 30 | 1 | | 1 | | |
| | CT | 178 | 38 | 1.203 | 0.745-1.941 | 0.450 | 0.532 | 1.183 | 0.733-1.913 | 0.491 | 0.608 |
| | CC | 50 | 20 | 2.436 | 1.383-4.29 | 0.002 | 0.018 | 2.209 | 1.251-3.902 | 0.006 | 0.040 |
| | CT+CC | 228 | 58 | 1.457 | 0.938-2.264 | 0.094 | 0.204 | 1.406 | 0.904-2.187 | 0.130 | 0.269 |
| rs2253526 | G | 642 | 125 | 1 | | 1 | | |
## TABLE 4

**Associations between tagSNP alleles, genotypes of SP‐D and grade ≥2 RP or grade ≥3 RP**

| Case No. | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|----------|---------------------|-----------------------|---------------------|-----------------------|
|          | HR  | 95% CI | P   |  | 95% CI | P   |  | 95% CI | P   |
|          | HR  |  |  |  | HR  |  |  | HR  |  |  |
| 46       | 1.000 | | | | | | | | |
| 30       | 0.987 | 0.623-1.563 | 0.955 | 0.962 | 0.928 | 0.585-1.472 | 0.750 | 0.974 |
| 15       | 1.000 | | | | | | | | |
| 16       | 0.791 | 0.391-1.599 | 0.513 | 0.785 | 0.714 | 0.351-1.453 | 0.352 | 0.654 |
| 7        | 1.080 | 0.44-2.649 | 0.867 | 0.962 | 0.965 | 0.391-2.381 | 0.938 | 0.974 |

| Case No. | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|----------|---------------------|-----------------------|---------------------|-----------------------|
|          | HR  | 95% CI | P   |  | 95% CI | P   |  | 95% CI | P   |
|          | HR  |  |  |  | HR  |  |  | HR  |  |  |
| 59       | 1.000 | | | | | | | | |
| 17       | 0.984 | 0.574-1.687 | 0.952 | 0.962 | 0.991 | 0.576-1.704 | 0.974 | 0.974 |
| 22       | 1.000 | | | | | | | | |
| 15       | 0.983 | 0.51-1.895 | 0.959 | 0.962 | 0.963 | 0.497-1.865 | 0.910 | 0.974 |
| 1        | 0.953 | 0.128-7.073 | 0.962 | 0.962 | 1.151 | 0.152-8.701 | 0.891 | 0.974 |

| Case No. | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|----------|---------------------|-----------------------|---------------------|-----------------------|
|          | HR  | 95% CI | P   |  | 95% CI | P   |  | 95% CI | P   |
|          | HR  |  |  |  | HR  |  |  | HR  |  |  |
| 62       | 1.000 | | | | | | | | |
| 14       | 0.793 | 0.444-1.416 | 0.432 | 0.750 | 0.926 | 0.516-1.663 | 0.798 | 0.974 |
| 26       | 1.000 | | | | | | | | |
| 10       | 0.635 | 0.306-1.316 | 0.222 | 0.412 | 0.728 | 0.349-1.518 | 0.397 | 0.666 |
| 2        | 1.128 | 0.268-4.752 | 0.870 | 0.962 | 1.809 | 0.408-8.022 | 0.435 | 0.666 |

| Case No. | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|----------|---------------------|-----------------------|---------------------|-----------------------|
|          | HR  | 95% CI | P   |  | 95% CI | P   |  | 95% CI | P   |
|          | HR  |  |  |  | HR  |  |  | HR  |  |  |
| 71       | 1.000 | | | | | | | | |
| 5        | 1.375 | 0.555-3.406 | 0.491 | 0.785 | 1.379 | 0.557-3.416 | 0.487 | 0.667 |
| 34       | 1.000 | | | | | | | | |
| 3        | 0.842 | 0.259-2.741 | 0.775 | 0.962 | 0.908 | 0.278-2.962 | 0.873 | 0.974 |
| 1        | 20.259 | 2.706-151.679 | 0.003 | 0.040 | 6.030 | 0.745-48.816 | 0.092 | 0.240 |

| Case No. | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|----------|---------------------|-----------------------|---------------------|-----------------------|
|          | HR  | 95% CI | P   |  | 95% CI | P   |  | 95% CI | P   |
|          | HR  |  |  |  | HR  |  |  | HR  |  |  |
| 54       | 1.000 | | | | | | | | |
| 22       | 0.557 | 0.339-0.915 | 0.021 | 0.077 | 0.587 | 0.357-0.965 | 0.036 | 0.176 |
| 21       | 1.000 | | | | | | | | |
| 12       | 0.377 | 0.186-0.767 | 0.007 | 0.046 | 0.380 | 0.187-0.774 | 0.008 | 0.074 |
| 5        | 0.451 | 0.17-1.196 | 0.109 | 0.219 | 0.510 | 0.19-1.372 | 0.183 | 0.365 |
| 17       | 0.396 | 0.209-0.751 | 0.005 | 0.040 | 0.410 | 0.216-0.781 | 0.007 | 0.074 |

| Case No. | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|----------|---------------------|-----------------------|---------------------|-----------------------|
|          | HR  | 95% CI | P   |  | 95% CI | P   |  | 95% CI | P   |
|          | HR  |  |  |  | HR  |  |  | HR  |  |  |
| 41       | 1.000 | | | | | | | | |
| 35       | 1.596 | 1.016-2.505 | 0.042 | 0.118 | 1.513 | 0.963-2.377 | 0.072 | 0.217 |
| 10       | 1.000 | | | | | | | | |
| 21       | 2.001 | 0.942-4.249 | 0.071 | 0.162 | 1.848 | 0.868-3.933 | 0.111 | 0.241 |
| 7        | 2.407 | 0.916-6.324 | 0.075 | 0.162 | 2.220 | 0.844-5.839 | 0.106 | 0.241 |
| 28       | 2.089 | 1.015-4.301 | 0.046 | 0.118 | 1.929 | 0.935-3.98 | 0.075 | 0.217 |

| Case No. | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|----------|---------------------|-----------------------|---------------------|-----------------------|
|          | HR  | 95% CI | P   |  | 95% CI | P   |  | 95% CI | P   |
|          | HR  |  |  |  | HR  |  |  | HR  |  |  |
| 52       | 1.000 | | | | | | | | |

(Continues)
3.3 | Univariate and multivariate analysis of tagSNPs association with grade ≥2 RP or grade ≥3 RP

The results of Pearson χ² goodness-of-fit test showed that allele and genotype frequencies of all tagSNPs were in Hardy-Weinberg equilibrium (P > 0.05) in both the patient and the control populations, indicating our samples were a random mating population without selection. With respect to grade ≥2 RP, the associations were statistically significant for rs1998374, rs911887 and rs2255326 in multivariate analysis after adjusting confounding factors and with correction with Q value (Table 4). The C mutant allele of rs1998374 was protective against grade ≥2 RP in dominant genetic model (HR, 0.575; 95% CI, 0.378-0.875) whereas the homozygous genotype of rs911887 increased the risk of grade ≥2 RP in the lung cancer patients (HR, 2.209; 95% CI, 1.251-3.902). For rs2255326, the mutant allele A was associated with an increased risk of grade ≥2 RP (HR, 1.777; 95% CI, 1.283-2.461) and these significant associations were still seen in both dominant (HR, 1.773; 95% CI, 1.251-2.696) and recessive models (HR, 3.320; 95% CI, 1.659-6.644). Although these associations were detected between grade ≥3 RP and rs1998374 or rs2255326 in multivariate analysis, they could not reach significance level after corrected with Q value (Table 4).

3.4 | SNPs and cumulative probability of grade ≥2 or ≥3 RP

The overall probabilities of grade ≥2 or grade ≥3 RP were assessed with the Kaplan-Meier curve and log-rank test (Figure 1). Patients bearing mutant allele C of rs1998374 showed lower risk for grade ≥2 RP and grade ≥3 RP compared to wild-type allele T. This protective effect was also seen in dominant model (Figure 1A,D). Patients with mutant allele of rs911887 (Figure 1B,E) or of rs2255326 (Figure 1C,F) had significantly higher risk for both grade ≥2 RP and grade ≥3 RP compared to wild-type allele. This risk effect was also seen in homozygous mutant genotype compared to wild-type genotype for grade ≥2 RP (Figure 1H,I) and in dominant model for grade ≥3 RP (Figure 1K,L).

3.5 | Haplotype association analysis

To further investigate the combined effect of the 3 tagSNPs (rs1998374, rs911887, and rs2255326) on the risk of RP, we estimated the frequencies of each haplotype by PHASE software and performed haplotype analysis using Cox proportional hazard regression model. There were only 4 haplotypes with over 10% frequency among eight haplotypes in the patients, other rare haplotypes were combined together as “others” in our statistical analysis (Table 5). The T-A-C haplotype was significantly

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### Table 4 (Continued)

| tagSNP allele/genotypes | All Radiotherapy patients | Grade ≥2 RP | All Radiotherapy patients | Grade ≥3 RP | All Radiotherapy patients | Grade ≥2 RP | All Radiotherapy patients | Grade ≥3 RP |
|-------------------------|--------------------------|------------|--------------------------|------------|--------------------------|------------|--------------------------|------------|
|                         | case No.                 | Univariate analysis | Multivariate analysis |
|                         | HR 95% CI                | P           | Q^d | HR 95% CI | P           | Q^d |
| A                       | 150 51                   | 1.817 1.312-2.516 | <0.001 0.013 | 1.777 1.283-2.461 | 0.001 0.009 |
| GG                      | 262 47                   | 1.086 0.945-2.34 | 0.240 | 1.540 0.978-2.425 | 0.063 0.161 |
| GA                      | 118 31                   | 1.487 0.945-2.34 | 0.086 | 1.540 0.978-2.425 | 0.063 0.161 |
| AA                      | 16 10                    | 4.058 2.048-8.041 | <0.001 | 3.320 1.659-6.644 | 0.001 0.009 |
| GA+AA                   | 134 41                   | 1.758 1.156-2.673 | 0.008 | 1.773 1.166-2.696 | 0.007 0.040 |

5% CI, 5% confidence interval; HR, hazard ratio; tagSNP, tag single-nucleotide polymorphisms.

aUnivariate analysis adopted Cox proportional hazard regression model with adjustment for no factor.

bMultivariate analysis adopted Cox regression model with adjustment for V30 (%).

^cMultivariate analysis adopted Cox regression model with adjustment for age group and V10 (%).

^dQ was the result of multiple comparison adjustment by Q test.
associated with increased risk for both grade ≥2 RP and grade ≥3 RP when compared the most common haplotype (C-G-T). Lung cancer patients bearing the T-A-C haplotype showed approximately twofold risk of RP than those bearing the C-G-T haplotype for both grade ≥2 RP (HR, 1.885; 95% CI, 1.284-2.765) and grade ≥3 RP (HR, 2.256; 95% CI, 1.248-4.080).

### DISCUSSION

In the present study, three tagSNPs (rs1998374, rs911887, rs2255326) were significantly associated with grade ≥2 RP and haplotype (T-A-C) in SP-D was significantly associated with both grade ≥2 RP and grade ≥3 RP in lung cancer patients treated with radiotherapy. To our knowledge, this is the first report on an association between SP-D and RP. It is worth noting that the eight tagSNPs tested captured 100% of 36 alleles in SP-D with mean $r^2$ of 0.959, which means 36 SNPs in SP-D were investigated in this study.

RP, a complication after radiotherapy, is involved clinical characteristics, therapy–related factors and follow–up tasks. Therefore, the design and measures of RP genetic association study would be more complex than common association studies of disease. One critical issue is the confounders. In our study, nongenetic factors including clinical information and radiotherapy–related factors were comprehensively considered before investigation of novel genetic risk factors. In addition to nongenetic factors usually analyzed in other RP genetic association studies, tumor location and more precise dosimetric parameters such as V5, V10 and V20 mentioned in some clinic studies as prediction factors for RP were also analyzed in this study. We found that PS, smoking status, age and dosimetric parameters were significantly associated with RP. Whether chemotherapy was performed or not was regarded as an influencing factor of RP in some studies, but in our study only 2.5% patients received radiotherapy alone and the results did not reach statistical significance ($P_{grade \geq 2} = 0.841$ and $P_{grade \geq 3} = 0.097$). Further analysis about different chemotherapy drugs used among patients showed no association with RP risk. Another critical issue is the stringency of RP diagnosis criteria. First, to ensure case group are strictly defined, RP diagnosis and grading should be defined properly and accurately recorded. In our study, 2 radiation oncologists diagnosed and graded RP in 2 radiation oncologists diagnosed and graded RP independently and were blinded to the genetic information. Second, our follow–up schedule was considered to record RP occurrence accurately. Lastly, to reduce the possibility of false non-RP phenotypes in the control group, the follow–up period in our study was extended to a median of 11.4 months, longer than normal occurrence time of RP (<6 months).

The allele and genotype analysis in this study consistently revealed that the genetic variants of SP-D were associated

| Grade ≥3 RP | Case No. | Univariate analysis | Multivariate analysis |
|------------|---------|--------------------|----------------------|
|            |         | HR  | 95% CI | P    | Q^d | HR  | 95% CI | P    | Q^d |
|            |         |     |        |      |     |     |        |      |     |
| 24         | 150     | 2.017 | 1.243-3.271 | 0.004 | 0.040 | 1.914 | 1.18-3.105 | 0.009 | 0.074 |
| 18         | 1       | 1    |        | 1    | 1    | 1    |        | 1    | 1    |
| 16         | 205     | 2.015 | 1.027-3.951 | 0.042 | 0.118 | 2.029 | 1.031-3.993 | 0.041 | 0.176 |
| 4          | 185     | 3.787 | 1.281-11.194 | 0.016 | 0.069 | 2.975 | 0.999-8.862 | 0.050 | 0.187 |
| 20         | 220     | 2.222 | 1.176-4.202 | 0.014 | 0.069 | 2.171 | 1.146-4.11 | 0.017 | 0.113 |
| 70         | 1       | 1    |        | 1    | 1    | 1    |        | 1    | 1    |
| 6          | 248     | 1.225 | 0.532-2.82 | 0.633 | 0.866 | 1.364 | 0.592-3.147 | 0.466 | 0.667 |
| 32         | 1       | 1    |        | 1    | 1    | 1    |        | 1    | 1    |
| 6          | 36      | 1.303 | 0.545-3.117 | 0.551 | 0.796 | 1.423 | 0.593-3.418 | 0.430 | 0.666 |

95% CI, 95% confidence interval; HR, hazard ratio; tagSNP, tag single–nucleotide polymorphisms.

aUnivariate analysis adopted Cox proportional hazard regression model with adjustment for no factor.
bMultivariate analysis adopted Cox regression model with adjustment for V30 (%).
cMultivariate analysis adopted Cox regression model with adjustment for age group and V10 (%).
dQ^d was the result of multiple comparison adjustment by Q test.
FIGURE 1 Kaplan-Meier curve and log-rank test comparing the cumulative RP-free probability of time to an adverse outcome between patients with alleles and genotypes of rs1998374, rs911887 and rs2255326. K-M allele analysis of rs1998374, rs911887 and rs2255326 for grade ≥2 RP were (A-C) and for grade ≥3 RP were (D-F) respectively. K-M genotype analysis of rs1998374, rs911887 and rs2255326 for grade ≥2 RP were (G-I) and for grade ≥3 RP were (J-L), respectively.
with RP. The mutant alleles of rs911887 and rs2255326 were risk factors for RP while the mutant allele of rs1998374 was a protect factor for RP. Their different effect for RP roused our interest to further analyze their combined effect for RP using haplotype analysis. Lung cancer patients treated with radiotherapy bearing C-G-T haplotype had about twofold higher risk of RP than those bearing T-A-C haplotype, strongly suggesting that the genetic variants of \(SP-D\) could be a genetic biomarker in predicting RP development among lung cancer patients.

In our cohort of 396 samples, there was only one patient with a homozygous mutant genotype TT of rs1923536. Although this patient developed high-grade RP, the association between rs1923536 and RP must be reinvestigated in a large sample. Therefore, this tagSNP was excluded from haplotype analysis.

Since RP susceptibility loci could be predictors before radiotherapy, screening RP susceptibility genes has attracted researchers’ interest lately. However, maybe for the reason that complex RP follow-up tasks limited the sample size, to explore RP susceptibility loci usually used not genome-wide association study (GWAS) but only candidate gene approach. Until now, RP susceptibility loci were identified in DNA repair-related, inflammation-related, angiogenesis-related and stress response-related pathways with different underlying mechanisms.\(^3^2\) \(SP-D\), a surfactant proteins known for its contribution to the host’s lung immunity,\(^3^9\) has been mentioned to be biomarker of severe RP after radiotherapy.\(^4^0\) This study expanded the range of RP candidate genes to a new gene encoded pulmonary surfactant protein D, suggesting another mechanism may underlying the pathogenesis of RP.

To further determine the effects of \(SP-D\) SNPs and its role in RP, the functional identification of SNPs linked to variable expression levels of \(SP-D\) and more biological studies would be performed later.

Although our patients were recruited from two hospitals, studies with larger sample size or more patients from multicenter studies are needed to validate our findings.

Despite its limitations, this study is the first to identify novel RP susceptibility gene \(SP-D\). Three tagSNPs (rs1998374, rs911887, rs2255326) were identified as significantly associated with RP risk in the lung cancer patients treated with thoracic radiotherapy. The findings of our study may be useful in the development of genetic testing for the prediction of RP.

### CONFLICT OF INTEREST

None declared.

### AUTHOR CONTRIBUTIONS

Study conception and design: Hua Li, Li Xu, Junhong Jiang, Zhen-zhou Yang, Bangxian Tan. Radiotherapy and RP
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REFERENCES

1. Tyldesley S, Boyd C, Schulze K, Walker H, Mackillop WJ. Estimating the need for radiotherapy for lung cancer: an evidence-based, epidemiologic approach. Int J Radiat Oncol Biol Phys. 2001;49:973-985.

2. Roach M, Gandara Dr, Yuo Hs, et al. Radiation pneumonitis following combined modality therapy for lung cancer: analysis of prognostic factors. J Clin Oncol. 1995;13:2606-2612.

3. Khalil AA, Hoffmann L, Moeller DS, Farr KP, Knap MM. New dose constraint reduces radiation-induced fatal pneumonitis in locally advanced non-small cell lung cancer patients treated with intensity-modulated radiotherapy. Acta Oncol. 2015;54:1343-1349.

4. Fleming C, Cagney DN, O’Keeffe S, Brennan SM, Armstrong JG, McClean B. Normal tissue considerations and dose-volume constraints in the moderately hypofractionated treatment of non-small cell lung cancer. Radiother Oncol. 2016;119:423-431.

5. Marks LB, Bentzen SM, Deasy JO, et al. Radiation dose-volume effects in the lung. Int J Radiat Oncol Biol Phys. 2010;76:S70-76.

6. Giridhar P, Mallick S, Rath GK, Julka PK. Radiation induced lung injury: prediction, assessment and management. Asian Pac J Cancer Prev. 2015;16:2613-2617.

7. Zhang Li, Yang M, Bi N, et al. ATM polymorphisms are associated with risk of radiation-induced pneumonitis. Int J Radiat Oncol Biol Phys. 2010;77:1360-1368.

8. Yin M, Liao Z, Liu Z, et al. Functional polymorphisms of base excision repair genes XRCC1 and APEX1 predict risk of radiation pneumonitis in patients with non-small cell lung cancer treated with definitive radiation therapy. Int J Radiat Oncol Biol Phys. 2011;81:e67-73.

9. Yang M, Zhang Li, Bi N, et al. Association of P53 and ATM polymorphisms with risk of radiation-induced pneumonitis in lung cancer patients treated with radiotherapy. Int J Radiat Oncol Biol Phys. 2011;79:1402-1407.

10. Mak RH, Alexander BM, Asomaning K, et al. A single-nucleotide polymorphism in the methylene tetrahydrofolate reductase (MTHFR) gene is associated with risk of radiation pneumonitis in lung cancer patients treated with thoracic radiation therapy. Cancer. 2012;118:3654-3665.

11. Tang Y, Liu Bo, Li J, et al. Genetic variants in PI3K/AKT pathway are associated with severe radiation pneumonitis in lung cancer patients treated with radiation therapy. Cancer Med. 2016;5:24-32.

12. Yuan X, Liao Z, Liu Z, et al. Single nucleotide polymorphism at rs1982073:T869C of the TGFbeta 1 gene is associated with the risk of radiation pneumonitis in patients with non-small-cell lung cancer treated with definitive radiotherapy. J Clin Oncol. 2009;27:3370-3378.

13. Hildebrandt M, Komaki R, Liao Z, et al. Genetic variants in inflammation-related genes are associated with radiation-induced toxicity following treatment for non-small cell lung cancer. PLoS ONE. 2010;5: e12402

14. Yin M, Liao Z, Yuan X, et al. Polymorphisms of the vascular endothelial growth factor gene and severe radiation pneumonitis in non-small cell lung cancer patients treated with definitive radiotherapy. Cancer Sci. 2012;103:945-950.

15. Korfhagen TR, Sheftelievich V, Burhans MS, et al. Surfactant protein-D regulates surfactant phospholipid homeostasis in vivo. J Biol Chem. 1998;273:28438-28443.

16. Ikegami M, Whitsett JA, Jobe A, Ross G, Fisher J, Korfhagen T. Surfactant metabolism in SP-D gene-targeted mice. Am J Physiol Lung Cell Mol Physiol. 2000;279:L468-476.

17. Ikegami M, Na CL, Korfhagen TR, Whitsett JA. Surfactant protein D influences surfactant ultrastructure and uptake by alveolar type II cells. Am J Physiol Lung Cell Mol Physiol. 2005;288:L552-561.

18. Wu X, Zhao G, Lin J, et al. The production mechanism and immunosuppression effect of pulmonary surfactant protein D via toll like receptor 4 signaling pathway in human corneal epithelial cells during Aspergillus fumigatus infection. Int Immunopharmacol. 2015;29:433-439.

19. Sapkota M, Kharbanda KK, Wyatt TA. Malondialdehyde-acetalddehyde-adducted surfactant protein alters macrophage functions through scavenger receptor A. Alcohol Clin Exp Res. 2016;40:2563-2572.

20. Liu Z, Shi Q, Liu J, et al. Innate immune molecule surfactant protein D attenuates sepsis-induced acute pancreatic injury through modulating apoptosis and NF-kappaB-mediated inflammation. Sci Rep. 2015;5:17798.

21. Nayak A, Dodagatta-Marri E, Tsolaki AG, Kishore U. An insight into the diverse roles of surfactant proteins, SP-A and SP-D in innate and adaptive immunity. Front Immunol. 2012;3:131.

22. Sasaki R, Soejima T, Matsumoto A, et al. Clinical significance of serum pulmonary surfactant proteins a and d for the early detection of radiation pneumonitis. Int J Radiat Oncol Biol Phys. 2001;50:301-307.

23. Takahashi H, Sano H, Chiba H, Kuroki Y. Pulmonary surfactant proteins A and D: innate immune functions and biomarkers for lung diseases. Curr Pharm Des. 2006;12:589-598.

24. Yamazaki H, Aibe N, Nakamura S, et al. Measurement of exhaled nitric oxide and serum surfactant protein D levels for monitoring radiation pneumonitis following thoracic radiotherapy. Oncol Lett. 2017;14:4190-4196.

25. Foreman MG, Kong X, DeMeo DL, et al. Polymorphisms in surfactant protein-D are associated with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol. 2011;44:316-322.

26. Ishii T, Hagiwara K, Ikeda S, et al. Association between genetic variations in surfactant protein D and emphysema, interstitial pneumonia, and lung cancer in a Japanese population. COPD. 2012;9:409-416.

27. Brandt EB, Mingler MK, Stevenson MD, et al. Surfactant protein D alters allergic lung responses in mice and human subjects. J Allergy Clin Immunol. 2008;121(1140–1147):e1142.
28. Thomas NJ, Diangelo S, Hess JC, et al. Transmission of surfactant protein variants and haplotypes in children hospitalized with respiratory syncytial virus. Pediatr Res. 2009;66:70-73.
29. Ampuero S, Luchsinger V, Tapia L, Palomino MA, Larrañaga CE. SP-A1, SP-A2 and SP-D gene polymorphisms in severe acute respiratory syncytial infection in Chilean infants. Infect Genet Evol. 2011;11:1368-1377.
30. Zhou F, He X, Liu H, et al. Functional polymorphisms of circadian positive feedback regulation genes and clinical outcome of Chinese patients with resected colorectal cancer. Cancer. 2012;118:937-946.
31. Kelsey CR, Rosenstein BS, Marks LB. Predicting toxicity from radiation therapy: it's genetic, right? Cancer. 2012;118:3450-3454.
32. Guo CX, Wang J, Huang LH, Li JG, Chen X. Impact of single-nucleotide polymorphisms on radiation pneumonitis in cancer patients. Mol Clin Oncol. 2016;4:3-10.
33. Wen J, Liu H, Wang Q, et al. Genetic variants of the LIN28B gene predict severe radiation pneumonitis in patients with non-small cell lung cancer treated with definitive radiation therapy. Eur J Cancer. 2014;50:1706-1716.
34. Yin M, Liao ZX, Liu ZS, et al. Genetic variants of the nonhomologous end joining gene LIG4 and severe radiation pneumonitis in nonsmall cell lung cancer patients treated with definitive radiotherapy. Cancer. 2012;118:528-535.
35. Palma DA, Senan S, Tsujino K, et al. Predicting radiation pneumonitis after chemoradiation therapy for lung cancer: an international individual patient data meta-analysis. Int J Radiat Oncol Biol Phys. 2013;85:444-450.
36. Oh D, Ahn YC, Park HC, Lim DH, Han Y. Prediction of radiation pneumonitis following high-dose thoracic radiation therapy by 3 Gy/fraction for non-small cell lung cancer: analysis of clinical and dosimetric factors. Jpn J Clin Oncol. 2009;39:151-157.
37. Tanabe S, Myojin M, Shimizu S, et al. Dose-volume analysis for respiratory toxicity in intrathoracic esophageal cancer patients treated with definitive chemoradiotherapy using extended fields. J Radiat Res. 2013;54:1085-1094.
38. Mehta V. Radiation pneumonitis and pulmonary fibrosis in non-small-cell lung cancer: pulmonary function, prediction, and prevention. Int J Radiat Oncol Biol Phys. 2005;63:5-24.
39. Carreto-Binaghi LE, el Aliouat M, Taylor ML. Surfactant proteins, SP-A and SP-D, in respiratory fungal infections: their role in the inflammatory response. Respir Res. 2016:17:66.
40. Yamashita H, Kubayashi-Shibata S, Terahara A, et al. Prescreening based on the presence of CT-scan abnormalities and biomarkers (KL-6 and SP-D) may reduce severe radiation pneumonitis after stereotactic radiotherapy. Radiat Oncol. 2010;5:32.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Xu L, Jiang J, Li Y, et al. Genetic variants of SP-D confer susceptibility to radiation pneumonitis in lung cancer patients undergoing thoracic radiation therapy. Cancer Med. 2019;8:2599–2611. https://doi.org/10.1002/cam4.2088