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FULL PAPER

A polymorphism in the DNA repair domain of *APEX1* is associated with the radiation-induced pneumonitis risk among lung cancer patients after radiotherapy

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Objective: To examine the association of tag single nucleotide polymorphisms (tagSNPs) (rs1130409, rs1760944, rs2307486 and rs3136817) in *APEX1* with the risk of severe radiation-induced pneumonitis (RP) after radiotherapy among Han Chinese patients with lung cancer.

Methods: A total of 168 patients with lung cancer who were receiving radiotherapy were prospectively recruited. RP was evaluated according to the Radiation Therapy Oncology Group. A case-control study was performed. The case group included patients with RP grade of ≥ 3 , while the control group comprised patients with RP grades < 3 . Four tagSNPs of *APEX1* were genotyped in 126 patients with complete follow-up by multi-SNaP-shot® (Genesky Biotechnologies Inc., Shanghai, China) genotyping assays. Results were assessed by a logistic regression model for RP risk and Mantel-Cox log-rank test for the cumulative RP probability by the genotypes.

Results: rs1130409 was associated with severe RP. GT genotype of rs1130409 was significantly higher in patients with RP than in those of the control group [68.8% vs 41.8%; $p = 0.025$; resulting odds ratio (OR), 5.98]. Patients with lung cancer bearing the G allele had a 5.83-fold higher risk of RP than those with the wild TT genotype [OR = 5.83; 95% confidence interval (CI), 1.27–26.90; $p = 0.024$], and this was further confirmed by the binary regression adjusted by some confounding factors, including Karnofsky performance scale, concurrent chemotherapy-radiotherapy and lung volume receiving > 30 Gy (OR = 6.96; 95% CI, 1.36–35.77; $p = 0.02$). rs1130409 was also associated with the time to occurrence of severe RP ($p = 0.04$). Three-dimensional model *APEX1* protein showed that rs1130409 is located in the random coil structure corresponding to the DNA repair function region.

Advances in knowledge: rs1130409 of *APEX1* can be a predictor of RP grades ≥ 3 among patients with lung cancer.

Radiation-induced pneumonitis (RP) is the most common acute radiation-induced normal tissue toxicity with an incidence of 13–37% within one year following treatment in patients with lung cancer.¹ It is the main dose-limiting factor² that limits the improvement of locoregional control in and the survival of patients with lung cancer. Some clinical characteristics,³ including age,⁴ smoking status,^{5,6} Karnofsky performance scale (KPS), tumour location,⁷ tumour stage, radiochemotherapy and dose-volume histogram parameters [$V(\text{dose})$]² are usually considered in radiation therapy planning to reduce the risk of RP. Nevertheless, the individual variability in sensitivity to radiation reactions among patients cannot be controlled by these factors, implying that genetic factors may play a role in the risk of RP.

Several association studies have confirmed that RP has a genetic basis.^{8–11} Transforming growth factor $\beta 1$ (TGF $\beta 1$) was the first RP susceptibility gene identified.⁹ Subsequently, ataxia-telangiectasia mutated gene, methylene tetrahydrofolate reductase and DNA ligase IV have been associated with RP in American patients.^{10,12,13} Also, p53 and vascular endothelial growth factor 9 were identified as RP susceptibility genes in Han Chinese patients.¹¹

RP is a radiation-caused sterile inflammatory response in which DNA repair and inflammation are both involved. APEX nuclease (multifunctional DNA repair enzyme) 1 (*APEX1*) is the primary enzyme responsible for the recognition and incision of apurinic/apyrimidinic (AP) sites in the damaged DNA fragments, which radiation causes in

the early stages of the DNA-damage response. In addition to its base excision repair function, APEX1 is also an important redox modulator, enabling the maintenance of multiple cellular transcription factors, such as nuclear factor κ B (NF- κ B), in an active reduced state.¹⁴ Through redox-mediated activation of NF- κ B-driven interleukin 8 expression, APEX1 has recently been shown to play a role in inflammatory processes.¹⁵ Therefore, we presumed that APEX1 might be the RP susceptibility gene among Han Chinese patients with lung cancer.

The single nucleotide polymorphism (SNP) rs1130409 (D148E) in the APEX1 was reported as a RP susceptibility loci in a mixed population mainly composed of Caucasians.¹⁶ However, in their previous study, only one polymorphism was mentioned and APEX1 genetic variants were not comprehensively investigated for an association with clinical factors or RP grades. Moreover, owing to the genetic heterogeneity in different ethnic populations, some RP susceptibility loci in one population have not been replicated in other populations.^{17–19} For example, the association between rs1982073 in TGF β 1 and RP grades ≥ 2 originally found at the Texas MD Anderson Cancer Center, Houston, TX,⁹ was not replicated in either a European cohort²⁰ or a Chinese non-small-cell lung cancer (NSCLC) cohort.²¹ Therefore, replications of these association studies are necessary.

In this study, we comprehensively analysed the linkage disequilibrium (LD) structure of all APEX1 polymorphisms using HapMap Han Chinese in Beijing data and selected tagSNPs to investigate whether these polymorphisms are associated with the risk of RP or the time to manifestation of RP in our patients with lung cancer. To reduce the influence of clinical factors on genetic analyses, we first investigated the clinical factor associated with RP or the time to RP occurrence and then adjusted these factors in the genetic association analysis. Finally, the RP susceptibility locus was mapped on the three-dimensional (3D) APEX1 protein model to show which functional domain of APEX1 influences the risk of RP.

METHODS AND MATERIALS

Patient population

A total of 168 patients with NSCLC undergoing radiotherapy were recruited at the Department of Radiation Oncology at Daping Hospital in Chongqing, China, from 2006 to 2012 and at the Cancer Center of Chengdu Military General Hospital in Chengdu, China, from 2009 to 2012, who received radiotherapy treatment from Z-ZY, JX and LZ. Finally, 126 patients completed (at least 3-month) follow-up information and provided whole-blood samples. Among the patients, 11 subjects were recruited during 2006–07 and the remainder were recruited during 2008–12. They were unrelated ethnic Han Chinese individuals, according to self-reported ancestry. Overall, 74.6% of the patients had a KPS > 70 before radiotherapy (Table 1). A total of 48.4% of the patients received radiation plus chemotherapy, whereas the others received radiation only. The median total irradiation dose was 60 Gy (range, 30.0–70.6 Gy) given at 1.5–3.0 Gy per fraction. Three of the patients with lung cancer received only 30 Gy, because they could not continue treatment owing to their physical condition; other reasons included distant complications and accidents. Although they received only 30 Gy, one of them developed RP and two did not. Three radiation

oncologists assessed RP according to the symptoms and imaging information available for each patient using the Common Terminology Criteria for Adverse Events v. 3.0.²² Written informed consent was obtained from all of the subjects before undergoing radiotherapy. Genomic DNA from the patients was extracted from ethylenediaminetetraacetic acid-treated whole blood using the Wizard® genomic DNA purification kit (Promega, Madison, WI) according to the manufacturer's instructions. The DNA samples were then stored at -20°C until use. The study was performed with the approval of the ethical committee of Daping Hospital and the Chengdu Military General Hospital, Chengdu, China.

Single nucleotide polymorphism selection and genotyping method

To select the tagSNPs of APEX1 on chromosome 14q11.2, the SNP data for the Han Chinese population Han Chinese in Beijing (CHB), China, was downloaded from HapMap (http://hapmap.ncbi.nlm.nih.gov/downloads/genotypes/latest_phaseII_ncbi_b36/rs_strand/non-redundant/) and analysed by HaploView v. 4.1 software (<http://broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/downloads>). We analysed the LD structure of the chromosome 14 region from 19977394 to 20019953, covering the APEX1 gene (shown in Supplementary Figure A). In the APEX1 gene region, there were only four SNPs (rs1130409, rs1760944, rs2307486 and rs3136817) with a minor allele frequency (MAF) $> 0.1\%$ in the Han Chinese population, which was quite different from the LD structure of the CEU population (Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain collection) (Supplementary Figure A). As for the LD structure, rs1760944 was in one block, including rs938882 (chr14 19986827), rs938881 (chr14 19987282), rs999692 (chr14 19987505), rs883037 (chr14 19987584) and rs3120067 (chr14 19989463), which covered the promoter region of APEX1. Therefore, rs1760944 was selected to represent this haplotype block. Multi-SNaPshot genotyping assays were performed by experts who were blinded to the clinical information. The genotype concordance rate was 100%, and the call rate was 99.4% (primer sequences for the SNP genotypes are supplied in Supplementary Table A).

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) test was undertaken by a Pearson χ^2 goodness-of-fit test. Binary logistical regression was used to examine the association between clinical characteristics or SNPs and severe RP using SPSS® v. 16.0 (SPSS Inc., Chicago, IL). Mantal–Cox log-rank test was performed to estimate the cumulative RP probability by the genotype or clinical factors using GraphPad Prism® software (GraphPad Software, Inc., La Jolla, CA). The odds ratios (ORs) were calculated with a 95% confidence interval (CI). All *p*-values refer to two-sided tests, with *p* ≤ 0.05 considered statistically significant.

Mapping protein polymorphisms onto APEX1 three-dimensional structures

The 3D structure of APEX1 was available from the National Center of Biotechnology Information (NCBI) Structure Summary Molecular Modeling database (<http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=94694>). The APEX1 gene

Table 1. Association between the clinical factors and the radiation-induced pneumonitis (RP) (grade ≥ 3) among 126 patients with lung cancer receiving radiotherapy with completed follow-up

Characteristics	Number (%)	Incidence (%)	The risk of RP (grade ≥3)			The time to occurrence of RP (grade ≥3)		
			Odds ratio	95% CI	<i>p</i> -value ^a	Hazard ratio	95% CI	<i>p</i> -value ^b
Gender								
Male	103 (81.7)	10.7	–	1	–	–	1	–
Female	23 (18.3)	21.7	2.32	0.72–7.50	0.160	2.36	0.66–8.53	0.230
Age (years)								
≤60	54 (42.9)	11.1	–	1	–	–	1	–
>60	72 (57.1)	13.9	1.29	0.44–3.80	0.640	0.54	0.19–.52	0.260
Smoking status								
Never	41 (32.5)	12.2	–	1	–	–	1	–
Ever	85 (67.5)	12.9	1.07	0.35–3.31	0.910	1.29	0.44–3.80	0.700
Tumour location								
Lower	32 (25.4)	6.3	–	1	–	–	1	–
Up and center	94 (74.6)	14.9	0.38	0.08–1.78	0.220	0.48	0.15–1.49	0.200
Histology								
Adenocarcinoma	56 (44.4)	14.3	–	1	–	–	1	–
Squamous cell	57 (45.2)	8.8	0.58	0.18–1.89	0.360	1.80	0.57–5.65	0.330
Others	13 (10.3)	23.1	1.80	0.41–8.00	0.440	0.53	0.11–2.51	0.410
Tumour stage at diagnosis								
T1–T2	65 (51.6)	9.2	–	1	–	–	1	–
≥T3	61 (48.4)	16.4	1.93	0.66–5.67	0.230	0.49	0.17–1.36	0.170
N0–2	56 (44.4)	10.7	–	1	–	–	–	–
N3	70 (55.6)	14.3	1.39	0.47–4.09	0.550	1.48	0.52–4.18	0.480
M0	85 (67.5)	15.3	–	1	–	–	–	–
M1	41 (32.5)	7.3	1.84	0.64–5.28	0.210	0.38	0.13–1.12	0.080
Karnofsky performance status								
≥70	94 (74.6)	7.4	–	1	–	–	1	–
<70	32 (25.4)	28.1	1.81	1.03–3.18	0.004	6.77	2.07–22.12	0.003
Chemotherapy								
No	65 (51.6)	21.5	–	1	–	–	1	–
Yes	61 (48.4)	3.3	4.29	1.16–15.87	0.002	0.19	0.07–0.51	0.001
Dose–volume histogram parameters								
V5 ≤60%	98 (77.8)	13.3	–	1	–	–	1	–
V5 >60%	28 (22.2)	10.7	1.21	0.41–3.56	0.720	0.57	0.17–1.94	0.370
V10 ≤40%	78 (61.9)	10.3	–	1	–	–	–	–
V10 >40%	48 (38.1)	16.7	1.75	0.61–5.02	0.300	1.28	0.44–3.78	0.620
V20 ≤25%	76 (60.3)	9.2	–	1	–	–	–	–
V20 >25%	50 (39.7)	18.0	2.16	0.75–6.25	0.150	1.65	0.57–4.83	0.330
V30 ≤15%	91 (72.2)	8.8	–	1	–	–	–	–
V30 >15%	35 (27.8)	22.9	3.07	1.05–8.98	0.040	2.40	0.72–7.92	0.140

(Continued)

Table 1. (Continued)

Characteristics	Number (%)	Incidence (%)	The risk of RP (grade ≥3)			The time to occurrence of RP (grade ≥3)		
			Odds ratio	95% CI	<i>p</i> -value ^a	Hazard ratio	95% CI	<i>p</i> -value ^b
Radiation dose (Gy)								
<60 Gy	45 (35.7)	13.3	–	1	–	–	1	–
≥60 Gy	81 (64.3)	12.3	0.92	0.31–2.71	0.870	0.94	0.33–2.67	0.930

CI, confidence interval; V5, lung volume receiving >5 Gy; V10, lung volume receiving >10 Gy; V20, lung volume receiving >20 Gy; V30, lung volume receiving >30 Gy.

^a*p*-values were obtained by logistic regression with SPSS® v. 13.0 software (SPSS Inc., Chicago, IL).

^b*p*-values were obtained by χ^2 (Gehan-Breslow-Wilcoxon test) (Mantel-Haenszel).

SNP information was available from the NCBI dbSNP Short Genetic Variations database (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=328). Polymorphisms were mapped onto the 3D structure of APEX1 through sequence alignment and displayed using Cn3D macromolecular structure viewer 4.3 (<http://ncbi.nlm.nih.gov/Structure/CN3D/cn3dinstall.shtml>).

RESULTS

Association between clinical characteristics and risk of radiation-induced pneumonitis with grade ≥ 3

By the time of the final analysis (August 2012), the median follow-up time of 126 patients was 39.5 months. According to the Radiation Therapy Oncology Group, RP ≥ 3 requires the administration of medication and oxygen therapy in hospital. Subjects were divided into a case group (RP of grade ≥ 3 and severe RP) and a control group (RP with grade 0–2). To determine whether any confounding factors were influencing the risk of RP, we analysed the associations between severe RP and clinical factors. We found KPS, concurrent chemotherapy–radiotherapy (CCRT) and lung volume receiving >30 Gy (V30) to be associated with severe RP (OR for KPS <70 vs KPS ≥ 70 , 1.81; 95% CI, 1.03–3.18; *p* = 0.004; OR for CCRT vs radiotherapy, only 4.29; 95% CI, 1.16–15.87; *p* = 0.002; OR for V30 >15% vs $\leq 15\%$, 3.07; 95% CI, 1.05–8.98; *p* = 0.04, shown in Table 1). None of the other clinical factors was associated with RP risk in our subjects.

Association between clinical characteristics and the time to occurrence of radiation-induced pneumonitis (grade ≥ 3)

To examine whether any clinical factor has an effect on the time to occurrence of RP, we performed Mantel–Cox log-rank test and found KPS and concurrent chemotherapy to be associated with the time to occurrence of severe RP [hazard ratio (HR) for KPS <70 vs ≥ 70 , 6.77; 95% CI, 2.07–22.12; *p* = 0.003; HR for CCRT vs radiotherapy, only 0.19; 95% CI, 0.07–0.51; *p* = 0.001, Table 1]. Other clinical factors had no effect on the time to occurrence of severe RP.

Association between APEX1 single nucleotide polymorphisms and risk of radiation-induced pneumonitis grade ≥ 3

To investigate whether APEX1 genetic variants were associated with severe RP, four SNPs were genotyped in 126 subjects. We found that none of the subjects had the mutant genotype GG for SNP rs2307486, and only one patient was homozygous for the

mutant genotype CC of rs3136817. All SNPs were in HWE (*p* > 0.05) in both patients and controls. SNP rs1130409 was associated with severe RP. GT genotype of rs1130409 was significantly higher in patients with RP than those in the control group (68.8% vs 41.8%; *p* = 0.025; resulting OR, 5.98). Patients with lung cancer bearing the G allele had a 5.83-fold higher risk than those with the wild TT genotype (OR = 5.83; 95% CI, 1.27–26.90; *p* = 0.024), and this was further confirmed by the binary regression adjusted by some confounding factors, including KPS, CCRT and V30 (OR = 6.96; 95% CI, 1.36–35.77; *p* = 0.02, Table 2). No association with severe RP was found for the other SNPs (Table 2).

Association between APEX1 single nucleotide polymorphisms and the time to occurrence of radiation-induced pneumonitis (grade ≥ 3)

To further investigate the association between SNPs and the time to occurrence of severe RP, the log-rank (Mantel–Cox) test was carried out using GraphPad Prism. As shown in Figure 1, rs1130409 was associated with the time to occurrence of severe RP (*p* = 0.04). Patients with lung cancer bearing the wild genotype TT developed less severe RP and had a later onset than the other patients with the genotypes GT or GG. (Figure 1a). However, we found no evidence of other tagSNPs being associated with the time to occurrence of severe RP (Figure 1b–d).

Mapping rs1130409 onto APEX1 three-dimensional structures

APEX1 has two separate functional domains: a DNA base excision repair domain and a redox regulator domain. rs1130409 is a missense SNP in exon 5 of the APEX1 gene (gene identity: 328), which arose our interest in investigating whether it is located within an active site of the APEX1 protein. The 3D APEX1 protein structure model (<http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=94694>) and the molecular structure of APEX1 when bound to DNA (<http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi>) were modelled. The sequence of rs1130409 (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=328) was compared with that of APEX1. rs1130409 is located between the active sites of APEX1, including the DNA-binding sites, the metal-binding sites and the putative catalytic sites (Figure 2A). The distance between rs1130409 and the nearest active site, a DNA-binding site, is approximately 18 aa in the primary structure of APEX1 (Figure 2B).

Table 2. Association between the *APEX1* single nucleotide polymorphisms (SNPs) and radiation-induced pneumonitis grade ≥ 3

SNP/minor allele	Genotype	Controls, n (%)	Cases, n (%)	Codominant model		Dominant model		Dominant model adjusted by clinical factors	
				OR (95% CI)	p-value ^a	OR (95% CI)	p-value ^a	OR (95% CI)	p-value ^b
rs1130409/G	TT	50 (45.5)	2 (12.5)	1	–				
	GT	46 (41.8)	11 (68.8)	5.98 (1.26–28.42)	0.025	5.83 (1.27–26.90)	0.024	6.96 (1.36–35.77)	0.020
	GG	14 (12.7)	3 (18.8)	5.36 (0.81–35.28)	0.081				
rs1760944/G	TT	36 (32.7)	3 (18.8)	1	–				
	GT	56 (50.9)	10 (62.5)	2.14 (0.55–8.32)	0.271	2.11 (0.57–7.87)	0.267	2.47 (0.58–10.44)	0.220
	GG	18 (16.4)	3 (18.8)	2.00 (0.37–10.92)	0.423				
rs2307486/G	AA	103 (93.6)	11 (100.0)	1	–				
	GA	7 (6.4)	2 (12.5)	0.48 (0.09–2.52)	0.383	0.48 (0.09–2.52)	0.383	9.79 (0.49–194.67)	0.140
	GG	0 (0.0)	0 (0.0)	–	–				
rs3136817/C	TT	91 (82.7)	13 (81.2)	1	–				
	CT	18 (16.4)	3 (18.8)	1.17 (0.30–4.52)	0.823	1.11 (0.29–4.26)	0.884	1.32 (0.30–5.76)	0.710
	CC	1 (0.9)	0 (0.0)	–	–				

CI, confidence interval; OR, odds ratio.

^ap-values were calculated by logistic regression model using univariate analysis.^bp-values were calculated with adjustment for gender, age, smoking status, tumour location, tumour histology and stage, chemotherapy, dose-volume histogram parameters (lung volume receiving >5, >10, >20 and >30 Gy), Karnofsky performance scale and radiation dose.

Figure 1. Percentage of grade ≥ 3 radiation-induced pneumonitis (RP) in 126 patients with lung cancer as a function of time from the start of radiation therapy and by tag single nucleotide polymorphism (tagSNP) genotype: (a) rs1130409, (b) rs1760944, (c) rs2307486 and (d) rs3136817. In each tagSNP, the solid line represents the wild homozygous genotype and the dashed lines represent the mutant homozygous genotype.

The 3D molecular model of APEX1 bound to DNA revealed that rs1130409 is located within the random coil structure near the DNA-binding site but not within the AP-binding or metal-binding sites (Figure 2B).

DISCUSSION

We investigated the association between clinical factors or genetic factors and severe RP in this study. It was found that the clinical factors, KPS, CCRT and V30 are associated with an increased risk of RP, whereas rs1130409 in the *APEX1* was associated not only with susceptibility to grade ≥ 3 RP but also with increased time to the occurrence of severe RP. Patients with NSCLC bearing the G allele had a 5.83-fold higher risk of severe RP than did other patients ($p = 0.024$; OR, 5.83; 95% CI, 1.27–26.9), even after adjusting for other confounding factors ($p = 0.02$), suggesting that the minor allele G of rs1130409 is a risk allele for grade ≥ 3 RP in Han Chinese patients with NSCLC. Mantel–Cox log-rank test also showed that this SNP was associated with the time to occurrence of RP grade ≥ 3 .

The redox and AP endonuclease activities of APEX1 reside in physically separate domains: the N-terminal 127 and C-terminal 157 amino acids, respectively.²³ The SNP rs1130409, a non-synonymous SNP in exon 5 of *APEX1*, lies within the AP endonuclease domain. Mapping rs1130409 onto the APEX1 3D model revealed that rs1130409 is located in the random coil region (Figure 2) rather than in the DNA-binding site, suggesting that the exchange of aspartic acid and glutamic acid may affect the coil structure of APEX1 rather than have a direct influence on DNA binding. This coincides with the results showing that the mutant with rs1130409 (D148E) had a slightly

lower incision activity of 0.94 relative to the wild type.²⁴ Radiation-produced DNA fragments may act as one of the damage-associated molecular pattern molecules that induce a non-infectious inflammatory response.²⁵ Individuals with mutant APEX1 with reduced DNA repair activity have increased RP susceptibility.

Our results in a Han Chinese population combined with the previous results in a mixed population (mainly Caucasians) show that this APEX1 polymorphism is a common risk factor for RP. However, it is associated with different grades of RP in different populations. In the mixed population (mainly Caucasians), this SNP was a RP grade ≥ 2 susceptibility locus, whereas in our Han Chinese patients, it was associated with a RP grade ≥ 3 instead. The divergent results may be attributed to the genetic heterogeneity of RP and differences in the genetic susceptibility to inflammation across the racial groups. The incidence of grade ≥ 2 RP in the study from the Texas MD Anderson Cancer Center was 28.57%, which was slightly higher than the result of 23.0% found in our patients.

No association was found between these SNPs and grade ≥ 3 RP, possibly owing to the low statistical power of the sample (the genetic power calculator²⁶). Also, we used a logistic regression model rather than Bonferroni correction to explore whether these SNPs were associated with severe RP in Han Chinese patients with lung cancer. Therefore, further association studies with larger sample sizes are needed to confirm whether the other three SNPs (rs1760944, rs2307486 and rs3136817) are associated with the risk of grade ≥ 3 RP.

In conclusion, the non-synonymous SNP rs1130409 in the *APEX1* gene was found to be associated with the risk of grade

Figure 2. Comparison of rs1130409 site and the active sites of the APEX1 protein on the sequence and three-dimensional model. (A) rs1130409 is indicated by a black arrow. The DNA binding sites, active sites, metal-binding sites and putative catalytic sites of the APEX1 protein are shown as red triangles. Non-synonymous single nucleotide polymorphisms (SNPs) are shown as green triangles, and synonymous SNPs are shown as yellow triangles. (B) Different secondary structures of APEX1 are shown in different colours: α -helices are shown in green, strands are shown in brown and random coils are shown in blue. Arrowheads indicate the N-to-C direction of the protein backbone. The SNP rs1130409 is highlighted in light yellow. Pink balls indicate the molecular structure of DNA. (a) rs1130409 on the APEX1 3D protein model. (b-d) are the APEX1 structures bound to DNA viewed from different angles: side, top and bottom. For colour image see online www.birpublications.org/doi/abs/10.1259/bjr.20140093.

≥ 3 RP in Han Chinese patients with lung cancer. The D148E mutant APEX1 protein may influence RP grade ≥ 3 susceptibility by reducing the DNA repair activity. APEX1 rs1130409 may be a potential biomarker for the prediction of severe RP.

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REFERENCES

- Roach M 3rd, Gandara DR, Yuo HS, Swift PS, Kroll S, Shrieve DC, et al. Radiation pneumonitis following combined modality therapy for lung cancer: analysis of prognostic factors. *J Clin Oncol* 1995; **13**: 2606–12.
- Rodrigues G, Lock M, D'Souza D, Yu E, Van Dyk J. Prediction of radiation pneumonitis by dose - volume histogram parameters in lung cancer—a systematic review. *Radiother Oncol* 2004; **71**: 127–38. doi: [10.1016/j.radonc.2004.02.015](https://doi.org/10.1016/j.radonc.2004.02.015)

3. Provatoopoulou X, Athanasiou E, Gounaris A. Predictive markers of radiation pneumonitis. *Anticancer Res* 2008; **28**: 2421–32.
4. Koga K, Kusumoto S, Watanabe K, Nishikawa K, Harada K, Ebihara H. Age factor relevant to the development of radiation pneumonitis in radiotherapy of lung cancer. *Int J Radiat Oncol Biol Phys* 1988; **14**: 367–71.
5. Kong FM, Ten Haken R, Eisbruch A, Lawrence TS. Non-small cell lung cancer therapy-related pulmonary toxicity: an update on radiation pneumonitis and fibrosis. *Semin Oncol* 2005; **32**: S42–54.
6. Johansson S, Bjerrmer L, Franzen L, Henriksson R. Effects of ongoing smoking on the development of radiation-induced pneumonitis in breast cancer and oesophagus cancer patients. *Radiother Oncol* 1998; **49**: 41–7.
7. Hope AJ, Lindsay PE, El Naqa I, Alaly JR, Vivic M, Bradley JD, et al. Modelling radiation pneumonitis risk with clinical, dosimetric, and spatial parameters. *Int J Radiat Oncol Biol Phys* 2006; **65**: 112–24.
8. Barnett GC, Elliott RM, Alsner J, Andreassen CN, Abdelhay O, Burnet NG, et al. Individual patient data meta-analysis shows no association between the SNP rs1800469 in TGF β 1 and late radiotherapy toxicity. *Radiother Oncol* 2012; **105**: 289–95. doi: [10.1016/j.radonc.2012.10.017](https://doi.org/10.1016/j.radonc.2012.10.017)
9. Yuan X, Liao Z, Liu Z, Wang LE, Tucker SL, Mao L, et al. Single nucleotide polymorphism at rs1982073:T869C of the TGF- β 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**: 3370–8.
10. Yin M, Liao Z, Liu Z, Wang LE, O'Reilly M, Gomez D, et al. Genetic variants of the non-homologous end joining gene LIG4 and severe radiation pneumonitis in non-small cell lung cancer patients treated with definitive radiotherapy. *Cancer* 2012; **118**: 528–35.
11. Yang M, Zhang L, Bi N, Ji W, Tan W, Zhao L, 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**: 1402–7. doi: [10.1016/j.ijrobp.2009.12.042](https://doi.org/10.1016/j.ijrobp.2009.12.042)
12. Mak RH, Alexander BM, Asomaning K, Heist RS, Liu CY, Su L, et al. A single-nucleotide polymorphism in the MTHFR (methylene tetrahydrofolate reductase) gene is associated with risk of radiation pneumonitis in lung cancer patients treated with thoracic radiation therapy. *Cancer* 2012; **118**: 3654–65.
13. Zhang L, Yang M, Bi N, Fang M, Sun T, Ji W, et al. ATM polymorphisms are associated with risk of radiation-induced pneumonitis. *Int J Radiat Oncol Biol Phys* 2010; **77**: 1360–8. doi: [10.1016/j.ijrobp.2009.07.1675](https://doi.org/10.1016/j.ijrobp.2009.07.1675)
14. Liu H, Colavitti R, Rovira II, Finkel T. Redox-dependent transcriptional regulation. *Circ Res* 2005; **97**: 967–74. doi: [10.1161/01.RES.0000188210.72062.10](https://doi.org/10.1161/01.RES.0000188210.72062.10)
15. Tell G, Fantini D, Quadrifoglio F. Understanding different functions of mammalian AP endonuclease (APE1) as a promising tool for cancer treatment. *Cell Mol Life Sci* 2010; **67**: 3589–608. doi: [10.1007/s00018-010-0486-4](https://doi.org/10.1007/s00018-010-0486-4)
16. Yin M, Liao Z, Liu Z, Wang LE, Gomez D, Komaki R, 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. doi: [10.1016/j.ijrobp.2010.11.079](https://doi.org/10.1016/j.ijrobp.2010.11.079)
17. Shaheen R, Faqih E, Alshammari MJ, Swaid A, Al-Gazali L, Mardawi E, et al. Genomic analysis of Meckel-Gruber syndrome in Arabs reveals marked genetic heterogeneity and novel candidate genes. *Eur J Hum Genet* 2013; **21**: 762–8. doi: [10.1038/ejhg.2012.254](https://doi.org/10.1038/ejhg.2012.254)
18. Zhang G, Karns R, Sun G, Indugula SR, Cheng H, Havas-Augustin D, et al. Finding missing heritability in less significant Loci and allelic heterogeneity: genetic variation in human height. *PLoS One* 2012; **7**: e51211.
19. Andersen V, Ernst A, Svventoraityte J, Kupcinskis L, Jacobsen BA, Krarup HB, et al. Assessment of heterogeneity between European Populations: a Baltic and Danish replication case-control study of SNPs from a recent European ulcerative colitis genome wide association study. *BMC Med Genet* 2011; **12**: 139.
20. Voets AM, Oberije C, Struijk RB, Reymen B, Ruyck KD, Thierens H, et al. No association between TGF- β 1 polymorphisms and radiation-induced lung toxicity in a European cohort of lung cancer patients. *Radiother Oncol* 2012; **105**: 296–8.
21. Wang L, Bi N. TGF- β 1 gene polymorphisms for anticipating radiation-induced pneumonitis in non-small-cell lung cancer: different ethnic association. *J Clin Oncol* 2010; **28**: e621–2. doi: [10.1200/JCO.2010.31.0458](https://doi.org/10.1200/JCO.2010.31.0458)
22. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol* 2003; **13**: 176–81. doi: [10.1016/S1053-4296\(03\)00031-6](https://doi.org/10.1016/S1053-4296(03)00031-6)
23. Xanthoudakis S, Miao GG, Curran T. The redox and DNA-repair activities of Ref-1 are encoded by nonoverlapping domains. *Proc Natl Acad Sci U S A* 1994; **91**: 23–7.
24. Hadi MZ, Coleman MA, Fidelis K, Mohrenweiser HW, Wilson DM. 3rd. Functional characterization of Ape1 variants identified in the human population. *Nucleic Acids Res* 2000; **28**: 3871–9.
25. Nakamura T, Ito T, Igarashi H, Uchida M, Hijioka M, Oono T, et al. Cytosolic double-stranded DNA as a damage-associated molecular pattern induces the inflammatory response in rat pancreatic stellate cells: a plausible mechanism for tissue injury-associated pancreatitis. *Int J Inflam* 2012; **2012**: 504128.
26. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003; **19**: 149–50.