ORIGINAL ARTICLE

Potentially Functional Variants of *ATG16L2* Predict Radiation Pneumonitis and Outcomes in Patients with Non–Small Cell Lung Cancer after Definitive Radiotherapy

Juyi Wen, PhD,[a,b] Hongliang Liu, PhD,[c,d] Lili Wang, PhD,[c,d] Xiaomeng Wang, PhD,[c,d] Ning Gu, MD,[c,d] Zhensheng Liu, PhD,[c,d] Ting Xu, PhD,[b] Daniel R. Gomez, MD,[b] Ritsuko Komaki, MD,[b] Zhongxing Liao, PhD,[b] Qingyi Wei, MD, PhD[c,d,*]

[a]*Department of Radiation Oncology, Navy General Hospital, Beijing, People's Republic of China*
[b]*Department of Radiation Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas*
[c]*Duke Cancer Institute, Duke University Medical Center, Durham, North Carolina*
[d]*Department of Population Health Sciences, Duke University School of Medicine, Durham, North Carolina*

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ABSTRACT

Introduction: Autophagy not only plays an important role in the progression of cancer but is also involved in tissue inflammatory response. However, few published studies have investigated associations between functional genetic variants of autophagy-related genes and radiation pneumonitis (RP) as well as clinical outcomes in patients with NSCLC after definitive radiotherapy.

Methods: We genotyped nine potentially functional single-nucleotide polymorphisms (SNPs) in four autophagy-related genes (autophagy related 2B gene [*ATG2B*], autophagy related 10 gene [*ATG10*], autophagy related 12 gene [*ATG12*], and autophagy related 16 like 2 gene [*ATG16L2*]) in 393 North American patients with NSCLC treated by definitive radiotherapy and assessed their associations with RP, local recurrence-free survival (LRFS), progression-free survival (PFS), and overall survival (OS) in multivariable Cox proportional hazard regression analyses.

Results: We found that patients with the *ATG16L2* rs10898880 CC variant genotype had a better LRFS, PFS, and OS (adjusted hazard ratio = 0.59, 0.64, and 0.64; 95% confidence interval: 0.45–0.79, 0.48–0.84, and 0.48–0.86; $p = 0.0004$, 0.002, and 0.003, respectively), but a greater risk for development of severe RP (adjusted hazard ratio = 1.80, 95% confidence interval: 1.04–3.12, $p = 0.037$) than did patients with AA/AC genotypes. Further functional analyses suggested that the *ATG16L2* rs10898880 C variant allele modulated expression of the *ATG16L2* gene.

Conclusion: This is the first report that one potentially functional SNP rs10898880 in *ATG16L2* may be a predictor of RP, LRFS, PFS, and OS in patients with NSCLC after definitive radiotherapy. Additional larger, prospective studies are needed to confirm these findings.

Keywords: Non–small cell lung cancer; Autophagy pathway; Radiation pneumonitis; Survival; Single-nucleotide polymorphisms; Cox regression

Introduction

Lung cancer ranks at the top for cancer-related mortality, with more than a million deaths each year worldwide.[1] In the United States, there were approximately 222,500 new cases and 155,870 deaths in 2017.[2] NSCLC is the most common histological type, accounting for approximately 90% of all patients with lung cancer.[3] Radiotherapy alone or chemoradiotherapy is still the standard treatment for unresectable, locally advanced NSCLC, and such a definitive therapy improves local control and overall survival (OS). Unfortunately, the

*Corresponding author.

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Address for correspondence: Qingyi Wei, MD, PhD, Duke Cancer Institute, Duke University Medical Center and Department of Population Health Sciences, Duke University School of Medicine, 905 LaSalle St., Durham, North Carolina, 27710. E-mail: qingyi.wei@duke.edu

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median survival remains less than a year, and the 2-year survival rate is still 15% to 20%.[4] Moreover, radiation pneumonitis (RP), which can occur after radiotherapy as a result of inflammation of normal lung tissues injured by radiation, has been identified as one of the most common dose-limiting complications of thoracic radiation. Among treated patients, approximately 10% to 20% have experienced severe RP (i.e., grade $\geq$3), almost half of whom died of this radiation complication.[5] Therefore, the discovery of suitable biomarkers is needed to predict RP and outcomes of NSCLC after definitive radiotherapy. Increasing evidence suggests that molecular and genetic factors may play an important role in RP development and subsequent clinical outcomes of NSCLC and are accessible molecular markers that may provide therapeutic benefits by predicting clinical outcomes of the patients with NSCLC on an individual basis.[6–8]

Recently, accumulating evidence has indicated that autophagy plays an important role in various stages of cancer development and progression, including in NSCLC.[9,10] Autophagy is a catabolic process that degrades intracellular components through the lysosomal machinery,[11] which usually functions at a low level but is upregulated in response to nutrient starvation, stress, or DNA damage. The role of autophagy in cancer development and progression is complex, because it can act as either a tumor suppressor by degradation of damaged proteins and organelles or as a growth promoter by a mechanism of cell survival depending on many factors like tissue type or tumor stage.[12,13] Generally, autophagy plays an important anticarcinogenic role at an early stage of carcinogenesis by clearing damaged mitochondria and aberrant protein aggregates that produce ROS. Autophagy-related genes (ATGs) play a key role in controlling autophagic formation, and the downregulation of ATG genes directly or indirectly accelerates cancer development and progression.[14]

The ATG family in yeast contains 35 members, of which 16 are currently known in humans (i.e., autophagy related 2A gene [*ATG2A*], autophagy related 2B gene [*ATG2B*], autophagy related 3 gene [*ATG3*], autophagy related 4A gene [*ATG4A*], autophagy related 4B gene [*ATG4B*], autophagy related 4C gene [*ATG4C*], autophagy related 4D gene [*ATG4D*], autophagy related 5 gene [*ATG5*], autophagy related 6 gene [*ATG6*] *(BECN1)*, autophagy related 7 gene [*ATG7*], autophagy related 9A gene [*ATG9A*], autophagy related 9B gene [*ATG9B*], autophagy related 10 gene [*ATG10*], autophagy related 12 gene [*ATG12*], autophagy related 14 gene [*ATG14*], autophagy related 16 like 1 gene [*ATG16L1*], and autophagy related 16 like 2 gene [*ATG16L2*]).[15,16] Studies have demonstrated that some frameshift mutations in ATG genes may have lead to premature cessation of amino acid synthesis of the affected proteins and thus may have inactivated their autophagy function.[17] For example, a mutation in *ATG2B* caused the loss of 1039 of the total 2078 amino acids, whereas a mutation in *ATG5* led to the loss of 41 of the total 275 amino acids.[17] Furthermore, a deficient *ATG5* in neural cells led to progressive neurodegeneration, accompanied by the accumulation of cytoplasmic inclusion bodies.[18] Although the exact roles of ATG10, ATG12, and ATG16 are not yet clear, an increased expression of ATG10 was reported to be significantly associated with lymph node metastasis and lymphovascular invasion in colorectal cancer,[19] and overexpression of ATG12 and ATG16 was demonstrated to inhibit autophagosome formation.[20] More recently, ATG12 silencing was shown to significantly reduce breast cancer cell growth in nude mice, which suggests that ATG12 may be an oncogenic protein.[21]

Recent clinical studies suggested that cancer radiotherapy induced autophagy directly or indirectly through DNA damage.[22] There are two primary opposing functions of radiation-induced autophagy: cytoprotective and cytotoxic. Although radiation-induced autophagy often serves a protective function in cell culture–based studies, the extent to which autophagy may be induced by radiation in human cancer cells is still unclear, although the reported functions of autophagy in response to radiation are inconsistent.[23] Some studies reported that autophagy promoted the anticancer effects of radiotherapy,[24] whereas others showed that upregulation of autophagy was associated with tumor resistance in radiation therapy and that blockade of autophagy contributed to the radiosensitization.[24] More recent reports suggested that inhibition of autophagy led to an increased expression of interleukin-1B and interleukin-6 and that autophagy played an important role in tissue inflammatory response.[25,26] However, the role of genetic variants of ATG genes in RP and outcomes of patients with NSCLC after radiotherapy is largely unknown.

To date, only two studies have reported that mRNA expression and genetic variants of ATG genes contributed to survival and brain metastasis in patients with NSCLC in Chinese populations,[8,27] but none of the published studies investigated functional genetic variants of ATG genes in association with RP and outcomes of patients with NSCLC in a North American population. Potentially functional genetic variants of autophagy genes may alter the host autophagic capacity and thus influence efficiency of therapies.[28] Inspired by these findings, we conducted the present study to test the hypothesis that potentially functional genetic variants in ATG genes are prognostic and predictive for radiation-induced pneumonitis and clinical outcomes in patients with NSCLC after definitive radiotherapy. In the present study, we evaluated the effects of nine functional

variants in important ATG genes (e.g., *ATGB2*, *ATG10*, *ATG12*, and *ATG16L2)* on clinical outcomes among 393 patients with NSCLC in a North American population.

Materials and Methods

Study Populations

Characteristic details of the study population used in the present study were described previously.[7] Briefly, the subjects included 474 patients with primary NSCLC who had been treated with definitive radiation and had available DNA samples as well as clinical follow-up data at a single institution between March 1998 and June 2009. Patients with an inoperable stage I to III disease and patients with oligometastatic stage IV disease (with solitary metastases to the bone or brain) were included. The final group analyzed for single-nucleotide polymorphisms (SNPs) and clinical outcomes consisted of 393 patients who received definitive radiotherapy in the initial treatment, some of whom also received chemotherapy either concurrent with or subsequent to the radiotherapy. Radiation toxicity events were scored according to the Common Terminology Criteria for Adverse Events v3.0, and the details on methods for evaluating local recurrence-free survival (LRFS), progression-free survival (PFS), OS, and RP were also described elsewhere.[7]

Briefly, we used computed tomography (CT) with or without positron emission tomography to evaluate RP at each follow-up visit. The time to RP development was calculated from the start of radiation therapy; patients not experiencing either end point were censored at the date of the last follow-up or death. For monitoring recurrence and scan interval, two primary surveillance strategies were used. The first was the chest CT scan at each of the surveillance visits every 3 to 6 months for the first 5 years and annually thereafter. The second was alternating positron emission tomography/CT and CT scans for 1 to 2 years, reserving positron emission tomography/CT for abnormal findings on the CT scan.[29] We also interviewed each of the 393 eligible patients to obtain data on tobacco smoking. Those who had smoked fewer than 100 cigarettes in their lifetime were considered never-smokers, and all others were considered ever-smokers. Techniques for treatment planning and delivery changed considerably during the study period. For example, the three-dimensional CT-based simulation and three-dimensional conformal radiation therapy were used before July 2004, but the four-dimensional CT-based simulation with respiratory motion management and intensity-modulated radiation therapy or proton beam radiation was used thereafter. The University of Texas M. D. Anderson Cancer Center institutional review board approved the present study, and the Health Insurance Portability and Accountability Act regulations were strictly followed.

Selection of SNPs and Genotyping Assays

The selection of candidate genes *ATGB2*,[17,30] *ATG10*,[8,31,32] *ATG12*,[21,33,34] and *ATG16L2*[35–37] was based on previously published studies, which show a positive association with risk of diseases, including cancers. The public HapMap SNP database (phase II plus III, August10, on NCBI B36 assembly, dbSNP b126) and HaploView 4.2 software were used to screen for common SNPs (minor allele frequency $\geq$0.05) in *ATG2B, ATG10*, *ATG12*, and *ATG16L2* in their gene regions (within these genes or $\pm$ 2-kb flanking regions) in Utah residents with Northern and Western European ancestry. After prediction by using the SNPinfo Web Server (http://snpinfo.niehs.nih.gov/) and RegulomeDB (http://regulomedb.org), candidate SNPs met at least two of the following three criteria: (1) minor allele frequency of 0.05 or higher in whites, (2) SNPs that were reported in previous association studies or with potential function (e.g., either causing amino acid change or affecting transcription factor binding site activity in the putative promoter, intron or 3′ untranslated [3′UTR] regions (FuncPred, http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm), and (3) not in high linkage disequilibrium (i.e., linkage disequilibrium $\geq$0.8). As a result, nine functional SNPs in the *ATG2B*, *ATG10*, *ATG12*, and *ATG16L2* genes were selected: *ATG2B* rs17784271 A>G (3′UTR) and rs4900321 A>T (3′UTR); *ATG10* rs10514231 C>T (intron 2), rs6884232 A>G (3′UTR), and rs4703533 C>G (the promoter region); *ATG12* rs26538 C>T (the promoter region) and rs1058600 C>T (3′UTR); and *ATG16L2* rs1126205 G>T (the promoter region) and rs10898880 A>C (the promoter region) (Supplementary Table 1).

We extracted genomic DNA from the buffy coat fraction of the whole blood samples by using a blood DNA mini kit (Qiagen, Valencia, CA) in a process according to the manufacturer's instructions. The DNA purity and concentration were determined by spectrophotometer measurement of absorbance at 260 and 280 nm. The genotyping was performed by using the TaqMan methodology in 384-well plates and read with the Sequence Detection Software on an ABI-Prism 7900 instrument according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). Primers and probes were supplied by Applied Biosystems. Each plate included four negative controls (no DNA), duplicated positive controls, and eight repeat samples. Amplification was done under the following conditions: 50ºC for 2 minutes, 95ºC for 10 minutes, and 60ºC for 1 minute for 40 cycles. The success rate of all the SNP assays was higher than 99%, and the repeated samples' results were 100% concordant.

Bioinformatic Analysis

Differential expression analysis was performed to evaluate the mRNA expression of *ATG16L2* in lung squamous carcinoma (LUSC), lung adenocarcinoma (LUAD), and paired adjacent normal tissues by using data generated by The Cancer Genome Atlas.[38] The Encyclopedia of DNA Elements (ENCODE) (https://genome.ucsc.edu/) was used to identify the regulatory potential of the region adjoining the SNP. We assessed the associations between SNPs and mRNA expression levels of *ATG16L2* by expression quantitative trait loci analysis by using the data on 338 whole blood cells of individuals of European descendants from the Genotype-Tissue Expression project (https://www.gtexportal.org/home/).

Statistical Analysis

We estimated the associations of the genotypes with RP and clinical outcomes in the patients by using a Cox proportional hazard regression model, with the time to event as described previously.[7] The Kaplan-Meier method was used to visualize RP, LRFS, PFS, and OS by genotype. RP intervals were calculated from the date on which treatment began to the time of incidence of severe RP (grade $\geq$3), LRFS intervals were calculated from the date on which treatment began to the time of tumor recurrence or death (i.e., LRFS) or the time of progression or death (i.e., PFS), and the patients were censored at the time of the final follow-up.[39] The OS interval was calculated from the date of diagnosis until death or the date of last contact. Multivariable Cox proportional hazard regression models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) of each genotype to estimate its effect on RP, LRFS, PFS, and OS with adjustment for confounding factors. Such factors were identified with stepwise Cox regression models that were used to estimate independent predictors of NSCLC prognosis, with a significance level of 0.050 for entering and 0.051 for removing the respective explanatory variables. Further stratified analysis was applied to assess stratified groups. All statistical tests were two sided with a p value of 0.05 considered significant, and all analyses were performed using SAS software version 9.2 (SAS Institute, Cary, NC). We also calculated the false-positive report probability (FPRP) to detect the false-positive association findings.[40] For all the significant results, we calculated FPRP with prior probabilities of 0.0001, 0.001, 0.01, 0.1, and 0.25. The HR was set close to 0.67 (protection) or 1.50 (risk), and a FPRP value less than 0.2 was considered noteworthy.

Results

Characteristics of the Study Population

Demographic and clinical characteristics of the 393 patients treated with definitive radiotherapy, with or without concurrent or subsequent chemotherapy, are presented in Table 1. There were more men (216 [55.0%]) than women (177 [45.0%]), with a median age of 65 years ranging between 35 and 88 years; 83.5% were self-reported non-Hispanic white; 85.5% had a stage III or IV disease (309 stage III and 27 stage IV)[41]; and 91.1% (358 patients) were treated with a combination of radiotherapy and chemotherapy. The median total radiation dose received by the patients was 66 Gy (range 50–88 Gy), with a median mean lung dose (MLD) of 19 Gy (range 2.7–30.6 Gy), a median of 35 fractions (range 15–58 fractions) a median gross tumor volume (GTV) of 108.1 cm^3, and a median follow-up time of 23 months (range 1.0–157.1 months). The overall median RP, LRFS, PFS, and OS times for all the 393 patients were 14.8, 15.2, 10.7, and 23.5 months, respectively. The median occurrence time for severe RP (grade $\geq$3) was 3.6 months (range 1.9–3.6 months) after radiation therapy. To identify potentially confounding factors, we evaluated potential associations of severe RP, LRFS, PFS, and OS with clinical and treatment-related characteristics in univariate Cox models for age, sex, ethnicity, Karnofsky performance status (KPS), disease stage, tumor histological grade, smoking status, use of chemotherapy, MLD, overall radiation time, GTV, and radiation dose, respectively. In the univariate analysis, we found that the MLD was significantly associated with severe RP, LRFS, PFS, and OS (MLD $\geq$19.0 Gy vs. MLD $<$19.0 Gy [crude HR = 3.12, 1.40, 1.35, and 1.45; 95% CI: 1.73–5.64, 1.11–1.76, 1.08–1.69, and 1.15–1.83; p = 0.0002, 0.005, 0.009, and 0.002 for RP, LRFS, PFS, and OS, respectively]) (see Table 1).

Associations of SNPs in ATG2B, ATG10, ATG12, and ATG16L2 with LRFS, PFS, OS, and Risk of RP

Table 2 and Supplementary Table 2 show genotype distributions of the nine SNPs, the results from the multivariable Cox regression analyses of the associations of the nine SNPs with severe RP, LRFS, and PFS as well as with OS in the patients with NSCLC. As shown in Table 2, in the multivariable Cox regression analyses, we found that the *ATG16L2* rs10898880 variant CC homozygotes had a better LRFS, PFS, and OS (adjusted HR [adjHR] = 0.54, 0.57, and 0.59; 95% CI: 0.39–0.76, 0.41–0.79, and 0.41–0.83; p = 0.0005, 0.0009, and 0.003; p_{trend} test = 0.0004, 0.0007, and 0.002, respectively) than did patients with the AA genotype; and these effects in a recessive genetic model also remained statistically significant for a better LRFS, PFS, and OS (adjHR = 0.59, 0.64, and 0.64; 95% CI: 0.45–0.79, 0.48–0.84, and 0.48–0.86; p = 0.0004, 0.002, and 0.003, respectively) after adjustment for potential confounders, including age, sex, histological grade, MLD, disease stage, KPS score, and GTV (see Table 2 and Fig. 1*A*–*C*). In contrast, we found that patients with the variant

Table 1. Demographics of 393 Patients with NSCLC And Their Associations with Hazard of Severe RP, and Clinical Outcomes after Definitive Radiotherapy

	RP (Grade ≥3)				LRFS				PFS				OS			
Parameter	0/1	HR	95% CI	*p* Value[a]	0/1	HR	95% CI	*p* Value[a]	0/1	HR	95% CI	*p* Value[a]	0/1	HR	95% CI	*p* Value[a]
Sex																
Male	181/35	1.00			36/180	1.00			32/184	1.00			41/175	1.00		
Female	154/23	0.76	0.45-1.28	0.300	33/144	0.89	0.72-1.11	0.309	24/153	0.98	0.79-1.21	0.845	42/135	0.88	0.70-1.10	0.263
Age, y																
<65	172/30	1.00			40/162	1.00			31/171	1.00			49/153	1.00		
≥65	163/28	1.03	0.61-1.72	0.922	29/162	1.15	0.93-1.43	0.206	25/166	0.98	0.79-1.21	0.815	34/157	1.18	0.94-1.47	0.147
Race																
White	280/48	1.00			61/267	1.00			49/279	1.00			74/254	1.00		
Black	55/10	1.05	0.53-2.08	0.879	8/57	1.13	0.85-1.51	0.399	7/58	1.13	0.85-1.50	0.395	9/56	1.13	0.85-1.51	0.407
KPS																
≥80	275/42	1.00			59/258	1.00			47/270	1.00			73/244	1.00		
<80	60/16	**1.80**	**1.01-3.20**	**0.046**	10/66	1.28	0.97-1.67	0.079	9/67	1.13	0.86-1.48	0.379	10/66	**1.36**	**1.03-1.79**	**0.028**
Stage groups[b]																
I, II	45/10	1.00			15/40	1.00			15/40	1.00			15/40	1.00		
III, IV	288/48	0.78	0.40-1.55	0.480	54/282	**1.51**	**1.08-2.10**	**0.015**	41/295	**1.80**	**1.29-2.51**	**0.0005**	68/268	**1.42**	**1.02-1.98**	**0.039**
Histological type																
Squamous	118/20	1.00			20/118	1.00			17/121	1.00			22/116	1.00		
Nonsquamous[c]	217/38	1.04	0.61-1.79	0.887	49/206	0.80	0.64-1.00	0.051	39/216	0.93	0.75-1.16	0.529	61/194	**0.79**	**0.63-1.00**	**0.046**
Smoking status																
Ever	305/54	1.00			64/295	1.00			54/305	1.00			74/285	1.00		
Never	30/4	0.85	0.31-2.36	0.760	5/29	1.10	0.75-1.62	0.614	2/32	1.26	0.88-1.82	0.210	9/25	0.95	0.63-1.44	0.821
Chemotherapy																
No	30/4	1.00			6/28	1.00			6/28	1.00			6/28	1.00		
Yes	304/54	1.27	0.46-3.50	0.648	62/296	0.95	0.64-1.39	0.776	49/309	1.03	0.70-1.51	0.895	76/282	0.94	0.64-1.39	0.759
MLD, Gy[b,d]																
<19.0	166/15	1.00			41/140	1.00			34/147	1.00			49/132	1.00		
≥19.0	140/41	**3.12**	**1.73-5.64**	**0.0002**	26/155	**1.40**	**1.11-1.76**	**0.005**	20/161	**1.35**	**1.08-1.69**	**0.009**	32/149	**1.45**	**1.15-1.83**	**0.002**
ORT																
IMRT	149/18	1.00			36/131	1.00			29/138	1.00			47/120	1.00		
3DCRT	139/38	2.09	1.20-3.67	0.010	22/155	1.02	0.81-1.29	0.880	18/159	1.06	0.84-1.34	0.606	24/153	1.17	0.92-1.48	0.215
Other	47/2	0.34	0.08-1.47	0.150	11/38	0.99	0.69-1.42	0.943	9/40	0.97	0.68-1.34	0.857	12/37	1.02	0.70-1.47	0.923

(continued)

Table 1. Continued

Parameter	RP (Grade ≥3) 0/1	HR	95% CI	p Value[a]	LRFS 0/1	HR	95% CI	p Value[a]	PFS 0/1	HR	95% CI	p Value[a]	OS 0/1	HR	95% CI	p Value[a]
GTV[b,e]																
<108 cm^3	151/22	1.00			43/130	1.00			35/138	1.00			53/120	1.00		
≥108 cm^3	142/32	1.68	0.98-2.89	0.062	22/152	**1.66**	**1.31-2.10**	**<0.0001**	18/156	**1.70**	**1.35-2.14**	**<0.0001**	25/149	**1.85**	**1.45-2.36**	**<0.0001**
Radiation dose[f]																
<66 Gy	159/26	1.00			27/158	1.00			24/161	1.00			34/151	1.00		
≥66 Gy	176/32	1.02	0.61-1.71	0.951	42/166	0.81	0.65-1.01	0.059	32/176 101	0.84	0.68-1.04	0.110	49/159	0.83	0.67-1.04	0.111

Note: Items in bold are p value <0.05.
[a]P values were calculated by using univariate Cox proportional hazard models.
[b]Some missing data.
[c]Included large cell, undifferentiated, and mixed cell carcinomas.
[d]Median 19 Gy.
[e]Median 108.1 cm^3.
[f]Median 66 Gy.
RP, radiation pneumonitis; LRFS, local recurrence free survival; PFS, progression-free survival; OS, overall survival; 0, the absence of events; 1, the presence of events; HR, hazard ratio; CI, confidence interval; KPS, Karnofsky performance status; MLD, mean lung dose; IMRT, intensity-modulated radiation therapy; 3DCRT, three-dimensional conformal radiotherapy; ORT, overall radiation time; GTV, gross tumor volume.

ATG16L2 rs10898880 CC genotype had a greater risk of severe RP (adjHR = 1.80, 95% CI: 1.04–3.12, $p = 0.037$) than did patients with AA/AC genotypes (a recessive genetic model) after adjustment for potential confounders, including MLD, disease stage, smoking status, and chemotherapy (see Table 2 and Fig. 1*D*). Our data also showed that patients with the *ATG10* rs4703533 GG variant genotype also had a better LRFS and PFS (adjHR = 0.62 and 0.63; 95% CI: 0.41–0.95 for both; $p = 0.029$ and 0.027 for LRFS and PFS, respectively) than did patients with the CC genotype; and the effect in a recessive genetic model also remained statistically significant for a better LRFS and PFS (adjHR = 0.59 and 0.60; 95% CI: 0.39–0.88 and 0.40–0.89; $p = 0.011$ for both, respectively). In contrast, the variant *ATG10* rs4703533 CG or GG genotype was associated with a greater risk of severe RP (adjHR = 2.04 and 2.49; 95% CI: 1.11–3.73 and 1.14–5.45; $p = 0.021$ and 0.023, respectively [p_{trend} test = 0.008]), and GG/CG genotypes were associated with a significantly increased hazard of severe RP (adjHR = 2.14, 95% CI: 1.21–3.80, $p = 0.009$) compared with the CC genotype (see Table 2). For the other five SNPs, we did not find any significant association with RP, LRFS, PFS, or OS under either additive or recessive genetic models (see Supplementary Table 2), except that the *ATG2B* rs17784271 AG genotype was associated with a poorer LRFS and PFS (adjHR = 1.40 and 1.38; 95% CI: 1.09–1.81 and 1.07–1.77; $p = 0.009$ and 0.012, respectively) than was the AA genotype in multivariable analysis and the *ATG12* rs1058600 TT genotype was associated with a poorer LRFS, PFS, and OS (adjHR = 1.51, 1.43, and 1.50; 95% CI: 1.08–2.10, 1.03–2.01, and 1.07–2.10; $p = 0.016$, 0.035, and 0.019, respectively) than were CC/CT genotypes (see Table 2). Furthermore, we also performed association analyses for each of the nine SNPs with RP, LFS, PFS, and OS in the presence of the other eight SNPs and significant clinical variables. As shown in Supplementary Table 3, only the *ATG16L2* rs10898880 showed a significant association with clinical outcomes, with two other SNPs (*ATG2B* rs17784271 and *ATG10* rs4703533) showing a significant association with RP. Finally, we calculated the correlation coefficients among SNPs. As shown in Supplementary Table 4, except for rs1058600, each SNP showed a significant correlation only with those in the same gene (rs17784271 and rs4900321 in *ATG2B*; rs10514231, rs6884232, and rs4703533 in *ATG10*; rs26538 and rs1058600 in *ATG12*; and rs10898880 and rs1126205 in *ATG16L2*), and rs1058600 also showed a significant correlation with rs4900321, rs6884232, and rs4703533.

Stratified Analyses by Selected Variables

We then performed stratified analyses to evaluate the effects of variant genotypes of *ATG16L2* rs10898880 and *ATG10 rs4703533* on LRFS, PFS, and OS by age, sex, KPS,

Table 2. Associations among Genotypes of Autophagy Related (*ATG*) Genes, Severe RP, and Clinical Outcomes in 393 Patients with NSCLC after Definitive Radiotherapy

	RP (Grade ≥3)				LRFS				PFS				OS			
Genotypes	0/1	HR	95% CI	*p* Value[a]	0/1	HR	95% CI	*p* Value[b]	0/1	HR	95% CI	*p* Value[b]	0/1	HR	95% CI	*p* Value[b]
ATG2B (rs17784271)																
AA	150/32	1.00			41/141	1.00			34/148	1.00			43/139	1.00		
AG	148/23	0.67	0.39-1.16	0.157	21/150	**1.40**	**1.09-1.81**	**0.009**	16/155	**1.38**	**1.07-1.77**	**0.012**	30/141	1.28	0.99-1.66	0.058
GG	37/3	0.45	0.14-1.49	0.192	7/30	1.04	0.67-1.61	0.863	6/34	1.14	0.74-1.74	0.565	10/30	0.90	0.57-1.43	0.657
P_{trend} test				0.078				0.148				0.082				0.513
GG/AG	185/26	0.64	0.37-1.08	0.094	28/180	**1.32**	**1.04-1.69**	**0.024**	22/189	**1.33**	**1.05-1.69**	**0.019**	40/171	1.21	0.94-1.54	0.142
AA/AG	298/55	1.00			62/191	1.00			50/303	1.00			73/280	1.00		
GG	37/3	0.55	0.17-1.75	0.310	7/30	0.89	0.59-1.35	0.577	6/34	0.98	0.65-1.48	0.922	10/30	0.80	0.51-1.25	0.325
ATG10 (rs4703533)																
CC	154/18	1.00			31/141	1.00			25/147	1.00			34/138	1.00		
CG	145/30	**2.04**	**1.11-3.73**	**0.021**	26/149	1.12	0.87-1.45	0.378	20/155	1.10	0.86-1.42	0.435	37/138	1.04	0.80-1.35	0.795
GG	36/10	**2.49**	**1.14-5.45**	**0.023**	12/34	**0.62**	**0.41-0.95**	**0.029**	11/35	**0.63**	**0.41-0.95**	**0.027**	12/34	0.68	0.44-1.04	0.077
P_{trend} test				**0.008**				0.165				0.151				0.199
GG/CG	181/40	**2.14**	**1.21-3.80**	**0.009**	38/183	0.98	0.77-1.25	0.883	31/190	0.97	0.77-1.24	0.829	49/172	0.94	0.74-1.21	0.646
CC/CG	299/48	1.00			57/290	1.00			45/302	1.00			71/276	1.00		
GG	36/10	1.70	0.85-3.37	0.131	12/34	**0.59**	**0.39-0.88**	**0.011**	11/35	**0.60**	**0.40-0.89**	**0.011**	12/34	0.67	0.44-1.01	0.054
ATG12 (rs1058600)																
CC	177/27	1.00			38/166	1.00			32/172	1.00			48/156	1.00		
CT	118/25	1.47	0.84-2.58	0.175	29/114	0.85	0.65-1.11	0.239	22/121	0.88	0.68-1.15	0.353	32/111	0.90	0.68-1.18	0.439
TT	40/6	0.79	0.33-1.93	0.610	2/44	1.41	0.99-2.00	0.054	2/44	1.36	0.96-1.94	0.085	3/43	1.43	1.00-2.05	0.050
P_{trend} test				0.900				0.369				0.361				0.227
TT/CT	158/31	1.26	0.74-2.13	0.398	31/158	0.98	0.77-1.24	0.834	24/165	0.99	0.78-1.25	0.925	35/154	1.02	0.79-1.31	0.885
CC/CT	295/52	1.00			67/280	1.00			54/293	1.00			80/267	1.00		
TT	40/6	0.67	0.29-1.57	0.358	2/44	**1.51**	**1.08-2.10**	**0.016**	2/44	**1.43**	**1.03-2.01**	**0.035**	3/43	**1.50**	**1.07-2.10**	**0.019**
ATG16L2 (rs10898880) T>C)																
AA	83/19	1.00			9/93	1.00			6/96	1.00			13/89	1.00		
AC	172/18	**0.47**	**0.24-0.91**	**0.024**	33/157	0.87	0.65-1.16	0.347	29/161	0.84	0.63-1.12	0.231	40/150	0.87	0.65-1.17	0.347
CC	80/21	1.14	0.60-2.18	0.693	27/74	**0.54**	**0.39-0.76**	**0.0005**	21/80	**0.57**	**0.41-0.79**	**0.0009**	30/71	**0.59**	**0.41-0.83**	**0.003**
P_{trend} test				0.645				**0.0004**				**0.0007**				**0.002**
CC/AC	252/39	0.68	0.38-1.19	0.177	60/231	**0.73**	**0.56-0.96**	**0.024**	50/241	**0.73**	**0.56-0.95**	**0.019**	70/221	**0.75**	**0.57-1.00**	**0.046**
AA/AC	255/37	**1.00**			42/250	1.00			35/257	1.00			53/239	1.00		
CC	80/21	**1.80**	**1.04-3.12**	**0.037**	27/74	**0.59**	**0.45-0.79**	**0.0004**	21/80	**0.64**	**0.48-0.84**	**0.002**	30/71	**0.64**	**0.48-0.86**	**0.003**

Note: Items in bold are *p* value <0.05.

[a]*P* values were calculated by using a Cox proportional model with adjustment for smoking status, mean lung dose, disease stage, and chemotherapy history.

[b]*P* values were calculated by using a Cox proportional model with adjustment for age, sex, mean lung dose, disease stage, Karnofsky performance status score, history, and gross tumor volume.

RP, radiation pneumonitis; LRFS, local recurrence-free survival; PFS, progression-free survival; OS, overall survival; 0, the absence of events; 1, the presence of events; HR, hazard ratio; CI, confidence interval

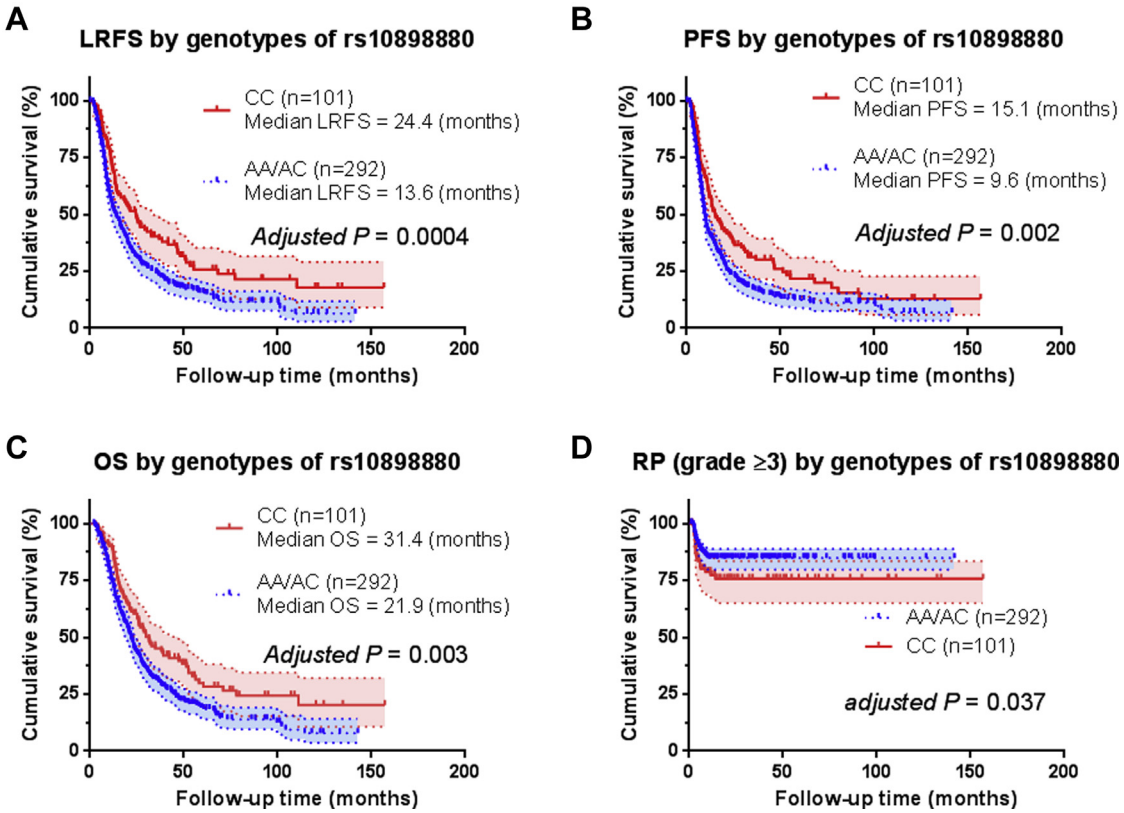


Figure 1. Kaplan-Meier analysis for severe radiation pneumonitis (RP) and clinical outcomes according to autophagy related 16 like 2 gene (*ATG16L2*) rs10898880 CC versus AA/AC genotypes (in a recessive model). The CC variant genotype was associated with local recurrence-free survival (LRFS) ($p = 0.0004$) (*A*), progression- free survival (PFS) ($p = 0.002$) (*B*), overall survival (OS) ($p = 0.003$) (*C*), and severe radiation pneumonitis (grade ≥3) (RP) ($p = 0.037$) (*D*).

disease stage, tumor histological grade, MLD, and GTV under recessive models by using multivariable Cox models for the main effects. As shown in Table 3, the *ATG16L2* rs10898880CC variant homozygotes had a better survival for LRFS, PFS, and OS in males (adjHR = 0.52, 0.54, and 0.53; 95% CI: 0.35–0.77, 0.37–0.79, and 0.35–0.79; $p = 0.001$, 0.002, and 0.002, respectively), KPS of 80 or higher (adjHR = 0.59, 0.64, and 0.63; 95% CI: 0.43–0.82, 0.47–0.88, and 0.45–0.88; $p = 0.002$, 0.006, and 0.006, respectively), and stages III and IV (adjHR = 0.59, 0.64, and 0.65; 95% CI: 0.44–0.80, 0.48–0.85, and 0.48–0.88; $p = 0.0006$, 0.002, and 0.005, respectively) compared with patients with AA/AC genotypes, whereas the CC variant genotype was more prominently associated with an increased risk of severe RP in those younger than 65 years (adjHR = 2.52, 95% CI: 1.18–5.37, $p = 0.016$) and those with stage III or IV disease (adjHR = 1.90, 95% CI: 1.04–3.48, $p = 0.038$) (see Table 3). Compared with CC/CG genotypes, the *ATG10* rs4703533GG variant genotype was more prominently associated with a better survival for LRFS, PFS, and OS in females (adjHR = 0.39, 0.42, and 0.48; 95% CI: 0.20–0.77, 0.22–0.80, and 0.24–0.96; $p = 0.007$, 0.009 and 0.037, respectively), those age 65 years or older (adjHR = 0.47, 0.48, and 0.54; 95% CI: 0.27–0.83, 0.28–0.82, and 0.31–0.95; $p = 0.009$, 0.007, and 0.032, respectively), those with squamous carcinoma (adjHR = 0.40, 0.38, and 0.44; 95% CI: 0.19–0.85, 0.18–0.81, and 0.20–0.93; $p = 0.018$, 0.012, and 0.032, respectively), and those with a GTV less than 108 cm^3 (adjHR = 0.37, 0.40, and 0.45; 95% CI: 0.20–0.69, 0.23–0.73, and 0.24–0.84; $p = 0.002$, 0.002, and 0.012, respectively) (Supplementary Table 5). In contrast, compared with CC/CT genotypes, the *ATG12* rs1058600TT variant genotype was more prominently associated with a poorer survival for LRFS, PFS, and OS in males (adjHR = 1.59, 1.68, and 1.71; 95% CI: 1.02–2.48, 1.08–2.62, and 1.09–2.67; $p = 0.040$, 0.022, and 0.019, respectively), in those 65 years or older (adjHR = 1.69, 1.67, and 1.80; 95% CI: 1.08–2.64, 1.07–2.62, and 1.15–2.83; $p = 0.022$, 0.024, and 0.011, respectively), and in those with a KPS of 80 or higher (adjHR = 1.69, 1.70, and 1.66, 95% CI: 1.17–2.44, 1.17–2.46, and 1.14–2.42; $p = 0.005$, 0.005, and 0.008, respectively) (Supplementary Table 6).

Because most of the significant findings were in the subgroup analyses, we calculated the FPRP values for all nine of the SNPs (Supplementary Table 7) and all the observed significant associations. As shown in Table 4,

Table 3. Stratified Analysis of Autophagy Related 16 Like 2 Gene (*ATG16L2*) rs10898880 Associated with RP, RFS, and OS under Recessive Genetic Models by Selected Patient Positive characteristics

	RP				LRFS				PFS				OS			
Variables	AA/AC (0/1)	CC (0/1)	Adjusted HR (95% CI)	*p* Value[a]	AA/AC (0/1)	CC (0/1)	Adjusted HR (95% CI)	*p* Value[b]	AA/AC (0/1)	CC (0/1)	Adjusted HR (95% CI)	*p* Value[b]	AA/AC (0/1)	CC (0/1)	Adjusted HR (95% CI)	*p* Value[b]
Overall	255/37	80/21	1.80 (1.04-3.12)	0.037	42/250	27/74	0.59 (0.45-0.79)	0.0004	35/257	21/80	0.64 (0.48-0.84)	0.002	53/239	30/71	0.64 (0.48-0.86)	0.003
Sex																
Male	139/23	42/12	1.52 (0.73-3.16)	0.259	21/141	15/39	**0.52 (0.35-0.77)**	**0.001**	19/143	13/41	**0.54 (0.37-0.79)**	**0.002**	25/137	16/38	**0.53 (0.35-0.79)**	**0.002**
Female	116/14	38/9	2.31 (0.96-5.58)	0.062	21/109	12/35	0.71 (0.47-1.08)	0.109	16/114	8/39	0.78 (0.52-1.17)	0.228	28/102 141	14/33	0.81 (0.53-1.25)	0.341
Age, y																
<65	134/19	38/11	**2.52 (1.18-5.37)**	**0.016**	26/127	14/35	**0.51 (0.33-0.78)**	**0.002**	20/133	11/38	**0.55 (0.37-0.82)**	**0.003**	34/119	15/34	**0.63 (0.41-0.96)**	**0.033**
≥65	121/18	42/10	1.41 (0.63-3.18)	0.403	16/123	13/39	**0.62 (0.41-0.92)**	**0.019**	15/124	10/42	**0.66 (0.45-0.99)**	**0.043**	19/120 171	15/37	**0.59 (0.39-0.90)**	**0.014**
KPS																
≥80	209/28	66/14	1.52 (0.78-2.95)	0.216	37/200	22/58	**0.59 (0.43-0.82)**	**0.002**	30/207	17/63	**0.64 (0.47-0.88)**	**0.006**	48/189	25/55	**0.63 (0.45-0.88)**	**0.006**
<80	46/9	14/7	2.39 (0.83-6.89)	0.106	5/50	5/16	0.57 (0.30-1.06)	0.077	5/50	4/17	**0.52 (0.28-0.98)**	**0.043**	5/50	5/16	0.62 (0.33-1.16)	0.134
Stage groups[c]																
I, II	37/7	8/3	1.74 (0.44-6.97)	0.433	11/33	4/7	1.31 (0.41-4.22)	0.651	11/33	4/7	1.14 (0.36-3.63)	0.824	11/33	4/7	1.17 (0.36-3.74)	0.796
III, IV	217/30	71/18	**1.90 (1.04-3.48)**	**0.038**	31/216	23/66	**0.59 (0.44-0.80)**	**0.0006**	24/223	17/72	**0.64 (0.48-0.85)**	**0.002**	42/205 248	26/63	**0.65 (0.48-0.88)**	**0.005**
Histological type																
Squamous	86/13	32/7	1.39 (0.52-3.73)	0.516	10/89	10/29	**0.52 (0.33-0.83)**	**0.006**	9/90	8/31	**0.55 (0.35-0.86)**	**0.009**	12/87	10/29	**0.56 (0.35-0.90)**	**0.017**
Nonsquamous[d]	169/24	48/14	**2.01 (1.02-3.97)**	**0.044**	32/161	17/45	**0.69 (0.48-1.00)**	**0.047**	26/167	13/49	0.73 (0.51-1.03)	0.073	41/152	20/42	0.74 (0.51-1.08)	0.117
MLD (Gy)[c]																
<19.0	123/10	43/5	1.75 (0.57-5.39)	0.326	25/108	16/32	**0.56 (0.37-0.86)**	**0.008**	20/113	14/34	**0.54 (0.36-0.82)**	**0.004**	32/101 138	17/31	**0.64 (0.42-0.99)**	**0.045**
≥19.0	109/26	31/15	1.84 (0.96-3.51)	0.065	16/119	10/36	**0.67 (0.46-0.99)**	**0.044**	14/121	6/40	0.77 (0.53-1.12)	0.173	20/115	12/34	0.67 (0.45-1.00)	0.052
GTV[c,e]																
<108 cm^3	111/12	40/10	2.14 (0.91-5.06)	0.082	28/95	15/35	**0.57 (0.37-0.88)**	**0.009**	23/100	12/38	**0.63 (0.43-0.95)**	**0.026**	36/87	17/33	**0.58 (0.38-0.90)**	**0.015**
≥108 cm^3	109/23	33/9	1.86 (0.83-4.18)	0.130	12/120	10/32	**0.61 (0.41-0.92)**	**0.019**	10/22	8/34	**0.62 (0.41-0.92)**	**0.017**	15/117	10/32	0.68 (0.45-1.02)	0.062

Note: Items in bold are *p* value <0.05.

[a]*P* values were calculated by using a Cox proportional model with adjustment for smoking status, mean lung dose, disease stage, and chemotherapy history.

[b]*P* values were calculated by using a Cox proportional model with adjustment for age, sex, mean lung dose, disease stage, KPS score, history, and gross tumor volume.

[c]Some missing data.

[d]Large cell, undifferentiated, and mixed cell carcinomas.

[e]Median 108.1 cm^3.

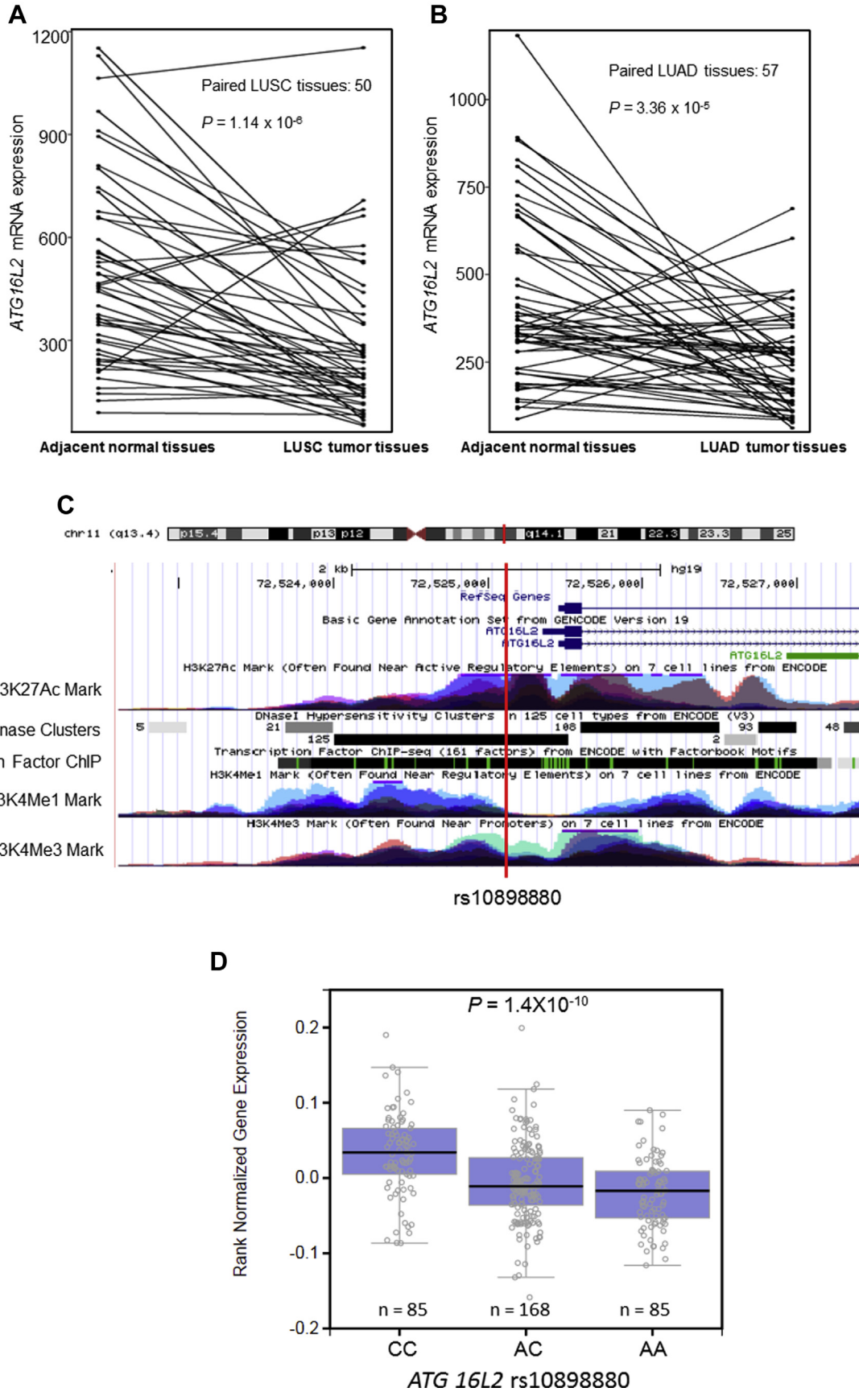
RP, radiation pneumonitis; LRFS, local recurrence-free survival; PFS, progression-free survival; OS, overall survival; 0, the absence of events; 1, the presence of events; HR, hazard ratio; CI, confidence interval; KPS, Karnofsky performance status; MLD, mean lung dose; GTV, gross tumor volume.

Table 4. False-Positive Report Probability (FPRP) Values for Associations between Frequencies of *ATG16L2* rs10898880 Genotypes (Recessive Genetic Model) and Clinical Outcomes in 393 Patients with NSCLC Who Received Definitive Radiotherapy

Genotype	Positive HR 95% CI[a]	*p* Value[b]	Statistical Power[c]	False-Positive Report Probability				
				0.25[d]	0.1[d]	0.01[d]	0.001[d]	0.0001[d]
RP	AA/AC vs. CC							
All subjects	1.80 (1.04-3.12)	0.036	0.646	**0.144**	0.335	0.847	0.982	0.998
Age <65 y	2.52 (1.18-5.37)	0.017	0.275	**0.154**	0.353	0.857	0.984	0.998
Stage III, IV	1.90 (1.04-3.48)	0.038	0.566	**0.166**	0.374	0.868	0.985	0.998
LRFS								
All subjects	0.59 (0.45-0.79)	0.0004	0.867	**0.001**	**0.004**	**0.043**	0.313	0.820
Males	0.52 (0.35-0.77)	0.001	0.587	**0.006**	**0.017**	**0.158**	0.654	0.950
Age <65 y	0.51 (0.33-0.78)	0.002	0.536	**0.010**	**0.031**	0.259	0.779	0.972
Age ≥65 y	0.62 (0.41-0.92)	0.018	0.857	**0.058**	**0.156**	0.670	0.953	0.995
KPS ≥80	0.59 (0.43-0.82)	0.002	0.838	**0.006**	**0.018**	**0.166**	0.667	0.953
Stage III, IV	0.59 (0.44-0.80)	0.0007	0.857	**0.002**	**0.007**	**0.073**	0.443	0.889
Squamous cell	0.52 (0.33-0.83)	0.006	0.565	**0.031**	**0.089**	0.518	0.915	0.991
Nonsquamous cell	0.69 (0.48-1.00)	0.050	0.956	**0.136**	0.320	0.838	0.981	0.998
MLD <19.0	0.56 (0.37-0.86)	0.008	0.698	**0.034**	**0.094**	0.534	0.920	0.991
MLD ≥19.0	0.67 (0.46-0.99)	0.044	0.929	**0.125**	0.301	0.825	0.979	0.998
GTV <108 cm^3	0.57 (0.37-0.88)	0.011	0.723	**0.044**	**0.122**	0.605	0.939	0.994
GTV ≥108 cm^3	0.61 (0.41-0.92)	0.018	0.829	**0.062**	**0.166**	0.687	0.957	0.996
PFS								
All subjects	0.64 (0.48-0.84)	0.001	0.962	**0.004**	**0.012**	**0.118**	0.574	0.931
Males	0.54 (0.37-0.79)	0.002	0.654	**0.007**	**0.020**	**0.185**	0.696	0.958
Age <65 y	0.55 (0.37-0.82)	0.003	0.680	**0.015**	**0.042**	0.328	0.831	0.980
Age ≥65 y	0.66 (0.45-0.99)	0.045	0.910	**0.128**	0.306	0.829	0.980	0.998
KPS ≥80	0.64 (0.47-0.88)	0.006	0.936	**0.019**	**0.055**	0.389	0.865	0.985
KPS <80	0.52 (0.28-0.98)	0.043	0.548	**0.191**	0.414	0.886	0.987	0.999
Stage III, IV	0.64 (0.48-0.85)	0.002	0.956	**0.006**	**0.019**	**0.175**	0.682	0.955
Squamous cell histological type	0.55 (0.35-0.86)	0.009	0.662	**0.038**	**0.106**	0.567	0.930	0.992
MLD <19.0	0.54 (0.36-0.82)	0.004	0.641	**0.018**	**0.051**	0.372	0.857	0.984
GTV <108 cm^3	0.63 (0.43-0.95)	0.027	0.865	**0.087**	0.222	0.759	0.969	0.997
GTV ≥108 cm^3	0.62 (0.41-0.92)	0.018	0.857	**0.058**	**0.156**	0.670	0.953	0.995
OS								
All subjects	0.64 (0.48-0.86)	0.003	0.949	**0.010**	**0.028**	0.243	0.764	0.970
Males	0.53 (0.35-0.79)	0.018	0.613	**0.009**	**0.026**	0.228	0.748	0.968
Age <65 y	0.63 (0.41-0.96)	0.032	0.859	**0.099**	0.249	0.784	0.973	0.997
Age ≥65 y	0.59 (0.39-0.90)	0.014	0.779	**0.052**	**0.142**	0.645	0.948	0.995
KPS ≥80 y	0.63 (0.45-0.88)	0.007	0.912	**0.022**	**0.062**	0.422	0.881	0.987
Stage III, IV	0.65 (0.48-0.88)	0.005	0.955	**0.016**	**0.048**	0.355	0.848	0.982
Squamous cell histological type	0.56 (0.35-0.90)	0.017	0.680	**0.068**	**0.180**	0.707	0.961	0.996
MLD <19.0	0.64 (0.42-0.99)	0.045	0.866	**0.135**	0.318	0.837	0.981	0.998
GTV <108 cm^3	0.58 (0.38-0.90)	0.015	0.746	**0.057**	**0.154**	0.667	0.953	0.995

[a]The adjusted HR.
[b]The omnibus chi-square test of the genotype frequency distributions.
[c]Calculated using study subjects to detect an OR of 2.00 with the common genotype used as the reference in FPRP level 0.2.
[d]Prior probability.
RP, radiation pneumonitis; LRFS, local recurrence-free survival; PFS, progression- free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; KPS, Karnofsky performance status; MLD, mean lung dose; GTV, gross tumor volume.

when the assumption of a prior probability was 0.25, the associations of severe RP and clinical outcomes with the *ATG16L2* rs10898880CC genotype (a recessive genetic model) were still noteworthy for all subjects (FPRP = 0.144, 0.001, 0.004, and 0.010 for RP, LRFS, PFS, and OS, respectively), those younger than 65 years (FPRP = 0.154, 0.010, 0.015, and 0.099 for RP, LRFS, PFS, and OS, respectively), and those with stage III or IV disease (FPRP = 0.166, 0.002, 0.006, and 0.016 for RP, LRFS, PFS, and OS, respectively). When the assumption of a prior probability was 0.1, the associations of clinical outcomes with the *ATG16L2* rs10898880CC genotype (a recessive genetic model) were still noteworthy for all subjects (FPRP = 0.004, 0.012, and 0.028 for LRFS, PFS, and OS, respectively), those younger than 65 years (FPRP = 0.031 and 0.042 for LRFS and PFS, respectively), and those with stage III or IV disease (FPRP = 0.007, 0.019, and 0.048 for LRFS, PFS, and OS, respectively).


A
ATG16L2 mRNA expression
1200
900
600
300
Paired LUSC tissues: 50
P = 1.14 x 10-6
Adjacent normal tissues
LUSC tumor tissues
B
ATG16L2 mRNA expression
1000
750
500
250
Paired LUAD tissues: 57
P = 3.36 x 10-5
Adjacent normal tissues
LUAD tumor tissues
C
chr11 (q13.4)
2 kb
hg19
72,524,000
72,525,000
72,526,000
72,527,000
RefSeq Genes
Basic Gene Annotation Set from GENCODE Version 19
ATG16L2
H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE
H3K27Ac Mark
DNaseI Hypersensitivity Clusters in 125 cell types from ENCODE (V3)
Dnase Clusters
Transcription Factor ChIP-seq (161 factors) from ENCODE with Factorbook Motifs
Txn Factor ChIP
H3K4Me1 Mark (Often Found Near Regulatory Elements) on 7 cell lines from ENCODE
H3K4Me1 Mark
H3K4Me3 Mark (Often Found Near Promoters) on 7 cell lines from ENCODE
H3K4Me3 Mark
rs10898880
D
P = 1.4X10-10
Rank Normalized Gene Expression
0.2
0.1
0.0
-0.1
-0.2
n = 85
n = 168
n = 85
CC
AC
AA
ATG 16L2 rs10898880

For *ATG10* rs4703533, when the assumption of a prior probability was 0.25, the associations of clinical outcomes with the *ATG10* rs4703533GG genotypes (a recessive genetic model) were still noteworthy for all subjects (FPRP = 0.035, 0.039, and 0.154 for LRFS, PFS, and OS, respectively), those age 65 years or older (FPRP = 0.194, 0.063, 0.047, and 0.139 for RP, LRFS, PFS, and OS, respectively), and a GVT less than 108 cm^3 (FPRP = 0.030, 0.035, and 0.090 for LRFS, PFS, and OS, respectively) (Supplementary Table 8). For *ATG12* rs1058600, when the assumption of a prior probability was 0.25, the associations of clinical outcomes with the *ATG12* rs1058600TT genotype (a recessive genetic model) were still noteworthy for all subjects and subgroups (Supplementary Table 9).

Differential mRNA Expression of ATG16L2 and Functional Analysis of ATG16L2 rs10898880

We also evaluated differential mRNA expression levels of *ATG16L2* in tumor tissues from 50 LUSCs, 57 LUADs, and paired adjacent normal tissues by using the data generated by The Cancer Genome Atlas. As shown in Figure 2*A* and *B*, the expression levels of *ATG16L2* were significantly lower in LUSC and LUAD than that in the adjacent normal tissues ($p = 1.14 \times 10^{-6}$ and 3.36×10^{-5}, respectively). To identify the putative functional role of *ATG16L2* rs10898880, functional annotations from the ENCODE data indicate that rs10898880 is situated at a locus with transcription factor binding, DNase hypersensitivity, and histone modification patterns that are characterized as the promoters in several cell types, which present strong signals of active enhancer and promoter functions (Fig. 2*C*). Moreover, the rs10898880 is within the *ATG16L2* promoter region and is predicted to be located at transcription factor binding sites by the SNPinfo online tool. To provide biologically plausible support for the observed association and prediction, we evaluated the correlation between the rs10898880 SNP and *ATG16L2* mRNA expression levels by genotype, using mRNA expression data of the blood cells from 338 European descendants by expression quantitative trait loci analysis with data from the Genotype-Tissue Expression project. The results showed that the rs10898880 variant C allele was associated with increased mRNA expression levels of *ATG16L2* ($p = 1.4 \times 10^{-10}$) (Fig. 2*D*). These data strongly suggest that the *ATG16L2* rs10898880 C variant allele may modulate gene expression levels of *ATG16L2*.

Discussion

In the present study, we have investigated the associations of nine potentially functional SNPs in *ATG2B*, *ATG10*, *ATG12,* and *ATG16L2* with severe RP, LRFS, PFS, and OS in 393 patients with NSCLC after definitive radiotherapy with or without chemotherapy. We found that the *ATG16L2* rs10898880CC variant genotype and *ATG10* rs4703533GG variant genotype contributed to a significantly better outcome but a greater risk of RP in patients with NSCLC; stratified analyses showed that the *ATG16L2* rs10898880CC variant genotype was more evident in subgroups younger than 65 years and stage III or IV disease. Although multiple tests had been performed in the present study, the results of FPRP indicated that the associations of the potentially functional C variant of *ATG16L2* rs10898880 with severe RP, LRFS, PFS, and OS were less likely to be false-positive. We also provided biological evidence that the mRNA expression levels of *ATG16L2* were lower in lung cancer tissue than that in normal lung tissue and that the *ATG16L2* rs10898880CC variant genotype was associated with higher mRNA expression levels of *ATG16L2* than were AA and AC genotypes in the whole blood cells. These data imply that the *ATG16L2* rs10898880 C variant may play a role in the severe RP and clinical outcomes of patients with NSCLC by modulating the mRNA expression levels of *ATG16L2*. Therefore, our results suggest that once these results have been validated by additional investigations, the *ATG16L2* rs10898880 functional C variant may be a useful biomarker for predicting severe RP and clinical outcomes of patients with NSCLC after definitive radiotherapy.

Although dysfunctional study of the ATG genes has addressed the roles of autophagy in both cell death and survival,[42] few studies to date have explored the role of genetic variants of ATGs in severe RP and clinical outcomes of lung cancer.[8] We selected *ATG2B* because it is a newly discovered gene that participates in the autophagy pathways.[43] As an important player in autophagy,

Figure 2. Differential mRNA expression of autophagy related 16 like 2 gene (*ATG16L2*) in lung tissues and functional analysis of *ATG16L2* rs10898880. (*A* and *B*) Differential mRNA expression analysis by using the data generated by The Cancer Genome Atlas showed a lower expression level of *ATG16L2* in lung squamous carcinoma (LUSC) and lung adenocarcinoma (LUAD) than that in paired adjacent normal lung tissue ($p = 1.14 \times 10^6$ and 3.36×10^5, respectively). (*C*) Location and functional prediction of the *ATG16L2* rs10898880 from Encyclopedia of DNA Elements data. The rs10898880 is located within *ATG16L2* promoter region and presented strong signals of active enhancer and promoter function (indicated by Dnase hypersensitivity, histone modification H3K27acetylation [H3K27Ac] and H3K4 methylation [H3K4Me1 and H3K4Me3], respectively). (*D*). Correlation of *ATG16L2*-related mRNA expression with genotypes of rs10898880 in 338 whole blood cells ($p = 1.4 \times 10^{10}$) by expression quantitative trait locus analysis using data from the Genotype-Tissue Expression project (http://www.gtexportal.org/home).

ATG2B is essential for autophagosome formation and necessary for closure of isolation membranes of autophagosomes.[44] The mutations in *ATG2B* have been shown to lead to the loss 1039 of the total 2078 amino acids, and loss-of-function mutations in *ATG2B*, which was overexpressed in primary hematopoietic cells from patients compared with in donor cells, have been identified in gastric and colorectal cancers.[17,45] However, the role of genetic variants of *ATG2B* in cancer susceptibility is largely unknown.

In a cohort of 192 patients with non–muscle-invasive bladder cancer treated with bacillus Calmette-Guérin, Buffen et al. demonstrated that the *ATG2B* rs3759601 SNP was correlated with progression and recurrence of bladder cancer after bacillus Calmette-Guérin intravesical instillation therapy.[30] In contrast, in another study of 238 patients with Paget disease of bone and 264 sex-matched controls, Usategui-Martín et al. found that genotype frequencies did not differ significantly between patients with Paget disease of bone and healthy subjects.[46] In the present study, we observed that *ATG2B* rs17784271 variant G allele was associated with a poor LRFS and PFS, but we did not find an association of the variant genotypes with risk of severe RP and OS in patients with NSCLC, suggesting that the *ATGB2* rs17784271 variant G allele may play a role in the LRF and PFS of NSCLC after definitive radiotherapy.

ATG10 has been mapped to chromosome 5q14, which codes for an autophagic E2-like enzyme essential for the autophagosome formation.[47] A previous study reported that increased ATG10 expression was strongly associated with tumor invasion and metastasis, and as an oncogenic gene, ATG10 may be a potential prognostic maker for colorectal cancer.[32] In one study of 1064 case patients with breast cancer and 1073 cancer-free controls in a Chinese population, the authors found that genetic variants in *ATG10* rs10514231 were associated with risk of breast cancer.[31] More recently, in another survival study of 1001 Chinese patients with NSCLC, Xie et al. observed that variant alleles of rs10514231 and rs1864182 were associated with a poor OS and that these variants were associated with the increased methylation levels of cg17942617.[8] The authors also observed that the elevated mRNA expression of *ATG10* predicted a shorter survival.[8] In the present study, we did not observe a significant association between genetic variant in rs10514231 and RP or clinical outcomes of patients with NSCLC. However, we found that the *ATG10* rs4703533 GG variant genotype contributed to a significantly increased risk of severe RP and a longer LRFS and PFS of patients with NSCLC. These findings need to be validated by other studies with large samples and in-depth functional experiments, and ethnic background should be carefully considered in further studies.

ATG12 plays a critical role in autophagy.[15,16] Recent studies have indicated that *ATG12* transcript is commonly up-regulated in trastuzumab-unresponsive erb-b2 receptor tyrosine kinase 2–overexpressing breast cancer cells and that *ATG12* silencing significantly reduced ATG12-shRNA/JIMT1 (breast cancer cell line) tumor growth induced by subcutaneous injection of trastuzumab in nude mice.[21] However, the role of *ATG12* variants in risk and clinical outcomes of cancer is largely unknown. One previously published study assessed the association between the *ATG12* rs26538 polymorphism and risk of breast cancer in 1064 case patients and 1073 controls in a Chinese population, but no significant association with cancer risk was found.[31] Other studies found that there was no significant association with survival and brain metastasis of NSCLC in Chinese patients.[8,27] In the present study, we genotyped two functional SNPs of this gene, and similarly, we also did not find a significant association between *ATG12* rs26538 SNP and RP or clinical outcome of white patients with NSCLC. However, our analysis of the *ATG12* rs1058600 SNP showed that the variant T allele was associated with a better LRFS, PFS, and OS, suggesting that the *ATG12* rs1058600 variant T allele may play a role in clinical outcomes of NSCLC after definitive radiotherapy. The functional rs1058600 T variant is located in the 3′UTR of the *ATG12* primary transcript and in the predicted microRNA binding site (microRNA-181 and microRNA-494) (http://snpinfo.niehs.nih.gov/snpfunc.htm). It has been reported that microRNA-181 functions as a tumor suppressor or a tumor promotor by influencing expression of the target genes at the posttranscriptional level[48] and microRNA-494 expression was correlated with lung cancer progression in mice.[49] These may partly explain the underlying biological and molecular mechanisms for the observed associations, which need to be further investigated.

It is known that autophagy-related protein 16 like (including autophagy-related protein 16 like 1 and autophagy-related protein 16 like 2 [ATG16L2]) is an important regulator of autophagy and plays key roles in the autophagy pathway and tumorigenesis.[50] However, the exact molecular mechanism of ATG16L2 is still unclear. Previously, one study reported that the isoform autophagy-related protein 16 like 1 was essential for autophagy, whereas ATG16L2 did not seem to be important.[51] Later, another study of cell responses to cisplatin treatment used the ATG16L2 antibody to monitor expression of autophagy and cell death–related genes,[52] which showed that ATG16L2 levels were reduced in response to cisplatin, a possible biomarker or target for tumor cell resistance to the platinum-based drugs.[52] Recently, *Atg16L2* mutants were found to have a more severe defect than *Atg16L1* mutants in *Caenorhabditis*

elegans.[53] *A* more recent study reported that *ATG16L2* was a ubiquitously expressed homologue of *ATG16L1* and the ATG16L2 interacts with ATG5 and codes for a protein related to autophagy and formation of autophagosome.[54] In one study of 54 patients with multiple sclerosis and 55 healthy controls in a Chinese population, Yin et al. found that *ATG16L2* might play an important role in autophagy (specifically, in T cells) and served as a potential biomarker to predict clinical relapse of multiple sclerosis.[36] In another study of a genome-wide association scan of 1174 case patients with systemic lupus erythematosus and 4248 controls in a Korean population, the authors observed that *ATG16L2* was involved in the association with systemic lupus erythematosus.[54] Similarly, in a Chinese population study of 363 case patients with Crohn's disease and 486 controls, the authors observed that mRNA expression levels of *ATG16L2* were lower in the T cells of the patients than in those of the controls and that an increasing mRNA expression level of *ATG16L2* in patients with the *ATG16L2* rs11235604CC genotype were associated with a decreased risk of Crohn's disease.[37] However, the exact role of *ATG16L2* in lung cancer development and progression remains unclear and needs to be further investigated. On the basis of the important role of *ATG16L2* in the autophagy pathway, we observed associations of two potentially functional SNPs in *ATG16L2* with severe RP and outcomes in 393 patients with NSCLC after definitive radiotherapy. Lower mRNA expression levels of *ATG16L2* in the lung cancer tissues and higher mRNA expression levels of *ATG16L2* correlated with the rs10898880CC variant genotype in blood cells were observed, whereas the rs10898880 variant CC genotype was associated with a better LRFS, PFS, and OS, suggesting that the functional genetic variant of *ATG16L2* rs10898880 may play a role in clinical outcomes of patients with NSCLC by modulating the mRNA expression levels of *ATG16L2*.

According to the ENCODE project data from the University of California Santa Cruz, rs10898880 was associated with considerable levels of histone modification of H3K27 acetylation (H3K27Ac), monomethylation of lysine 4 (H3K4Me1), and trimethylation of lysine 4 of the H3 (H3K4Me3) histone protein enrichment, which may be associated with promoter function, and histone modifications may influence gene expression.[55] More interestingly, our results also showed that among patients with NSCLC, severe RP was more likely to occur in those carrying the rs10898880CC genotype than in those carrying the rs10898880 AA/AC genotypes. This finding is consistent with previous reports showing that overexpression of ATG16 inhibited autophagosome formation and resulted in tissue inflammatory response.[20,25,26] However, this hypothesis needs to be tested in future studies.

To the best of our knowledge, the present study is the first focusing only on associations of the potentially functional SNPs of *ATGB2*, *ATG10*, *ATG12,* and *ATG16L2* with severe RP, LRFS, PFS, and OS in patients with NSCLC after definitive radiotherapy. However, there were some limitations in the present study. First, the present study included patients treated over a 10-year period, during which the standard of care in terms of both staging and treatment (especially the changes in chemotherapy dose and duration) may have changed, and information on these was not available and could not be controlled for in the multivariate analysis. Second, we were unable to explore the exact mechanisms by which the ATG SNPs influence severe RP and outcomes of patients with NSCLC, because we did not have the access to the target tissues. Third, we used the reported common functional SNPs, which may not include all representative SNPs in the entire gene. Some other rare functional SNPs, which could influence survival, may have been missed and need to be investigated in future larger studies. Fourth, genetic variants of *AGT16L2* were suggested to predict the response to radiotherapy, which may be merely a prognostic biomarker for patients with NSCLC after definitive radiotherapy. Fifth, the impact of *EGFR* and ALK receptor tyrosine kinase gene (*ALK*) were not evaluated in the present study, because we did not have the mutation data of these genes, although our previous study had suggested that vascular endothelial growth factor gene (*VEGF*) mutation −460 C genotypes may be associated with a better survival of patients with NSCLC after chemoradiotherapy.[6] However, this should be further investigated in the future. Sixth, we did not find a suitable and accessible patient population for the validation of our results. Last, additional larger validation studies with multiethnic groups are needed to confirm our results, because our prognosis-predicting model was based on a North American patient population.

In conclusion, we found that potentially functional genetic variants of *ATG16L2* predicted risk of RP and better LRFS, PFS, and OS in patients with NSCLC treated with definitive radiotherapy with or without chemotherapy. Whether the SNPs have an impact on the prognosis of NSCLC directly through alteration of autophagy or by other, possibly indirect mechanisms should be further investigated in the future. Once our results have been validated, patients who at a high risk for development of RP should receive a closer follow-up and should be considered for adjuvant therapy in the consideration of individualized treatment.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2018.01.028.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61:69–90.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67:7–30.
3. Goldstraw P, Ball D, Jett JR, et al. Non-small-cell lung cancer. *Lancet*. 2011;378:1727–1740.
4. Dagoglu N, Karaman S, Arifoglu A, Kucucuk S, Oral EN. Definitive radiotherapy in locally advanced non-small cell lung cancer: dose and fractionation. *Balkan Med J*. 2014;31:278–285.
5. Roach M 3rd, 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.
6. Guan X, Yin M, Wei Q, et al. Genotypes and haplotypes of the VEGF gene and survival in locally advanced non-small cell lung cancer patients treated with chemoradiotherapy. *BMC Cancer*. 2010;10:431.
7. Li P, Wang X, Liu Z, et al. Single nucleotide polymorphisms in CBLB, a regulator of T-cell response, predict radiation pneumonitis and outcomes after definitive radiotherapy for non-small-cell lung cancer. *Clin Lung Cancer*. 2016;17:253–262.e5.
8. Xie K, Liang C, Li Q, et al. Role of ATG10 expression quantitative trait loci in non-small cell lung cancer survival. *Int J Cancer*. 2016;139:1564–1573.
9. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. *Cell*. 2011;147:728–741.
10. Rao S, Yang H, Penninger JM, Kroemer G. Autophagy in non-small cell lung carcinogenesis: A positive regulator of antitumor immunosurveillance. *Autophagy*. 2014;10:529–531.
11. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature*. 2008;451:1069–1075.
12. Liu JL, Chen FF, Lung J, et al. Prognostic significance of p62/SQSTM1 subcellular localization and LC3B in oral squamous cell carcinoma. *Br J Cancer*. 2014;111:944–954.
13. Avalos Y, Canales J, Bravo-Sagua R, Criollo A, Lavandero S, Quest AF. Tumor suppression and promotion by autophagy. *Biomed Res Int*. 2014;2014:603980.
14. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell*. 2008;132:27–42.
15. Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol*. 2007;8:931–937.
16. Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y. Dynamics and diversity in autophagy mechanisms: lessons from yeast. *Nat Rev Mol Cell Biol*. 2009;10:458–467.
17. Kang MR, Kim MS, Oh JE, et al. Frameshift mutations of autophagy-related genes ATG2B, ATG5, ATG9B and ATG12 in gastric and colorectal cancers with microsatellite instability. *J Pathol*. 2009;217:702–706.
18. Hara T, Nakamura K, Matsui M, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature*. 2006;441:885–889.
19. Jo YK, Kim SC, Park IJ, et al. Increased expression of ATG10 in colorectal cancer is associated with lymphovascular invasion and lymph node metastasis. *PLoS One*. 2012;7:e52705.
20. Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol Biol Cell*. 2008;19:2092–2100.
21. Cufi S, Vazquez-Martin A, Oliveras-Ferraros C, et al. Autophagy-related gene 12 (ATG12) is a novel determinant of primary resistance to HER2-targeted therapies: utility of transcriptome analysis of the autophagy interactome to guide breast cancer treatment. *Oncotarget*. 2012;3:1600–1614.
22. Hu L, Wang H, Huang L, Zhao Y, Wang J. Crosstalk between autophagy and intracellular radiation response (review). *Int J Oncol*. 2016;49:2217–2226.
23. Saleh T, Cuttino L, Gewirtz DA. Autophagy is not uniformly cytoprotective: a personalized medicine approach for autophagy inhibition as a therapeutic strategy in non-small cell lung cancer. *Biochim Biophys Acta*. 2016;1860:2130–2136.
24. Yang Y, Yang X, Zhu H, et al. Autophagy and its function in radiosensitivity. *Tumour Biol*. 2015;36:4079–4087.
25. Crisan TO, Plantinga TS, van de Veerdonk FL, et al. Inflammasome-independent modulation of cytokine response by autophagy in human cells. *PLoS One*. 2011;6:e18666.
26. Yang S, Imamura Y, Jenkins RW, et al. Autophagy inhibition dysregulates TBK1 signaling and promotes pancreatic inflammation. *Cancer Immunol Res*. 2016;4:520–530.
27. Li QX, Zhou X, Huang TT, et al. The Thr300Ala variant of ATG16L1 is associated with decreased risk of brain metastasis in patients with non-small cell lung cancer. *Autophagy*. 2017:1–11.
28. Plantinga TS, van de Vosse E, Huijbers A, et al. Role of genetic variants of autophagy genes in susceptibility for non-medullary thyroid cancer and patients outcome. *PLoS One*. 2014;9:e94086.
29. Reddy JP, Tang C, Shih T, et al. Influence of surveillance PET/CT on detection of early recurrence after definitive radiation in stage iii non-small-cell lung cancer. *Clin Lung Cancer*. 2017;18:141–148.

30. Buffen K, Oosting M, Quintin J, et al. Autophagy controls BCG-induced trained immunity and the response to intravesical BCG therapy for bladder cancer. *PLoS Pathog*. 2014;10:e1004485.
31. Qin Z, Xue J, He Y, et al. Potentially functional polymorphisms in ATG10 are associated with risk of breast cancer in a Chinese population. *Gene*. 2013;527:491–495.
32. Jo YK, Roh SA, Lee H, et al. Polypyrimidine tract-binding protein 1-mediated down-regulation of ATG10 facilitates metastasis of colorectal cancer cells. *Cancer Lett*. 2017;385:21–27.
33. Pan B, Feng B, Chen Y, et al. MiR-200b regulates autophagy associated with chemoresistance in human lung adenocarcinoma. *Oncotarget*. 2015;6:32805–32820.
34. Kunanopparat A, Kimkong I, Palaga T, Tangkijvanich P, Sirichindakul B, Hirankarn N. Increased ATG5-ATG12 in hepatitis B virus-associated hepatocellular carcinoma and their role in apoptosis. *World J Gastroenterol*. 2016;22:8361–8374.
35. Dunwell T, Hesson L, Rauch TA, et al. A genome-wide screen identifies frequently methylated genes in haematological and epithelial cancers. *Mol Cancer*. 2010;9:44.
36. Yin L, Liu J, Dong H, et al. Autophagy-related gene16L2, a potential serum biomarker of multiple sclerosis evaluated by bead-based proteomic technology. *Neurosci Lett*. 2014;562:34–38.
37. Ma T, Wu S, Yan W, Xie R, Zhou C. A functional variant of ATG16L2 is associated with Crohn's disease in the Chinese population. *Colorectal Dis*. 2016;18:O420–O426.
38. Liu H, Liu Z, Wang Y, et al. Functional variants in DCAF4 associated with lung cancer risk in European populations. *Carcinogenesis*. 2017;38:541–551.
39. Lopez Guerra JL, Gladish G, Komaki R, Gomez D, Zhuang Y, Liao Z. Large decreases in standardized uptake values after definitive radiation are associated with better survival of patients with locally advanced non-small cell lung cancer. *J Nucl Med*. 2012;53:225–233.
40. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst*. 2004;96:434–442.
41. Greene FL. The American Joint Committee on Cancer: updating the strategies in cancer staging. *Bull Am Coll Surg*. 2002;87:13–15.
42. Debnath J, Baehrecke EH, Kroemer G. Does autophagy contribute to cell death? *Autophagy*. 2005;1:66–74.
43. Reggiori F, Tucker KA, Stromhaug PE, Klionsky DJ. The Atg1-Atg13 complex regulates Atg9 and Atg23 retrieval transport from the pre-autophagosomal structure. *Dev Cell*. 2004;6:79–90.
44. Velikkakath AK, Nishimura T, Oita E, Ishihara N, Mizushima N. Mammalian Atg2 proteins are essential for autophagosome formation and important for regulation of size and distribution of lipid droplets. *Mol Biol Cell*. 2012;23:896–909.
45. Plo I, Bellanne-Chantelot C, Vainchenker W. ATG2B and GSKIP: 2 new genes predisposing to myeloid malignancies. *Mol Cell Oncol*. 2016;3:e1094564.
46. Usategui-Martín R, Garcia-Aparicio J, Corral-Gudino L, Calero-Paniagua I, Del Pino-Montes J, Gonzalez Sarmiento R. Polymorphisms in autophagy genes are associated with Paget disease of bone. *PLoS One*. 2015;10:e0128984.
47. Lamb CA, Yoshimori T, Tooze SA. The autophagosome: origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol*. 2013;14:759–774.
48. Cao Y, Zhao D, Li P, et al. MicroRNA-181a-5p impedes IL-17-induced nonsmall cell lung cancer proliferation and migration through targeting VCAM-1. *Cell Physiol Biochem*. 2017;42:346–356.
49. Faversani A, Amatori S, Augello C, et al. miR-494-3p is a novel tumor driver of lung carcinogenesis. *Oncotarget*. 2017;8:7231–7247.
50. Zheng H, Ji C, Li J, et al. Cloning and analysis of human Apg16L. *DNA Seq*. 2004;15:303–305.
51. Ishibashi K, Fujita N, Kanno E, et al. Atg16L2, a novel isoform of mammalian Atg16L that is not essential for canonical autophagy despite forming an Atg12-5-16L2 complex. *Autophagy*. 2011;7:1500–1513.
52. Huang Y, Chuang AY, Ratovitski EA. Phospho-DeltaNp63alpha/miR-885-3p axis in tumor cell life and cell death upon cisplatin exposure. *Cell Cycle*. 2011;10:3938–3947.
53. Zhang H, Wu F, Wang X, Du H. The two C. elegans ATG-16 homologs have partially redundant functions in the basal autophagy pathway. *Autophagy*. 2013;9:1965–1974.
54. Lessard CJ, Sajuthi S, Zhao J, et al. Identification of a systemic lupus erythematosus risk locus spanning ATG16L2, FCHSD2, and P2RY2 in Koreans. *Arthritis Rheumatol*. 2016;68:1197–1209.
55. Barski A, Cuddapah S, Cui K, et al. High-resolution profiling of histone methylations in the human genome. *Cell*. 2007;129:823–837.