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ORIGINAL RESEARCH

Association between single nucleotide polymorphisms of *NOTCH* signaling pathway-related genes and the prognosis of NSCLC

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Objective: In this study, we analyzed the association between genetic variants of genes in the NOTCH signaling pathway and the prognosis of non-small-cell lung cancer (NSCLC) in the Chinese population. We also explored the interaction between genetic and epidemiological factors for the test group.

Methods: We performed genotyping of 987 NSCLC patients. Then, we used Cox proportional hazard models to analyze the associations between single-nucleotide polymorphisms (SNPs) and the prognosis of NSCLC. We employed Stata software to test the heterogeneity of associations between subgroups, and we analyzed the additive and multiplicative interactions between SNPs and epidemiologic factors.

Results: This work revealed the important prognostic and predictive value of rs915894 in the *NOTCH4* gene, which may be regarded as a promising prognosis biomarker of NSCLC. Cox regression analysis indicated that the C allele of rs915894 is associated with longer survival and decreased risk of death in NSCLC (codominant model: adjusted HR =0.83, 95% CI =0.70–0.99; dominant model: adjusted HR =0.83, 95% CI =0.71–0.98). Additional stepwise regression analysis suggested that this SNP is an independently favorable factor for the prognosis of NSCLC (dominant model: adjusted HR =0.85, 95% CI =0.72–0.99). This protective effect is more pronounced for patients who are not smokers, have a history of other lung diseases, or have a family history of cancer. We also detected statistically significant additive and multiplicative interactions between rs915894 and smoking, rs915894 and history of lung diseases, and rs915894 and family history of cancer, which all affect NSCLC survival.

Conclusion: This study demonstrated that rs915894 in *NOTCH4* may be a genetic marker for NSCLC prognosis in the Chinese population and that rs915894 may have an interactive relationship with epidemiologic factors.

Keywords: NOTCH signaling pathway, single-nucleotide polymorphism, non-small-cell lung cancer, interaction, prognosis

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Introduction

Lung cancer is the most common cancer and the first leading cause of malignancy worldwide.[1] In China, 781,000 new cases of lung cancer and 626,000 deaths were reported in 2014, accounting for 27.26% of all cancer-related deaths.[2] In spite of advances made in the early detection and treatment, the five-year survival rate for lung cancer is only 16.1%.[3] Non-small-cell lung cancer (NSCLC) is the major pathological subtype of lung cancer, accounting for 80–85% of all cases.

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Epidemiological studies have shown that the prognosis of patients with NSCLC is affected by many factors, including pathological type,[4] clinical stage,[5] gender,[6] and age.[7] Patients with the same clinical features and demographic characteristics often demonstrate extensive heterogeneity in treatment sensitivity, disease recurrence, and survival outcomes after receiving the same treatment.[8,9] Therefore, it is necessary to identify new biomarkers to predict patients' prognosis to improve the efficacy of cancer treatment.

In recent years, with the introduction of expanded knowledge of stem cells into oncology research, and the successful isolation and identification of tumor stem cells in various tumor tissues and cancer cell lines, the theory of cancer stem cells has received increasing attention.[10] Recent studies have found that cancer stem cells are resistant to chemoradiotherapy, and although traditional chemoradiotherapy can kill most tumor cells and reduce tumor volume, some cancer stem cells remain and become the source of tumor resistance, recurrence, and metastasis.[11,12]

As one of the main signaling pathways of cancer stem cells, the NOTCH signaling pathway plays an important role in the maintenance of self-renewal, multidirectional differentiation, and apoptosis of various cancer stem cells and thus participates in the regulation of cancer stem cells.[13] Deregulation or mutation of NOTCH receptors, ligands, and signaling regulators is implicated in the occurrence, development, and progression of several human solid tumors, for example, breast cancer,[14] colon cancer,[15] and liver cancer,[16] as well as NSCLC.[17] Previous reports have demonstrated that NOTCH signaling is activated aberrantly and plays a critical role in the initiation, progression, and metastasis of NSCLC.[18]

These characteristics of cancer stem cells are closely related to their unique signaling mechanisms. Mutations, including SNPs, in some key genes in these signaling pathways cause disorder or overactivation of the entire signaling pathway, leading to tumor development. Our previous study identified several SNPs in the NOTCH signaling pathway associated with the susceptibility of NSCLC in patients of the Chinese population.[19] In the present study, we further explored the prognostic roles of candidate SNPs of the NOTCH signaling pathway in NSCLC patients and analyzed the interaction between genes and epidemiologic factors. The goal of this research was to improve the prediction of the prognosis of NSCLC and provide a basis for the development of treatment measures.

Materials and methods

Study subjects

We recruited 987 patients with primary NSCLC diagnosed by pathological histology from three hospitals (First Affiliated Hospital of Fujian Medical University, Fujian Medical University Union Hospital, and Fuzhou General Hospital of Nanjing Military Command) between January 2006 and December 2012. All NSCLC patients were of the Chinese population. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Fujian Medical University Ethics Committee. Written informed consent was obtained from all subjects before their enrolment in the study.

Data and sample collections

We obtained demographic data by in-person interviews using a standardized questionnaire that included gender, age, phone number, smoking status, history of lung diseases, and family history of cancer. We collected clinical information through hospital medical records, including pathological diagnosis, clinical stage, metastasis, the presence or absence of pleural effusion at the time of diagnosis, treatment information, date of diagnosis, and date of discharge. We conducted a standard follow-up by trained investigators using medical records or telephone interviews. The latest follow-up data in this analysis were obtained by December 1, 2017.

Before treatment, we collected a 5 mL blood sample from each participant for genomic DNA extraction. The sample was taken in the laboratory using the Oragene DNA self-collection kit (DNA Genotek, OraSure Technologies, Inc., Ottawa, ON, Canada).

Selection and genotyping of SNPs

On the basis of data in Genebank (http://www.ncbi.nlm.nih.gov/Genebank), dbSNP (http://www.ncbi.nlm.nih.gov/SNP), and the International HapMap database (http://www.hapmap.org), we selected SNPs with the minimum allele frequency (MAF) threshold of 0.10, r2>0.8 to explore the association between SNPs in *NOTCH* signaling and the prognosis of NSCLC (Table 1). We conducted SNP genotyping using matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF-MS) with the Sequenom platform according to the manufacturer's iPLEX Application Guide (Sequenom Inc, San Diego, CA, USA). We analyzed results using the MassArrayTyper 4.0 Software. After we processed all of

Table 1 Information about selected SNPs in NOTCH signaling

Gene	SNP	Chromoome position	Location	MAF-CHB	Allele Change
NOTCH3	rs3815188	19p13.12	Exon3	0.451	G→A
NOTCH4	rs915894	6p21.32	Exon3	0.478	A→C
NOTCH4	rs520692	6p21.32	Exon5	0.098	A→G
JAG1	rs8708	20p12.2	Exon26	0.183	A→G
DLL1	rs1033583	6q27	Exon11	0.280	A→C
HES2	rs11364	1p36.31	Exon4	0.159	G→A
HEY1	rs1046472	8q21.13	Exon5	0.305	C→A
HEY2	rs3734637	6q22.31	Exon5	0.195	A→C

Abbreviations: SNP, single-nucleotide polymorphism; MAF, minor allele frequency; JAG, Jagged; DLL, Delta-like; HES, hairy and enhancer of split; HEY, hairy/enhancer of split related with YRPW motif family members.

the test samples, we selected 10% of the samples at random and reran the samples for quality control purposes. Genotyping call rates were greater than 90%.

Statistical methods

We defined overall survival (OS) as the time from the date of diagnosis until the date of death from any cause or to the date of the last follow-up. We applied four genetic models (codominant, dominant, additive, and recessive) to assess the association between the SNPs and the clinical outcomes of the sample NSCLC patients. We considered the model with the smallest *P*-value to be the best-fitting model. We employed the Kaplan-Meier method to draw the overall survival curves of the SNPs and used the log-rank test to compare the differences between groups. We fitted univariate and multivariate analyses with Cox proportional hazard regressions to calculate crude or adjusted hazard ratios (HR*s*) and their 95% confidence intervals (CIs). We used the stepwise Cox regression model to determine independent predictive factors of the NSCLC prognosis. In addition, we analyzed the additive and multiplicative interactions between the SNPs and epidemiologic factors, for which we found heterogeneity in the stratification analysis. All statistical tests were two-sided, and statistical significance was set at $P<0.05$. All statistical analyses were carried out by SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA) and Stata software version 14.0 (Stata Corp, College Station, TX, USA).

Results

Characteristics and survival status of NSCLC patients

Demographic characteristics for the 987 NSCLC patients are provided in Table 2. The median age at the time of NSCLC diagnosis was 59 years (range 23–86 years). Most patients were male (71.6%). The overall median survival time (MST) for the entire cohort of patients was 25.0 months. The associations between clinical pathology characteristics and outcomes in NSCLC patients are also summarized in Table 2. The log-rank test showed that the MST varied significantly based on gender, smoking status, history of lung diseases, clinical stage, metastasis, and pleural effusion, as well as surgical treatment (log-rank $P<0.05$). We used the Cox proportional hazards regression model to identify the prognostic factors of overall survival (OS). Univariate analysis showed that patients who accepted surgical treatment (HR =0.35, 95% CI =0.30–0.41) were associated with significantly better OS. Males (HR =1.39, 95% CI =1.18–1.64), smokers (HR =1.29, 95% CI =1.11–1.50), patients with a history of lung diseases (HR =1.33, 95% CI =1.15–1.55), patients at clinical stage III or IV (HR =2.72, 95% CI =2.33–3.18), patients with metastasis (HR =2.74, 95% CI =2.31–3.25), and patients with pleural effusion at the time of diagnosis (HR =1.56, 95% CI =1.34–1.82) showed significant association with poor OS. We did not find any association, however, between other tested clinical characteristics and outcomes. Therefore, further adjustment was made for gender, clinical stage, metastasis, pleural effusion, and surgery in the multivariate Cox regression to control for possible confounding of the main effects of the rs915894 in the prognosis of NSCLC.

Association between genotypes and survival of NSCLC

From the univariate Cox regression analysis, we found that patients who carried the rs11364 AG variant genotype (general model: $P=0.028$, HR =1.21, 95% CI =1.02–1.43) and AG+AA variant genotype (dominant model: $P=0.036$, HR =1.19, 95% CI =1.01–1.40) had a significantly shorter MST

Table 2 Distribution of characteristics in Chinese patients with non-small-cell lung cancer (n=987) and prognosis analysis

Variable	Patients (n=987) N (%)	Deaths (n=749) N (%)	MST (months)	Log-rank *P*	HR (95% CI)
Gender					
Female	280 (28.4)	190 (25.4)	34.5	**<0.001**	1.00
Male	707 (71.6)	559 (74.6)	23.1		**1.39 (1.18–1.64)**
Age (years)					
<60	492 (49.8)	360 (48.1)	26.5	0.055	1.00
≥60	495 (50.2)	389 (51.9)	23.7		1.15 (1.00–1.33)
Smoking					
No	371 (37.6)	261 (34.8)	29.9	**0.001**	1.00
Yes	616 (62.4)	488 (65.2)	23.0		**1.29 (1.11–1.50)**
History of lung diseases					
No	651 (66.0)	476 (63.6)	27.6	**<0.001**	1.00
Yes	336 (34.0)	273 (36.4)	22.3		**1.33 (1.15–1.55)**
Family history of cancer					
No	788 (79.8)	601 (80.2)	25.5	0.695	1.00
Yes	199 (20.2)	148 (19.8)	22.8		1.04 (0.87–1.24)
Pathological types					
Adenocarcinoma	579 (58.7)	432 (57.7)	26.7	0.304	1.00
Squamous cell carcinoma	305 (30.9)	241 (32.2)	23.2		1.13 (0.97–1.32)
Others	103 (10.4)	76 (10.1)	21.8		1.08 (0.85–1.38)
Clinical stage					
I+II	413 (41.8)	241 (32.2)	60.3	**<0.001**	1.00
III+IV	574 (58.2)	508 (67.8)	18.1		**2.72 (2.33–3.18)**
Metastasis					
No	327 (33.1)	174 (23.2)	75.2	**<0.001**	1.00
Yes	660 (66.9)	575 (76.8)	19.9		**2.74 (2.31–3.25)**
Pleural effusion					
No	698 (70.7)	502 (67.0)	29.5	**<0.001**	1.00
Yes	289 (29.3)	247 (33.0)	18.7		**1.56 (1.34–1.82)**
Surgery					
No	444 (45.0)	407 (54.3)	16.6	**<0.001**	1.00
Yes	543 (55.0)	342 (45.7)	46.5		**0.35 (0.30–0.41)**
Chemotherapy					
No	297 (30.1)	219 (29.2)	22.9	0.699	1.00
Yes	690 (69.9)	530 (70.8)	26.1		0.97 (0.83–1.14)
Radiotherapy					
No	802 (81.3)	592 (79.0)	24.3	0.696	1.00
Yes	185 (18.7)	157 (21.0)	29.1		1.04 (0.87–1.24)

Note: Bold values indicate significance.

compared with those with the GG genotype. We did not observe a significant effect for SNP rs11364 when the analysis was adjusted by gender, age, smoking, history of lung diseases, family history of cancer, pathological types, clinical stage, metastasis, pleural effusion, surgery, chemotherapy, and radiotherapy. In contrast, we did not find any association between rs915894 and the prognosis of NSCLC before adjustment (Figure 1). Adjusting possible confounding factors, we observed that compared with patients carrying the rs915894 homozygous AA genotype, those with the AC genotype (adjusted HR =0.83, 95% CI =0.70–0.99, adjusted *P*=0.039) and the AC+CC genotype (adjusted HR =0.83, 95% CI =0.71–0.98, adjusted *P*=0.030) had a significantly decreased risk of death from lung cancer (Table 3).

The dominant model with the smallest *P*-value (*P*=0.003) was the best model for rs915894. Further multivariate Cox regression analysis showed that rs915894 was an independent predictive factor for NSCLC (AC/CC versus AA, HR =0.85, 95% CI =0.72–0.99, *P*=0.041). Other factors, including male gender (HR =1.23, 95% CI =1.03–1.47; *P*=0.020), clinical stage III or IV (HR =1.46, 95% CI =1.17–1.83, *P*=0.001), metastasis (HR =1.82, 95% CI =1.44–2.30, *P*<0.001), and pleural effusion at the time of diagnosis (HR =1.25, 95% CI =1.06–1.47, *P*=0.009) were associated with shorter survival times, whereas receiving surgery (HR =0.49, 95% CI =0.41–0.58, *P*<0.001) decreased the risk of death, as shown in Table 4.

Associations between rs915894 and the prognosis of NSCLC stratified by patient characteristics

We conducted stratified analyses to evaluate the associations between genotype of rs915894 and the OS of NSCLC patients by gender, age, smoking, history of lung diseases, family history of cancer, pathological types, clinical stage, metastasis, pleural effusion, surgery, chemotherapy and radiotherapy. The significant protective effects conferred by rs915894 were more prominent in some subgroups with an HR ranging from 0.49 to 0.80 (Figure 2). As shown in Figure 2, we observed a significantly decreased risk of death associated with rs915894 AC/CC genotype in patients who were female (HR =0.71, 95% CI =0.51–0.99), were of less than 60 years old (HR =0.74, 95% CI =0.59–0.94), were not smokers (HR =0.63, 95% CI =0.48–0.84), had a history of lung diseases (HR =0.58, 95% CI =0.44–0.77), had a family history of cancer (HR =0.49, 95% CI =0.34–0.73), were without pleural effusion at time of diagnosis (HR =0.80, 95% CI =0.65–0.98), were without surgery (HR =0.77, 95% CI =0.62–0.96), or were accepting chemotherapy (HR =0.78, 95% CI =0.64–0.95).

The heterogeneity test found that the protective effect of the rs915894 AC/CC genotype on the prognosis of NSCLC was more pronounced in smokers, patients with a history of lung disease, and patients with a family history of cancer (Figure 2).

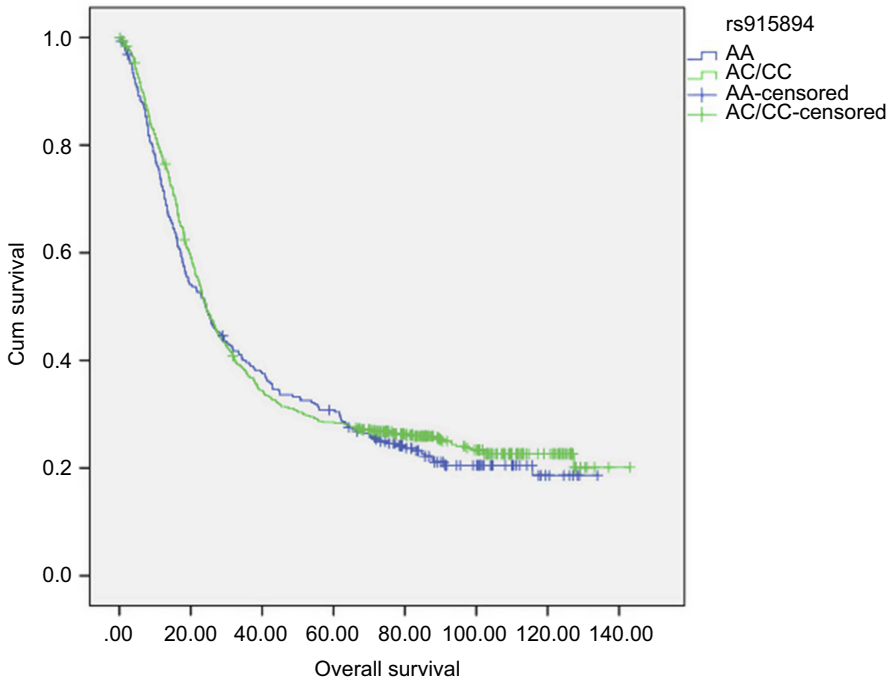

Figure 1 Kaplan–Meier survival curves for NSCLC patients by the rs915894 in overall analysis in the dominant model.

Table 3 Association between SNPs in the NOTCH signal pathway and clinical outcomes in Chinese patients with NSCLC

SNP	Genotype	Death/Total	MST (months)	Univariate		Multivariate	
				HR (95% CI)	*P*-value	HR (95% CI)[a]	*P*-value[a]
rs3815188	GG	356/277	22.93	1.00	0.522	1.00	0.669
	AG	358/265	25.00	0.91 (0.77–1.08)	0.286	0.95 (0.80–1.13)	0.569
	AA	114/89	23.20	1.00 (0.79–1.27)	0.992	0.90 (0.70–1.15)	0.396
	Dominant	472/354	24.43	0.93 (0.80–1.09)	0.389	0.94 (0.80–1.10)	0.436
	Recessive	714/542	24.37	1.05 (0.84–1.31)	0.681	0.92 (0.73–1.16)	0.489
	Additive			0.98 (0.87–1.09)	0.680	0.95 (0.85–1.06)	0.370
rs915894	AA	287/223	24.27	1.00	0.724	1.00	0.095
	AC	428/321	24.57	0.94 (0.79–1.11)	0.468	**0.83 (0.70–0.99)**	**0.039**
	CC	167/125	22.17	0.93 (0.75–1.16)	0.521	0.84 (0.67–1.05)	0.122
	Dominant	595/446	24.33	0.94 (0.80–1.10)	0.424	**0.83 (0.71–0.98)**	**0.030**
	Recessive	715/544	24.43	0.97 (0.80–1.17)	0.732	0.94 (0.77–1.14)	0.529
	Additive			0.96 (0.86–1.07)	0.471	0.90 (0.81–1.01)	0.075
rs520692	AA	753/572	24.03	1.00	0.270	1.00	0.342
	AG	180/137	26.93	0.95 (0.78–1.14)	0.550	1.06 (0.88–1.28)	0.542
	GG	7/4	73.60	0.47 (0.17–1.24)	0.127	0.52 (0.19–1.39)	0.191
	Dominant	187/141	28.07	0.92 (0.76–1.10)	0.363	1.03 (0.86–1.24)	0.758
	Recessive	933/709	24.37	0.47 (0.18–1.26)	0.132	0.51 (0.19–1.38)	0.183
	Additive			0.90 (0.76–1.07)	0.225	1.00 (0.84–1.19)	0.967
rs8708	AA	638/493	24.87	1.00	0.227	1.00	0.307
	AG	273/197	25.37	0.90 (0.76–1.06)	0.200	0.92 (0.78–1.09)	0.312
	GG	38/31	21.37	1.20 (0.83–1.72)	0.330	1.21 (0.84–1.75)	0.310
	Dominant	311/228	24.43	0.93 (0.79–1.09)	0.361	0.95 (0.81–1.11)	0.517
	Recessive	911/690	24.90	1.24 (0.86–1.77)	0.248	1.24 (0.86–1.79)	0.245
	Additive			0.97 (0.85–1.11)	0.693	0.99 (0.86–1.13)	0.873
rs1033583	AA	578/447	24.33	1.00	0.675	1.00	0.919
	AC	296/218	25.47	0.93 (0.79–1.10)	0.396	0.97 (0.82–1.14)	0.696
	CC	55/43	28.80	0.94 (0.69–1.28)	0.694	1.01 (0.74–1.39)	0.953
	Dominant	351/261	25.67	0.93 (0.80–1.09)	0.376	0.98 (0.84–1.14)	0.743
	Recessive	874/665	24.43	0.96 (0.71–1.31)	0.803	1.02 (0.75–1.40)	0.901
	Additive			0.95 (0.84–1.08)	0.421	0.99 (0.87–1.12)	0.830
rs11364	GG	663/487	25.40	1.00	0.089	1.00	0.057
	AG	236/193	24.03	**1.21 (1.02–1.43)**	**0.028**	1.16 (0.98–1.37)	0.093
	AA	29/21	23.30	1.04 (0.67–1.61)	0.858	0.71 (0.46–1.11)	0.136
	Dominant	265/214	23.77	**1.19 (1.01–1.40)**	**0.036**	1.09 (0.93–1.29)	0.286
	Recessive	899/680	24.87	0.99 (0.64–1.53)	0.965	0.68 (0.44–1.06)	0.089
	Additive			1.13 (0.99–1.30)	0.076	1.02 (0.89–1.17)	0.800
rs1046472	CC	622/481	24.43	1.00	0.829	1.00	0.362
	AC	272/197	24.33	0.95 (0.81–1.12)	0.560	1.05 (0.88–1.24)	0.605
	AA	47/38	27.33	1.02 (0.73–1.42)	0.918	1.27 (0.91–1.78)	0.162
	Dominant	319/235	24.37	0.96 (0.82–1.12)	0.626	1.08 (0.92–1.26)	0.375
	Recessive	894/678	24.37	1.03 (0.75–1.43)	0.849	1.25 (0.90–1.75)	0.183
	Additive			0.98 (0.86–1.11)	0.747	1.09 (0.95–1.24)	0.221
rs3734637	AA	581/445	25.53	1.00	0.225	1.00	0.391
	AC	311/237	23.13	1.06 (0.91–1.24)	0.464	1.11 (0.95–1.31)	0.185
	CC	54/37	32.43	0.78 (0.56–1.10)	0.155	0.98 (0.70–1.38)	0.911
	Dominant	365/274	24.03	1.01 (0.87–1.18)	0.872	1.09 (0.94–1.28)	0.247

(*Continued*)

Table 3 (Continued).

SNP	Genotype	Death/Total	MST (months)	Univariate		Multivariate	
				HR (95% CI)	*P*-value	HR (95% CI)[a]	*P*-value[a]
	Recessive Additive	892/682	24.77	0.77 (0.55–1.07) 0.97 (0.86–1.09)	0.118 0.614	0.94 (0.68–1.32) 1.05 (0.93–1.19)	0.737 0.418

Notes: [a]Adjusted by gender, age, smoking, history of lung diseases, family history of cancer, pathological types, clinical stage, metastasis, pleural effusion, surgery, chemotherapy, and radiotherapy. Bold values indicate significance.

Table 4 COX regression analysis of the prognosis of non-small-cell lung cancer patients

Variables	*β*	SE	HR	95% CI	*P*
Gender	0.208	0.089	1.23	**1.03–1.47**	**0.020**
Clinical stage	0.381	0.114	1.46	**1.17–1.83**	**0.001**
Metastasis	0.598	0.119	1.82	**1.44–2.30**	**<0.001**
Pleural effusion	0.219	0.084	1.25	**1.06–1.47**	**0.009**
Surgery	−0.711	0.088	0.49	**0.41–0.58**	**<0.001**
Radiotherapy	−0.162	0.096	0.85	0.71–1.03	0.090
rs915894	−0.169	0.082	0.85	**0.72–0.99**	**0.041**

Note: Bold values indicate significance.

Joint effect between rs915894 and smoking, history of lung diseases, and family history of cancer on OS

Prompted by these findings, we carried out an interaction analysis for the following combinations of factors: gene and smoking, gene and history of lung diseases, and gene and family history of cancer. We observed statistically significant multiplicative interactions. As presented in Table 5, there were significant interactions between the genotype rs915894 and smoking, history of lung diseases, and family history of cancer (all $P_{\text{interaction}}$ <0.001). Compared with nonsmokers who carried AC+CC genotypes, patients with the AAgenotype who were not smokers had a significantly increased death risk (HR =1.62, 95% CI =1.24–2.13). We found similar results in patients carrying the AA genotype who also had a history of lung diseases (HR =1.52, 95% CI =1.18–1.96) and in patients carrying the AA genotype who had a family history of cancer (HR =1.77, 95% CI =1.29–2.42) as compared with the corresponding reference groups (Table 5).

Discussion

In this study, we investigated the association of genetic polymorphisms in the genes of the NOTCH signaling pathway with the prognosis of NSCLC patients. We demonstrated that a variant genotype (AC+CC) of rs915894 in the *NOTCH4* gene was significantly associated with a better prognosis of NSCLC when compared with AA genotypes, whereas we did not observe a significant association between other SNPs selected and the clinical outcomes of NSCLC patients. Moreover, multivariate COX regression analysis showed that rs915894 affected the prognosis of NSCLC patients. The favorable prognostic effect of rs915894 was more evident in specific subgroups of NSCLC patients. Additionally, the joint analysis found a significant interaction between genes and epidemiological elements. To the best of our knowledge, this study is the first to report the association between *NOTCH4* gene polymorphisms and NSCLC prognosis. Once validated, *NOTCH4* SNP rs915894 may be used as a prognostic marker in combination with epidemiological factors to make decisions about individualized treatment of NSCLC.

In the stratified analysis, with respect to SNP rs915894, we observed that more significant HR was shown for patients in the nonsmoking subgroup and for patients who had a history of lung diseases or a family history of cancer. These results indicated that SNP rs915894 might be a promising biomarker for specific subgroups, and thus it could contribute to individualized prediction in specific populations. In addition, joint analysis revealed strong effects from the combinations of rs915894 and smoking, rs915894 and history of lung diseases, and rs915894 and family history of

Subgroup	Genotypes (Death/Tatal)		*HR (95% CI)*[a]	Heterogeneity *P*
	CC	AA/AC		
Gender				
Female	103/161	68/90	0.71(0.51-0.99)	0.255
Male	343/434	155/197	0.89(0.73-1.08)	
Age(years)				
<60	209/291	114/148	0.74(0.59-0.94)	0.232
≥60	237/304	109/139	0.91(0.72-1.15)	
Smoking				
No	139/211	89/114	0.63(0.48-0.84)	0.014
Yes	307/384	134/173	0.98(0.80-1.21)	
History of lung diseases				
No	285/385	141/194	0.98(0.79-1.20)	0.003
Yes	161/210	82/93	0.58(0.44-0.77)	
Family history of cancer				
No	356/467	178/235	0.94(0.79-1.13)	0.003
Yes	90/128	45/52	0.49(0.34-0.73)	
Pathological types				
Adenocarcinoma	256/350	129/167	0.81(0.65-1.01)	0.833
Squamous cell carcinoma	142178	72/91	0.86(0.64-1.14)	
Others	48/67	22/29	0.98(0.55-1.77)	
Clinical Stage				
I+II	139/247	81/131	0.84(0.63-1.12)	0.897
III+IV	307/348	142/156	0.82(0.67-1.01)	
Metastasis				
No	97/191	56/101	0.77(0.55-1.08)	0.648
Yes	349/404	167/186	0.84(0.70-1.02)	
Pleural effusion				
No	291/413	153/207	0.80(0.65-0.98)	0.978
Yes	155/182	70/80	0.80(0.60-1.08)	
Surgery				
No	248/274	119/126	0.77(0.62-0.96)	0.176
Yes	198/321	104/161	0.97(0.76-1.24)	
Chemotherapy				
No	146/192	55/78	0.97(0.71-1.33)	0.248
Yes	300/403	168/209	0.78(0.64-0.95)	
Radiotherapy				
No	360489	168/226	0.84(0.70-1.01)	0.800
Yes	86106	55/61	0.89(0.61-1.29)	

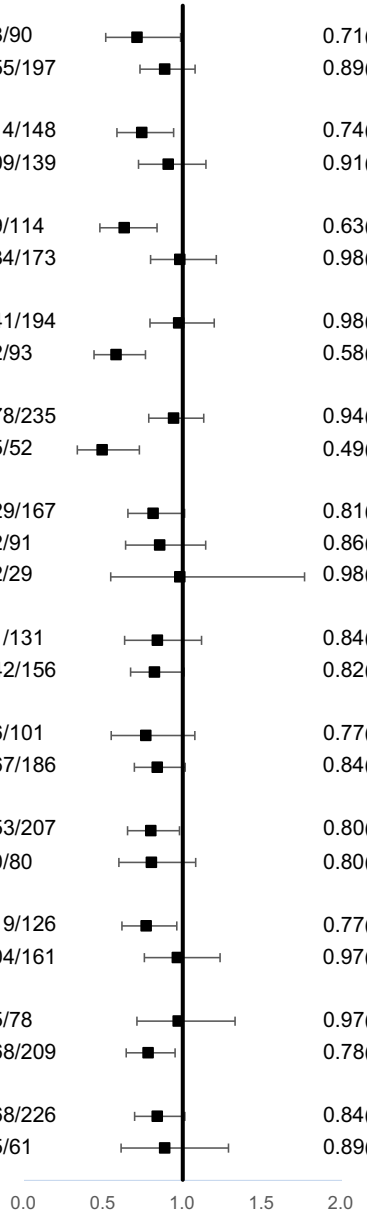

Figure 2 Stratified analysis of the association between SNPs and the prognosis of NSCLC in the Chinese population. [a]Adjusted by gender, age, smoking, history of lung diseases, family history of cancer, pathological types, clinical stage, metastasis, pleural effusion, surgery, chemotherapy, and radiotherapy.

cancer. Smoking, history of lung diseases, and family history of cancer were defined as influencing factors for the prognosis of NSCLC.[20,21]

Although previous studies have not provided related reports about the interaction of the rs915894 polymorphism and epidemiological factors, data revealed by the additive and multiplicative analyses have indicated that it would be promising to study the association of the rs915894 SNP with these factors. Cigarette by-products, especially nicotine, are potent mutagens and can be a direct cause of mutations in oncogenes or tumor suppressor genes.[22,23] Li et al.[24] conducted in vitro and in vivo experiments to investigate the association of smoking and mutations in the NOTCH signaling pathway in NSCLC. They found a direct effect of smoking on the NOTCH signaling pathway that leads to the progression of lung cancer. In addition, they further discovered that smoking significantly changed the SNPs in the signaling pathway, and specific SNPs

Table 5 Joint effect on OS of rs915894 genotypes and smoking, history of lung diseases, and family history of cancer

Genotype		Death/Total	HR (95% CI)	HR (95% CI)[a]	*P*-value[a]
rs915894	Smoking				
AC+CC	No	139/211	1.00	1.00	
AC+CC	Yes	307/384	**1.46 (1.19–1.78)**	1.11 (0.83–1.47)	0.494
AA	No	89/114	**1.41 (1.08–1.84)**	**1.62 (1.24–2.13)**	**<0.001**
AA	Yes	134/173	**1.34 (1.05–1.69)**	1.12 (0.83–1.53)	0.458
P interaction					**0.007**
rs915894	History of lung diseases				
AC+CC	No	285/385	1.00	1.00	
AC+CC	Yes	161/210	1.11 (0.92–1.35)	0.91 (0.74–1.11)	0.326
AA	No	141/194	**0.93 (0.76–1.14)**	1.00 (0.82–1.23)	0.980
AA	Yes	82/93	**1.66 (1.30–2.12)**	**1.52 (1.18–1.96)**	**0.001**
P interaction					**0.003**
rs915894	Family history of cancer				
AC+CC	No	356/467	1.00	1.00	
AC+CC	Yes	90/128	0.88 (0.70–1.10)	0.84 (0.67–1.07)	0.157
AA	No	178/235	0.96 (0.80–1.15)	1.06 (0.88–1.27)	0.543
AA	Yes	45/52	**1.53 (1.12–2.09)**	**1.77 (1.29–2.42)**	**<0.001**
P interaction					**0.001**

Note: [a]Adjusted by gender, age, smoking, history of lung diseases, family history of cancer, pathological types, clinical stage, metastasis, pleural effusion, surgery, chemotherapy, and radiotherapy. Bold values indicate significance.

modulated the expression levels of downstream molecules significantly. Prior investigation[25] indicated that aberrant NOTCH signaling contributed to the pathophysiology of diverse lung disorders, such as chronic obstructive pulmonary disease, asthma, and pulmonary fibrosis. Moreover, a family history of cancer may provide a synergy with the NOTCH signaling pathway at the gene level, which in turn amplifies the effect on the prognosis of NSCLC. We must consider, however, that genetic variations among various populations and residents of different regions may have contributed to this paper's finding that only rs915894 was related to NSCLC. A multicenter prospective study with a larger sample is needed to verify whether correlations exist between other NOTCH pathway SNPs and NSCLC.

NOTCH4, a transmembrane receptor of the Notch signaling pathway, is involved in the regulation of blood vessel formation and the remodeling and maturation of vascular networks.[26] Accumulating evidence has illustrated the specificity of NOTCH4 for different cancers and has suggested the potential for NOTCH4 to be used as a marker for the diagnosis and prognosis of cancers. Zhang et al.[15] reported that NOTCH4 might be an independent marker of relapse and prognosis in colorectal cancer patients, because overexpression of NOTCH4 in colorectal cancer cell lines suppressed tumor cell proliferation, migration, and invasion, while it induced apoptosis. Consistent with colorectal cancer, previous results demonstrated that high expression of NOTCH4 in ovarian cancer is correlated with a better prognosis.[27,28] On the contrary, *NOTCH4* is an oncogene of breast tumors, and patients with high NOTCH4 expression had significantly lower OS rates compared with the NOTCH4 low expression group in breast cancer, especially luminal breast cancer.[28,29] Studies have shown that NOTCH inhibitors slow cell proliferation and trigger apoptosis in ER-positive breast cancer cells.[30] Studies[31,32] also have demonstrated that the overexpression of NOTCH4 should promote the process of invasion and metastasis and meant poor prognosis of NSCLC, although a few studies showed no association between them.[17,18] Thus, further work is needed to determine the association between the expression of NOTCH4 and the prognosis of NSCLC.

Currently, only a handful of studies have focused on the association between *NOTCH4* polymorphisms and the prognosis of cancer, especially NSCLC. In previous studies, there was no exploration of the association between rs915894 and the overall survival of NSCLC, which we investigated for the first time in our study. Located in

exon3, rs915894 of *NOTCH4* is a missense mutation. We speculated that rs915894 could influence NOTCH4 expression activity and transcription of downstream proteins, resulting in a different NSCLC prognosis. Unfortunately, we did not collect the corresponding lung cancer tissue. Therefore, further investigation is needed to elucidate whether the rs915894 SNP in an exon could affect the expression of *NOTCH4* and NSCLC prognosis.

In addition, our data showed the association between rs11364 and the prognosis of NSCLC, even though the *P*-value did not reach statistical significance after adjustment. This result could be a function of the location of rs11364 in exon4 of *HES2*, the expression of which is closely related to the occurrence and development of cancer.[33] Further study is needed to understand the effects of the rs11364 polymorphism on tumor progression.

In summary, our research discovered the important prognostic and predictive value of rs915894 in the *NOTCH4* gene, which may be regarded as a promising prognosis biomarker of NSCLC in the Chinese population. To the best of our knowledge, this study was the first to examine the association of *NOTCH4* polymorphisms with the overall survival of NSCLC patients. Nevertheless, some limitations of this study should be acknowledged. First, our sample size, although adequate for overall analysis, may be limited in some strata in the stratified analysis. This constraint might decrease the ability to detect the statistical associations between genotypes and strata. Second, all the subjects were recruited from Fujian Province, primarily from the city of Fuzhou, which could introduce selection bias. Third, because the lung tissue specimens were not collected in the previous study, gene expression analysis could not be performed, which failed to explore the mechanism of the effect of rs915894 on the prognosis of NSCLC. Future studies should include larger populations and other races to allow for a wider evaluation of the significance of the SNPs for NSCLC patients. Additional functional assays are warranted to reveal the underlying mechanisms implied in our findings.

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Disclosure

The authors report no conflicts of interest in this work.

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