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Single nucleotide polymorphisms in NOS2A and NOS3 genes are not associated with treatment response of non-small cell lung cancer patients following the definitive radiochemotherapy

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Nitric oxide (NO), is endogenously synthesized from L-arginine by nitric oxide synthase (NOS), exhibits a dual role in sensitivity to radiotherapy and chemotherapy of cancer cells. The aim of this study was to evaluate the influence of polymorphisms in NOS genes on treatment response of non-small-cell lung cancer (NSCLC) patients after radiochemotherapy.

A cohort of 198 NSCLC patients treated with radiochemotherapy between 2009 and 2011 were included in this study. Genotyping analyses of 35 SNPs (NOS2A, 21 and NOS3, 14) in each sample were conducted by using the Sequenom MassArray system. Unconditional logistic regression was performed to assess the association between treatment response and each genotype while adjusting or not for other covariates.

Of 198 patients, 87 (43.9%) had objective responses, and 111(56.1%) did not respond. We observed no significant associations between treatment response and each genotype. While adjusting for other covariates, the associations were also not significant.

Our results suggest that genetic variations within the NOS2A and NOS3 genes may not influence the treatment response in NSCLC patients with radiochemotherapy. Future studies in this problem are required to confirm our findings.

Key words: single nucleotide polymorphism, nitric oxide synthase, non-small cell lung cancer, radiochemotherapy, treatment response

Worldwide, lung cancer is the most common cause of cancer-related death in men and women, and is responsible for 1.4 million deaths annually, as of 2008 [1]. Most patients diagnosed with non-small cell lung cancer (NSCLC) presented with advanced disease. For these patients, chemotherapy and radiotherapy were often the primary choices of treatment. Although platinum-based chemotherapy alone or in combination with radiotherapy is effective in treating some patients, insensitivity to radiochemotherapy is still a major problem in cancer treatment [2] and usually predicted shorter overall survival [3]. The basis behind treatment resistance either as primary or secondary, i.e., occurring after an initial treatment response, is still challenging to understand [4]. In previous studies, genetic factors were proved to influence the effectiveness of lung cancer treatment [5-14].

Nitric oxide (NO), a reactive radical, is endogenously synthesized from L-arginine by nitric oxide synthase (NOS), which exists as three isoforms: neuronal NOS (nNOS/NOS1), inducible NOS (iNOS/NOS2), endothelial NOS (eNOS/NOS3) [15]. The nNOS and eNOS isoforms are constitutively expressed in a variety of cell types including the endothelium, platelets, and neurons [16]. However, iNOS is absent in resting cells, but is capable of being rapidly expressed in response to proinflammatory stimuli such as cytokines and the HIF-1 mediated pathway [17]. In cancer biology and treatment, NO may have dual effects [18], cytoprotective and cytotoxic, which depended upon NO concentrations [19] or the specific gene, such as P53 [20]. On the one hand, NO is considered as an efficient hypoxic radiosensitizer [21-27] or a chemosensitizer [28-30]. Further studies suggested that

iNOS overexpression by adenoviral gene delivery enhances the radioresponsiveness of colorectal cancer via p53 activation and caspase-dependent apoptotic mechanism [30-33]. On the other hand, NO radicals could contribute to the induction of radioresistance [34] and chemotherapeutic resistance [35,36]. In lung cancer patients, several cytokines (IL-1β, IL-6, IFN-γ, TNF-α, TGF-β, etc.) are produced to enhance the production of NO [37]. These studies suggest that NO and NOS may be a novel therapeutic strategy for NSCLC treatment.

Given the uncertainty between NOS and treatment response, we speculated that genetic variants in NOS genes may alter their expression, activity or functions and in turn influence the effects of cancer treatment. To test this hypothesis, we genotyped 198 specimens collected from a cohort of NSCLC patients for 35 single nucleotide polymorphisms (SNPs) in NOS2A and NOS3 genes by using the Sequenom MassArray system, and then evaluated their associations with radiochemotherapy sensitivity. The result is expected to guide individualized NSCLC therapy.

Patients and Methods

Patient Population and Clinical Data Collection. In this study, a total of 198 DNA samples available from newly diagnosed lung cancer patients were prospectively collected between December 2009 and January 2011 at Radiation Oncology Department in Shandong Cancer Hospital (Jinan). All patients were Chinese and were diagnosed with histologically confirmed locally advanced (stage III) or advanced (stage IV) NSCLC, Karnofsky performance status (KPS) ≥60, and an expected survival of >6 months. Each patient signed an informed consent and was entered into the clinical research database prior to study entry. This study was approved by Shandong Cancer Hospital institutional review board. The clinical information including sex, age, histology, performance status, smoking status, clinical stage, tumor location, total radiation dose, radiotherapy technique (two- or three-dimension), chemotherapy or other factors were collected. Patient responses to treatment including complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) were determined by the Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1 [38]. For data analysis, CR and PR were combined as responders, and SD and PD were grouped as non-responders.

Radiotherapy. All patients received radiotherapy with 6-MV X-rays from linear accelerators (21EX, 23EX or Trilogy; Varian Inc., CA, USA). Target volumes were defined according to the report of International Commission on Radiological Units. The gross tumor volume (GTV) included the primary disease plus any involved regional lymph nodes. The planning target volume (PTV) was considered to include the GTV plus a 10- to 15-mm margin. 95% isodose line encompassed the PTV. Planning objective for total lung receiving > 20 Gy (V_{20}) was limited to ≤ 35%. Treatment planning was optimized using Philips Pinnacle^3 planning system (Philips Radiation Oncology Systems, Milpitas, CA, USA).

Chemotherapy Regimens. Patients were treated with one of the following regimens: (1) GP regimens (1000 mg / m^2 of gemcitabine on day 1 and day 8, plus 25mg/m^2 of cisplatin on day 1 through day 3, repeated every 3 weeks); (2) TP regimens (135mg/ m^2 of taxol on day 1, plus 25mg/m^2 of cisplatin on day 1 through day 3 or carboplatin at a dose calculated to produce an area under the serum concentration-time curve of 6.0 min·mg/mL, repeated every 3 weeks) or DP regimens (docetaxel 60 mg/m^2 followed by cisplatin 25 mg/m^2 on day 1 through day 3, repeated every 3 weeks); (3) NP regimens (25mg/ m^2 of vinorelbine on day 1 and day 8, plus 25mg/ m^2 of cisplatin on day 1 through day 3, repeated every 3 weeks) (4) irinotecan 60 mg/m^2 on days 1, 8, and 15 and cisplatin 80 mg/m^2 on day 1, repeated every 4 weeks. Each treatment was repeated for more than two cycles unless the patient met the criteria for PD or experienced unacceptable toxicity. Chemotherapy dosage was modified by toxicities in subsequent courses.

SNP Selection. We chose tag SNPs according to a similar approach in the previous article [39]. Tag SNPs were selected based on the ability to tag surrounding variants (iNOS, chr17 and eNOS, chr7) in the Han Chinese panel (Beijing, China) of the International HapMap project, NCBI build B36 assembly HapMap phase II+III (http://www.hapmap.org). The region used in tagSNP selection included a region from 3,000 bp 5' upstream to 1000 bp 3' downstream of NOS2A or NOS3. Tagger software included in Haploview software 4.2 was used to select tag SNPs. The pairwise algorithm of the Tagger program was used to select the tags. An r^2 of 0.8 was defined as the coefficient threshold for tag selection and minor allele frequency (MAF) was 0.05 [40]. In addition, common SNPs based on previous report of other diseases in NOS were included in this study. The characteristics of 35 selected SNPs were listed in Table 1.

DNA Collection and Genotyping. A 1mL whole-blood sample was obtained from each patient before treatment. The blood samples were collected in EDTA vacutainer tubes and stored at −80°C until analysis. Genomic DNA was extracted from whole-blood cells using AxyPrep Blood Genomic DNA Miniprep Kit (Axygen, USA) according to the manufacturer's instructions. The DNA purity and concentration were determined by spectro- photometric measurement of absorbance at 260 and 280 nm, respectively. Approximately 25 ng of genomic DNA was used to genotype each sample using the Sequenom MassArray system (San Diego, USA). The sample DNA was amplified by multiplex PCR reaction and the PCR products were then used for single-base extension reaction. The resulting products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was performed using MALDI-TOF MS. The mass spectrograms were analysed by the Sequenom MassARRAY TYPER software (San Diego, USA). The whole SNPs genotyping was conducted at Shandong Provincial Key Lab for Dermatovenereology (Jinan, China).

Table 1. Characteristics of 35 SNPs in NOS2A and NOS3

Gene	SNP ID	Chromosome	Position	Allele	Domain	
NOS3	rs7830	7	150340504	A/C	Intron	tagSNP
NOS3	rs743507	7	150338421	A/G	Intron	tagSNP
NOS3	rs1800781	7	150323377	A/G	Intron	tagSNP
NOS3	rs3918188	7	150333714	A/C	Intron	tagSNP
NOS3	rs3918227	7	150331879	A/C	Intron	tagSNP
NOS3	rs891512	7	150339022	A/G	Intron	tagSNP
NOS3	rs11771443	7	150318620	C/T	Promoter	tagSNP
NOS2A	rs2297515	17	23117460	A/C	Intron	tagSNP
NOS2A	rs2297518	17	23120724	A/G	CDS-nonsynonymous	tagSNP
NOS2A	rs2779248	17	23151959	C/T	Promoter	tagSNP
NOS2A	rs3794763	17	23135353	A/G	Intron	tagSNP
NOS2A	rs4795067	17	23130802	A/G	Intron	tagSNP
NOS2A	rs8072199	17	23140975	C/T	Intron	tagSNP
NOS2A	rs2314809	17	23119505	C/T	Intron	tagSNP
NOS2A	rs7208775	17	23109235	C/G	Intron	tagSNP
NOS2A	rs16949	17	23148826	C/T	Intron	tagSNP
NOS2A	rs3730013	17	23150045	C/T	Intron	tagSNP
NOS2A	rs9906835	17	23113501	A/G	Intron	tagSNP
NOS2A	rs11080344	17	23128638	C/T	Intron	tagSNP
NOS3	rs1549758	7	150326659	C/T	CDS-synonymous	
NOS3	rs1799983	7	150327044	G/T	CDS-nonsynonymous	
NOS3	rs1800779	7	150320876	A/T	Intron	
NOS3	rs1800780	7	150329812	A/G	Intron	
NOS3	rs1800783	7	150320330	A/T	Intron	
NOS3	rs3918174	7	150328227	A/G	Intron	
NOS3	rs4496877	7	150311439	G/T	5'UTR	
NOS2A	rs944722	17	23116164	C/T	Intron	
NOS2A	rs944725	17	23133698	C/T	Intron	
NOS2A	rs1137933	17	23130059	C/T	CDS-synonymous	
NOS2A	rs2072324	17	23141023	A/C	Intron	
NOS2A	rs2248814	17	23124448	A/G	Intron	
NOS2A	rs2255929	17	23112094	A/T	Intron	
NOS2A	rs2314810	17	23128237	C/G	Intron	
NOS2A	rs10459953	17	23151645	C/G	5'UTR	
NOS2A	rs17722851	17	23134963	A/T	Intron	

Abbreviations: SNP, single nucleotide polymorphism; 5'UTR, 5' untranslational region; CDS, Coding Sequence.

Statistical Analysis. The frequencies of different genotypes were compared between patients with and without treatment response through the Chi-square test. In multivariate analyses, unconditional logistic regression was performed to assess the association between treatment response and each genotype while adjusting for other covariates, including radiation dose, sex, age, histology, performance status, smoking status, clinical stage, tumor location, chemo-radiotherapy type. In the regression analysis, the dependent variable was patient response to treatment; patients who did not respond to treatment (SD + PD) were compared to patients who responded to treatment (CR + PR). Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated using the logistic regression model. *P* value <0.05 was considered statistically significant. All tests were two-sided. Statistical analysis was performed using IBM SPSS statistical version 19.0 software.

Results

Clinical Characteristics. Table 2 shows the baseline clinical characteristics of the 198 NSCLC patients (150 men and 48 women; median age, 60 years; range, 25 to 87 years), of whom

Table 2. Baseline Clinical Characteristics of Patients (n=198)

Characteristics	No.	%
Sex		
Female	48	24.2
Male	150	75.8
KPS		
90-100	105	53.0
80	66	33.3
<80	27	13.6
Histopathology		
Squamous cell	78	39.4
Adenocarcinoma	58	29.3
NSCLC, NOS	62	31.3
Tumor location		
Peripheral	128	64.6
Central	70	35.4
Clinical stage		
III	106	53.5
IV	92	46.5
Smoking status		
Nonsmoker	48	24.2
CSI≥600	89	44.9
CSI<600	61	30.8
Treatment		
Concurrent chemoradiation	94	47.5
Sequential chemoradiation	104	52.5
Radiation technique		
2-dimensional radiotherapy	25	12.6
3-dimensional radiotherapy	123	57.1
IMRT	60	30.3

Abbreviations: KPS, karnofsky performance status; NSCLC, NOS, non–small-cell lung carcinoma, not otherwise specified; CSI, cigarette-smoke index; IMRT, intensity-modulated radiation therapy.

54.5% had stage III disease, and 75.8% were cigarette smokers. Histological types included squamous cell carcinoma (39.4%), adenocarcinoma (29.3%), and large cell or not otherwise specified (31.3%). The median radiation dose was 60.0 Gy (range, 45 to 80.6 Gy); 83.3% (n=165) of patients received 50 to 70 Gy. Of the 198 patients, 100% received platinum-based chemotherapy, 94 (47.5%) were given concurrent chemoradiation, and 104 (52.5%) had sequential chemoradiation. Seventy-four (37.4%) patients were given GP regimens, 70 (35.4%) received TP regimens, 40 (20.2%) had NP regimens, and 14 (7.1%) were treated with other combinations. All patients were treated for three to six cycles, with a median of four cycles.

Patient Response to Treatment. Patient response to treatment was evaluated 1 to 2 months after completion of treatment. For 198 patients, 87 (43.9%) had objective responses (CR + PR), and 111 (56.1%) had not (SD + PD). For patients who received sequential chemoradiation, 43 (41.3%) had treatment responses and 61 (58.7%) showed no response. More patients treated with concurrent chemoradiation had higher response rates (46.8%), but the difference between the two groups was not significant (P = 0.439). Patients with radiotherapy dose (≥60 Gy) was shown to have a higher response than those treated with lower ones (53.8% versus 29.6%, P = 0.001). The response rates among all the treatment groups with different chemotherapy regimens were not significantly different (P = 0.221). The response rate to treatment for the patients with stage III NSCLC was higher than those with stage IV, but the difference was not statistically significant (49.1% versus 38%, P = 0.119). Associations between clinical characteristics and treatment response were shown in Table 3.

Genotypes and Treatment Responses. Successful genotype call rates in 35 SNPs were 97-100% and no SNP was excluded from analysis. Genotype distributions of 35 SNPs in NOS3 and NOS2A genes and association analysis results were listed in Table 4, Table 5, respectively. We observed no significant associations between treatment response and each genotype. It was noted that treatment responders and non-responders differed obviously with respect to radiation dose. While adjusting for covariates in the multiple logistic regression analysis, the associations were also not significant.

Discussion

The aim of this study is to investigate the association between genetic variants of NOS and treatment response. Our results suggest that single nucleotide polymorphisms in NOS2A and NOS3 genes are not associated with treatment response of NSCLC patients following radiochemotherapy. Even after adjusting for other covariates, no significant associations were found. On one hand, this negative result was possibly due to: (i) There was a small cohort of patients analyzed. Whenever a negative association study is reported, the power of the study is normally questioned. On this regard we speculate that smaller sample size may be a limiting factor for this study especially for the analysis of treatment effects where we are comparing 87 responders against 111 non-responders. (ii) The role of genetic variants of NOS2A and NOS3 on treatment response may be covered by other clinical or dosimetric factors, such as stage, chemotherapy regimens, radiation dose, etc. On the other hand, since NO and NOS might have totally diverse biological effects on chemoradiotherapy and the evidence in cancer is also conflicting, the hypothesis that NOS gene is responsible for the differences in treatment response of NSCLC patients may be incorrect. In view of the two possible aspects, the large sample validation study is necessary in the future.

In previous studies, genetic factors had been proved to influence the effectiveness of lung cancer treatment [5-14]. Over the past few years, associations between NOS polymorphisms and clinical prognosis of patients with breast cancer, vulvar cancer and NSCLC had also been reported [14, 41-43]. The polymorphism of the 27-bp variable number of tandem repeats (VNTR) in intron 4, not G894T in exon 7 (rs1799983) of the eNOS gene was found to be an independent prognostic factor for survival in advanced stage NSCLC patients treated with standard platinum-based chemotherapy [14]. However, the

Table 3. Associations between clinical characteristics and treatment response

Parameter	Crude OR	95% CI	*P* value	Adjusted OR	95% CI	*P* value
Sex						
Female	1.000			1.000		
Male	0.904	0.47-1.74	0.761	0.915	0.45-1.90	0.861
Age, years			.			
<65	1.000			1.000		
≥65	1.033	0.58-1.85	0.914	0.904	0.46-1.79	0.773
KPS						
90-100	1.000			1.000		
80	0.646	0.35-1.21	0.172	0.632	0.31-1.29	0.208
<80	0.728	0.31-1.71	0.468	0.684	0.27-1.76	0.431
Stage						
IV	1.000			1.000		
III	1.568	0.90-2.77	0.120	1.083	0.56-2.02	0.816
Hypertension						
No	1.000			1.000		
Yes	2.036	0.98-4.24	0.057	2.459	0.98-6.16	0.055
COPD						
No	1.000			1.000		
Yes	1.324	0.58-3.03	0.505	1.476	0.56-3.92	0.434
Tumor location						
Central	1.000			1.000		
Peripheral	0.626	0.35-1.13	0.117	0.766	0.40-1.52	0.446
Histology						
Squamous cell	1.000			1.000		
Adenocarcinoma	0.743	0.37-1.48	0.396	1.031	0.47-2.30	0.941
NSCLC, NOS	0.711	0.36-1.40	0.322	0.747	0.35-1.60	0.451
Tobacco use						
Never	1.000			1.000		
CSI<600	0.922	0.46-1.90	0.821	0.922	0.43-1.98	0.835
CSI≥600	0.878	0.41-1.88	0.738	0.843	0.36-2.00	0.693
CRT						
Yes	1.000			1.000		
No	0.801	0.46-1.41	0.440	0.812	0.44-1.51	0.512
Radiation dose, Gy						
<60	1.000			1.000		
≥60	2.771	1.52-5.10	0.001	2.601	1.35-5.00	0.004

Abbreviations: OR, odds ratios; 95%CI, 95% confidence intervals; KPS, karnofsky performance status; COPD, chronic obstructive pulmonary disease; NSCLC, NOS, non–small-cell lung carcinoma, not otherwise specified; CSI, cigarette-smoke index; CRT, concurrent chemoradiation.

author continued to analyze the treatment response among patients without definitive thoracic radiotherapy, indicating that the effect of the polymorphism on survival was not associated with treatment response [14]. The present study, revealed that there was no association between rs1799983 of the eNOS gene and treatment response in NSCLC patients, was consistent with this study.

Strength of this study is the inclusion of a considerable number of SNPs for which an association with cancer is biologically plausible and/or has been previously reported. However, our study has several limitations. Firstly, because we selected the most informative SNPs with high MAF and r^2, some causal variants might be missed. Secondly, the single-institutional study design contains the potential to introduce a selection bias. Thirdly, the analysis based upon a small number of enrolled patients could potentially lead to false-negative results. Finally, because all patients in our study were Han Chinese, it is uncertain whether these

Table 4. Genotypes in NOS3 and its association with treatment response for NSCLC patients

SNPs	Genotype	CR + PR		SD + PD		Adjusted OR	95% CI	Adjusted P value
		N	%	N	%			
rs11771443	CC	31	36.0	42	39.3	1		
(n = 193)	CT	41	47.7	43	40.2	1.419	0.71-2.83	0.320
	TT	14	16.3	22	20.6	0.863	0.40-2.11	0.746
rs1800779	AA	73	84.9	93	83.8	1		
(n = 197)	AG	11	12.8	15	13.5	0.898	0.40-2.30	0.817
	GG	2	2.3	3	2.7	0.901	0.12-6.60	0.918
rs1800781	AA	1	1.1	3	2.7	1		
(n = 197)	AG	12	13.8	14	12.7	2.580	0.18-36.50	0.483
	GG	74	85.1	93	84.5	2.531	0.21-31.10	0.468
rs1799983	GG	70	81.4	85	77.3	1		
(n = 196)	GT	16	18.6	23	20.9	0.900	0.42-1.94	0.788
	TT	0	0	2	1.8	0.000	0.00-	0.999
rs3918174	AA	74	86.0	90	84.1	1		
(n = 193)	AG	11	12.8	14	13.1	1.040	0.41-2.64	0.934
	GG	1	1.2	3	2.8	0.402	0.03-4.91	0.475
rs3918227	AA	0	0	1	0.9	1		
(n = 192)	AC	9	10.8	12	11.0	5.638E8	0.00-	1.000
	CC	74	89.2	96	88.1	5.637E8	0.00-	1.000
rs891512	AG	4	4.7	11	10.0	1		
(n = 196)	GG	82	95.3	99	90.0	1.913	0.53-6.92	0.323
rs7830	AA	15	17.2	19	17.3	1		
(n = 197)	AC	36	41.4	54	49.1	0.915	0.40-2.21	0.844
	CC	36	41.4	37	33.6	1.635	0.70-4.01	0.283
rs4496877	GG	74	85.1	92	83.6	1		
(n = 195)	GT	12	13.8	15	13.6	0.964	0.40-2.40	0.937
	TT	1	1.1	3	2.7	0.394	0.03-4.83	0.466
rs1800783	AA	2	2.3	3	2.7	1		
(n = 197)	AT	11	12.6	15	13.6	1.001	0.12-8.64	1.000
	TT	74	85.1	92	83.6	1.156	0.16-8.40	0.886
rs1549758	CC	61	70.1	75	67.6	1		
(n = 198)	CT	24	27.6	31	27.9	0.840	0.42-1.70	0.622
	TT	2	2.3	5	4.5	0.515	0.10-2.90	0.447
rs1800780	AA	10	11.5	11	9.9	1		
(n = 198)	AG	38	43.7	57	51.4	0.695	0.25-1.95	0.489
	GG	39	44.8	43	38.7	0.887	0.31-2.54	0.824
rs3918188	AA	11	12.6	10	9.0	1		
(n = 198)	AC	32	36.8	57	51.4	0.442	0.15-1.27	0.130
	CC	44	50.6	44	39.6	0.786	0.28-2.20	0.643
rs743507	AA	40	40.6	60	54.1	1		
(n = 198)	AG	42	48.3	49	44.1	1.458	0.78-2.74	0.240
	GG	5	5.7	2	1.8	3.730	0.60-23.12	0.157

Abbreviations: NSCLC, non–small-cell lung carcinoma; SNPs, single nucleotide polymorphisms; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

results can be generalized to other populations. Because ethnic differences in the genotypes in NOS genes may exist, other studies in different population are required to confirm such results.

Conclusions

To our knowledge, this is the comprehensive report investigating the association between genetic polymorphisms of

Table 5. Genotypes in NOS2A and its association with treatment response for NSCLC patients

SNP	Genotype	CR+PR		SD+PD		Adjusted OR	95% CI	Adjusted *P* value
		N	%	N	%			
rs2255929	AA	50	57.5	59	53.6	1		
(n=197)	AT	32	36.8	44	40.0	0.750	0.39-1.44	0.384
	TT	5	5.7	7	6.4	0.876	0.23-3.29	0.844
rs9906835	AA	26	30.2	38	35.2	1		
(n=194)	AG	40	46.5	48	44.4	1.324	0.65-2.68	0.436
	GG	20	23.3	22	20.4	1.269	0.54-3.00	0.582
rs944722	CC	4	4.7	5	4.7	1		
(n=193)	CT	32	37.2	40	37.4	0.632	0.14-2.81	0.547
	TT	50	58.1	62	57.9	0.761	0.18-3.30	0.715
rs2248814	AA	6	7.0	9	8.4	1		
(n=193)	AG	32	37.2	36	33.6	1.195	0.36-3.95	0.770
	GG	48	55.8	62	57.9	1.164	0.36-3.80	0.799
rs11080344	CC	34	39.1	54	49.1	1		
(n=197)	CT	41	47.1	46	41.8	1.221	0.62-2.40	0.561
	TT	12	13.8	10	9.1	2.011	0.70-5.80	0.194
rs4795067	AA	58	67.4	67	64.1	1		
(n=195)	AG	24	27.9	37	31.3	0.922	0.47-1.83	0.815
	GG	4	4.7	5	4.6	1.192	0.25-5.61	0.824
rs944725	CC	31	36.0	46	42.2	1		
(n=195)	CT	42	48.8	47	43.1	1.471	0.75-2.87	0.258
	TT	13	15.1	16	14.7	1.269	0.47-3.41	0.637
rs17722851	AT	6	7.0	11	10.1	1		
(n=195)	TT	80	93.0	98	89.9	1.824	0.60-5.60	0.294
rs8072199	CC	68	79.1	92	84.4	1		
(n=195)	CT	16	18.6	17	15.6	1.387	0.60-3.20	0.443
	TT	2	2.3	0	0	4.596E9	0.00-	0.999
rs3730013	CC	30	34.9	38	35.5	1		
(n=193)	CT	41	47.7	48	44.9	1.148	0.57-2.31	0.700
	TT	15	17.4	21	19.6	0.935	0.38-2.33	0.885
rs10459953	CC	16	18.4	24	22.2	1		
(n=195)	CG	39	44.8	50	46.3	1.384	0.60-3.21	0.448
	GG	32	36.8	34	31.5	1.874	0.77-4.55	0.165
rs7208775	CG	84	96.6	107	97.3	1		
(n=197)	GG	3	3.4	3	2.7	1.219	0.22-6.73	0.820
rs2297515	AA	66	75.9	94	84.7	1		
(n=198)	AC	21	24.1	17	15.3	1.885	0.88-4.06	0.105
rs2314809	CC	4	4.6	5	4.5	1		
(n=198)	CT	32	36.8	43	38.7	0.573	0.13-2.55	0.465
	TT	51	58.6	63	56.8	0.745	0.17-3.23	0.694
rs2297518	AA	4	4.6	3	2.7	1		
(n=197)	AG	15	17.2	25	22.7	0.428	0.07-2.74	0.371
	GG	68	78.2	82	74.5	0.489	0.09-2.77	0.418
rs2314810	CC	5	5.7	6	5.4	1		
(n=198)	CG	29	33.3	38	34.2	0.720	0.18-2.92	0.646
	GG	53	60.9	67	60.4	0.850	0.22-3.36	0.816
rs1137933	CC	69	79.3	80	72.1	1		
(n=198)	CT	17	19.5	30	27.0	0.898	0.42-1.93	0.783
	TT	1	1.1	1	0.9	0.670	0.03-15.90	0.804

Table 5. Continued

SNP	Genotype	CR+PR		SD+PD		Adjusted OR	95% CI	Adjusted *P* value
		N	%	N	%			
rs3794763	AA	11	12.6	15	13.5	1		
(n=198)	AG	45	51.7	44	39.6	1.438	0.55-3.73	0.456
	GG	31	35.6	52	46.8	0.829	0.31-2.19	0.706
rs2072324	AA	5	5.7	12	10.8	1		
(n=198)	AC	40	46.0	43	38.7	2.370	0.69-8.14	0.170
	CC	42	48.3	56	50.5	1.971	0.57-6.80	0.283
rs16949	CC	4	4.6	3	2.7	1		
(n=197)	CT	19	21.8	26	23.6	0.747	0.11-4.91	0.761
	TT	64	73.6	81	73.6	0.585	0.10-3.55	0.560
rs2779248	TT	65	75.6	80	72.1	1		
(n=197)	CT	21	24.4	27	24.3	0.938	0.45-1.96	0.865
	CC	0	0	4	3.6	0.000	0.00-	0.999

Abbreviations: NSCLC, non–small-cell lung carcinoma; SNPs, single nucleotide polymorphisms; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

NOS and treatment response. Our results indicate that genetic variations within the NOS2A and NOS3 genes may not influence the treatment response in locally advanced or advanced NSCLC patients with radiochemotherapy.

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