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# Polymorphism in ASCL1 target gene DDC is associated with clinical outcomes of small cell lung cancer patients

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## Abstract

**Background:** Achaete-scute homolog 1 (ASCL1) is a basic helix-loop-helix transcription factor and is essential in the differentiation of neuroendocrine cells and neural tissues. ASCL1 is frequently overexpressed in small cell lung cancer (SCLC) and plays a crucial role in the pathogenesis of SCLC.

**Methods:** This study was conducted to identify the association between single nucleotide polymorphisms (SNPs) in ASCL1 target genes and clinical outcomes of patients with SCLC after chemotherapy. A total of 261 patients diagnosed with SCLC were enrolled in this study. The association between 103 SNPs in 58 ASCL1 target genes and the response to chemotherapy and survival of patients with SCLC were analyzed.

**Results:** Among the 103 SNPs, 10 SNPs were significantly associated with the response to chemotherapy, and 19 SNPs were associated with OS in multivariate analyses. Among these, *Dopa Decarboxylase (DDC)* rs12666409A>T was significantly associated with both a worse response to chemotherapy and worse OS (adjusted odds ratio [aOR] = 0.40, 95% CI = 0.18–0.90, $P = 0.03$; adjusted hazard ratio [aHR] = 1.52, 95% CI = 1.10–2.10, $P = 0.01$, respectively, under a dominant model). In a stage-stratified analysis, the association was significant only in the extensive disease subgroup (aOR = 0.19, 95% CI = 0.06–0.60, $P = 0.01$; aHR = 1.73, 95% CI = 1.16–2.56, $P = 0.01$, respectively, under a dominant model), but not in the limited disease subgroup.

**Conclusion:** The results of our study suggest that *DDC* rs12666409A>T may be useful markers for predicting the clinical outcomes of patients with SCLC undergoing chemotherapy.

## Introduction

In spite of intensive clinical efforts to develop new therapies, lung cancer is one of the leading causes of cancer-related death globally.[1] Lung cancer is divided into two types; non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC, for which surgery is the best treatment option in the early stages, accounts for approximately 85% of all lung cancers.[2] SCLC is a highly

aggressive carcinoma, accounting for approximately 15% of all lung cancers.[3] SCLC is thought to originate from neuro-endocrine cells and is characterized by rapid tumorigenesis, metastasis and frequent relapse.[4] Previous research over several decades has led to only modest progress in the treatment and survival of patients with SCLC until recently when PD-1 inhibitor atezolizumab, in addition to etoposide/carboplatin was introduced, and showed a longer progression-free and overall survival, becoming the standard first-line therapy for patients with extensive disease.[5,6]

Achaete-scute homolog 1 (ASCL1) is a basic helix-loop-helix (BHLH) transcription factor which is essential in the differentiation of neuroendocrine cells and neural tissues.[7–10] ASCL1 is overexpressed in classic SCLC and in NSCLC with neuroendocrine features, suggesting its role in the pathogenesis of those malignancies.[10] It has been reported that ASCL1 expression correlated with the tumor-initiating capacity of SCLC.[10] Studies showed that inhibition of *ASCL1* gene led to the loss of pulmonary neuroendocrine cells, and induced growth inhibition and apoptosis in SCLC.[10,11] ASCL1 plays a crucial role in promoting SCLC carcinogenesis through the interaction with Notch signaling. ASCL1 upregulates the expression of its transcriptional target DLL3, a nonfunctioning Notch ligand which acts as a Notch inhibitor,[12] leading to the inhibition of Notch pathway, and the Notch pathway inhibition has been shown to promote neuroendocrine cell fate decisions.[13,14] Because Notch functions as a tumor suppressor in neuroendocrine tumors including SCLC,[14,15] ASCL1 overexpression in SCLC results in tumor progression. In addition, given that HES1, an important target gene of Notch signaling, is a strong repressor of ASCL1,[16] inhibition of Notch pathway by ASCL1 overexpression may lead to decreased *HES1* activity and reduced repression of ASCL1, thus further promoting SCLC. Therefore, ASCL1 and its target genes may be potential new therapeutic targets in SCLC.[3,17]

In the present study, we hypothesized that functional SNPs of ASCL1 target genes may have an influence on the pathogenesis of SCLC, and consequently on the clinical outcomes. To test this hypothesis, we searched 103 SNPs from 58 target genes of ASCL1 using web-based database and published literature,[17] and evaluated the associations between those SNPs and the clinical outcomes of the patients with SCLC who received chemotherapy.

## Methods

### Study populations of patients

This study included 261 patients who were diagnosed with SCLC from 1997 to 2017 at Kyungpook National University Hospital (KNUH), had received chemotherapy, and for which genomic DNA was available. Patients who underwent radiotherapy concurrently with chemotherapy as a first treatment modality were excluded to avoid the confounding effect of radiation on the response to chemotherapy. The biospecimens and clinical information used for this study were provided by Korea National Biobank of Kyungpook National University Hospital under institutional review board (IRB)-approved protocols. Limited disease (LD) was defined as tumor confined to the ipsilateral hemithorax and regional nodes that could be included in a single tolerable radiotherapy port. Extensive disease (ED) was tumor beyond the boundaries of LD including distant metastases, malignant pericardial, or pleural effusions, and contralateral supraclavicular and contralateral hilar involvement. All patients consented to enrollment in this study and ethnically Korean. The patients received either etoposide 100 mg/m$^2$ administered i.v. on day 1–3, and cisplatin 60 mg/m$^2$ on day one, every three weeks, or irinotecan 60 mg/m$^2$ administered i.v. on days one, eight, 15, and cisplatin 60 mg/m$^2$ on day one, every four weeks. Treatment was discontinued in cases of disease progression, major toxicities, or according to the decision of the patient or physician. Assessment of tumor response was carried out by computed tomography scan every two cycles. Responses were assessed using Response Evaluation Criteria in Solid Tumors.[18] The best overall response for each patient was reported and all responses were reviewed by an independent radiologist. Patients having complete response (CR) and partial response (PR) to first-line chemotherapy were considered as responders, and those having stable disease (SD) and progressive disease (PD) as nonresponders.

### Selection of single nucleotide polymorphisms (SNPs) and genotyping

We searched the list of 58 ASCL1 target genes from a published research article,[19] and collected all the SNPs (*n* = 35 995) in those genes using the public database (https://www.ncbi.nlm.nih.gov/SNP) to find the potentially functional polymorphisms. Using the FuncPred utility for functional SNP prediction in the SNPinfo web server (https://snpinfo.niehs.nih.gov/), 331 SNPs with predicted functions were collected. The SNPs with low minor allele frequency (<0.1 in HapMap-JPT data, *n* = 95) were excluded based on the NCBI SNP database (https://snpinfo.niehs.nih.gov/), and 236 SNPs remained. Next, 95 SNPs were excluded because of linkage disequilibrium (LD, r$^2$ > 0.8) based on the TagSNP utility in the SNPinfo web server, and 141 SNPs remained that were evaluated for further study. We designed primers of 24plex in multiplex level and seven SNPs were excluded during the primer combination, and we then processed the remaining SNPs

**Table 1** Univariate analysis for response to chemotherapy and overall survival by clinical variables

| Variables | No. of cases | Responders (CR + PR)† | Nonresponders (SD + PD)† | OR (95% CI) | P-value | MST (months) | 95% CI | Log-rank P-value | HR (95% CI) | P-value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Response to chemotherapy | | | | Overall survival | | | | |
| Overall | 261 | 190 (72.8) | 71 (27.2) | | | 10.5 | 9.3–11.4 | | | |
| Age (years) | | | | | | | | | | |
| <68 | 129 | 100 (77.5) | 29 (22.5) | 1.00 | | 11.8 | 11.0–13.5 | | 1.00 | |
| ≥68 | 132 | 90 (68.2) | 42 (31.8) | 0.62 (0.36–1.08) | 0.09 | 7.9 | 7.1–9.4 | 0.0004 | 1.59 (1.23–2.05) | 0.0004 |
| Gender | | | | | | | | | | |
| Male | 226 | 163 (72.1) | 63 (27.9) | 1.00 | | 10.5 | 9.2–11.4 | | 1.00 | |
| Female | 35 | 27 (77.1) | 8 (22.9) | 1.30 (0.56–3.02) | 0.54 | 11 | 6.4–15.3 | 0.99 | 1.00 (0.68–1.46) | 0.99 |
| Smoking status | | | | | | | | | | |
| Never | 19 | 16 (84.2) | 3 (15.8) | 1.00 | | 11.4 | 6.4–15.5. | | 1.00 | |
| Ever | 242 | 174 (71.9) | 68 (28.1) | 0.48 (0.14–1.70) | 0.26 | 10.3 | 9.2–11.3 | 0.80 | 1.07 (0.65–1.75) | 0.80 |
| Clinical stage | | | | | | | | | | |
| LD | 66 | 46 (69.7) | 20 (30.3) | 1.00 | | 13 | 10.7–15.5 | | 1.00 | |
| ED | 195 | 144 (73.8) | 51 (26.2) | 1.23 (0.66–2.27) | 0.51 | 9.6 | 8.2–10.9 | 0.0004 | 1.73 (1.27–2.35) | 0.001 |
| PS ECOG | | | | | | | | | | |
| 0–1 | 215 | 162 (75.4) | 53 (24.6) | 1.00 | | 10.9 | 10.0–11.7 | | 1.00 | |
| 2 | 46 | 28 (60.9) | 18 (39.1) | 0.51 (0.26–0.99) | 0.05 | 7.2 | 4.3–9.2 | 0.0003 | 1.82 (1.31–2.53) | 0.0004 |
| Weight loss | | | | | | | | | | |
| No | 185 | 139 (75.1) | 46 (24.9) | 1.00 | | 11.2 | 10.2–12.2 | | 1.00 | |
| Yes | 76 | 51 (67.1) | 25 (32.9) | 0.68 (0.38–1.21) | 0.19 | 8.1 | 7.2–10.2 | 0.03 | 1.36 (1.03–1.79) | 0.03 |
| Chemotherapy regimen | | | | | | | | | | |
| EP | 134 | 90 (67.2) | 44 (32.8) | 1.00 | | 10.9 | 9.0–12.4 | | 1.00 | |
| CC | 127 | 100 (78.7) | 27 (21.3) | 1.81 (1.04–3.16) | 0.04 | 10.2 | 8.9–11.4 | 0.98 | 1.00 (0.77–1.29) | 0.98 |
| Second-line chemotherapy | | | | | | | | | | |
| Yes | 140 | | | | | 12.2 | 11.2–13.7 | | 1.00 | |
| No | 121 | | | | | 7.2 | 6.2–8.4 | 0.0003 | 1.62 (1.24–2.10) | 0.0003 |
| Radiation to tumor | | | | | | | | | | |
| Yes | 34 | | | | | 16.7 | 13.0–30.8 | | 1.00 | |
| No | 227 | | | | | 9.6 | 8.2–10.8 | $7 \times 10^{-6}$ | 2.78 (1.75–4.43) | $2 \times 10^{-5}$ |

†Row percentage. CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; MST, median survival time; OR, odds ratio; PS, performance status.

with three-step PCR with a total of 134 SNPs. Genotyping was performed using Sequenom's MassARRAY iPLEX assay (Sequenom Inc., Sandiego, USA) following the manufacturer's instructions. Of the 134 SNPs, 103 SNPs excluding those with call rate (CR) < 95% and Hardy-Weinberg equilibrium (HWE) < 0.05 were processed for statistical analysis (Table S1).

## Statistical analysis

All statistical analyses were performed using statistic software (SAS, version 9.4, SAS institute, Cary, NC, USA). The Hardy-Weinberg equilibrium was tested using goodness-of-fit $\chi^2$ test. Differences in the distribution of genotypes according to patient characteristics were compared using a chi-square test. For survival assessment, the time between the date of first chemotherapy and the date of death was measured as overall survival (OS). The estimated survival according to different genotypes and clinical variables was analyzed using the Kaplan-Meier method and log-rank test. Hazard ratio (HR) and 95% confidence interval (CI) were analyzed using Cox proportional hazard models. Through logistic regression analysis, adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated to identify the associations between response to chemotherapy and genotypes or clinical factors. A cutoff P-value of 0.05 was adopted for all statistical analyses. All clinical factors were adjusted; gender (male vs. female), smoking status (never-smoker vs. ex–/current smoker), clinical stage (LD vs. ED), ECOG PS (0–1 vs. 2), weight loss (yes vs. no), second-line chemotherapy (yes vs. no), radiation to tumor (yes vs. no).

## Results

### Patient characteristics and clinical predictors

In this study, 261 patients diagnosed with SCLC were included (Table 1). The baseline characteristics are shown in Table 1. Median age of the patients was 68 (range 34–86). Of all patients, 226 were males and 242 were ever smokers. A total of 66 patients had LD and 195 patients had ED. ECOG 0–1 PS was recorded in 215 patients, and 76 patients experienced weight loss. As first-line chemotherapy, 134 patients received etoposide/cisplatin and 127 patients received irinotecan/cisplatin. A total of 140 patients received second-line chemotherapy, and 34 of 66 patients with LD underwent sequential radiotherapy.

**Table 2** DDC rs12666409 A>T and the association with the response to chemotherapy and overall survival

|  | Genotype | Responders (%)† | Nonresponders (%)† | OR (95% CI)‡ | P-value ‡ | No. of cases (%)§ | MST (months) | 95% CI | L-R-P | HR (95% CI)¶ | P-value ¶ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DDC |  |  |  |  |  |  |  |  |  |  |  |
| rs12666409 | AA | 51 (85.0) | 9 (15.0) | 1.00 |  | 60 (23.5) | 11.5 | 10.3–14.8 | 0.05 | 1.00 |  |
| A>T | AT | 94 (68.6) | 43 (31.4) | 0.37 (0.17–0.85) | 0.02 | 137 (53.7) | 9.4 | 8.0–11.0 |  | 1.62 (1.16–2.26) | 0.01 |
|  | TT | 44 (75.9) | 14 (24.1) | 0.50 (0.19–1.29) | 0.15 | 58 (22.8) | 11.9 | 8.5–13.7 |  | 1.32 (0.89–1.97) | 0.17 |
|  | Dominant |  |  | 0.40 (0.18–0.90) | 0.03 |  | 9.7 | 8.6–11.4 | 0.02 | 1.52 (1.10–2.10) | 0.01 |
|  | Recessive |  |  | 1.02 (0.51–2.08) | 0.95 |  | 11.9 | 8.5–13.7 | 0.97 | 0.95 (0.69–1.30) | 0.75 |
|  | Codominant |  |  | 0.74 (0.48–1.14) | 0.17 |  |  |  |  | 1.14 (0.95–1.38) | 0.16 |

†Row percentage. ‡ORs, 95% CI, and their corresponding P-values were calculated using multivariate regression analysis, adjusted for age, sex, smoking status, stage, ECOG performance status, weight loss, and chemotherapy regimen. §Column percentage. ¶HRs, 95% CI and their corresponding P-values were calculated using multivariate Cox proportional hazard models, adjusted for age, sex, smoking status, stage, ECOG performance status, weight loss, chemotherapy regimen, second-line chemotherapy and radiotherapy. CI, confidence interval; L-R-P, Log-rank P; HR, hazard ratio; OR, odds ratio.

## Association between clinical factors and clinical outcomes

The clinical outcomes were estimated according to the clinical characteristics of the patients (Table 1). The number of responders to first-line chemotherapy was 190 among 261 patients (72.8%). ECOG PS 2 was significantly associated with worse response to chemotherapy compared with ECOG PS 0–1 ($P = 0.05$) and first-line irinotecan/cisplatin was associated with a significantly better response compared with etoposide/cisplatin ($P = 0.04$). However, there was no significant difference in response rate according to age, gender, smoking status, stage, and weight loss. The estimated median survival time (MST) was 10.5 months (95% CI = 9.3–11.4 months). Clinical factors that were significantly associated with worse OS were patients age 68 or higher ($P = 0.0004$), ED ($P = 0.001$), ECOG PS 2 ($P = 0.0004$), weight loss ($P = 0.03$), not receiving second-line chemotherapy ($P = 0.0003$), and not receiving radiotherapy ($P = 2 \times 10^{-5}$). However, gender, smoking status, and first-line chemotherapy regimen were not associated with OS.

## Association between *ASCL1* target gene polymorphisms and clinical outcomes

The SNP ID, call rates (%), and minor allele frequencies are shown in Table S1. In multivariate analysis, 10 SNPs were significantly associated with the response to chemotherapy (*DDC* rs12666409, *BEND5* rs1385152, *FOXG1* rs12589013, *DBH* rs1611131, *DBH* rs1611114, *CACNA1C* rs10466907, *CACNA1A* rs2248069, *DDC* rs3735273, *SLC8A3* rs3809401, *GABRB3* rs4906902), and 19 SNPs were associated with OS (*DDC* rs12666409, *CACNA1A*

rs7254351, *SNAP25* rs3746544, *GABRB3* rs751994, *GRIP1* rs4617664, *GRIP1* rs17827030, *KCNK16* rs4714238, *LFNG* rs755179, *ADCYAP1* rs1610037, *FOXA2* rs1055080, *DLL3* rs3212275, *GNAO1* rs3790112, *SLC8A3* rs8022091, *DBH* rs5320, *TPH1* rs10488682, *JAG2* rs1057744, *MYT1* rs2427625, *BARX1* rs4563951, *SLC8A3* rs8018340) (Table S2). Among those 28 SNPs, *Dopa Decarboxylase* (*DDC*) rs12666409A>T was significantly associated with both a worse response to chemotherapy and worse OS under a dominant model (adjusted odds ratio [aOR] = 0.40, 95% CI = 0.18–0.90, $P = 0.03$; adjusted hazard ratio [aHR] = 1.52, 95% CI = 1.10–2.10, $P = 0.01$, respectively), but the association was not significant under recessive and codominant models (Table 2 and Fig 1). The *DDC* rs12666409 was not significantly associated with patient- or tumor-related factors, such as age, gender, smoking status, stage, performance status, weight loss, chemotherapy regimen, second-line chemotherapy, and radiation therapy (data not shown). The multivariate analysis showed that *DDC* rs12666409 AT+TT genotype and etoposide/cisplatin regimen were independent risk factors for worse chemotherapy response, and that *DDC* rs12666409 AT+TT genotype, age, ED, no second-line chemotherapy, and no radiation to tumor were independent risk factors for worse OS (Table 3). In stage-stratified analyses, the association was significant only in patients with ED under a dominant model (aOR = 0.19, 95% CI = 0.06–0.60, $P = 0.01$; aHR = 1.73, 95% CI = 1.16–2.56, $P = 0.01$, respectively), although not under recessive and codominant models. However, the association was not significant in those with LD (aOR = 0.99, 95% CI = 0.26–3.73, $P = 0.99$; aHR = 1.20, 95% CI = 0.61–2.35, $P = 0.60$, respectively, under a dominant model) (Table 4). When stratified according to the chemotherapy regimen, the association was significant only in
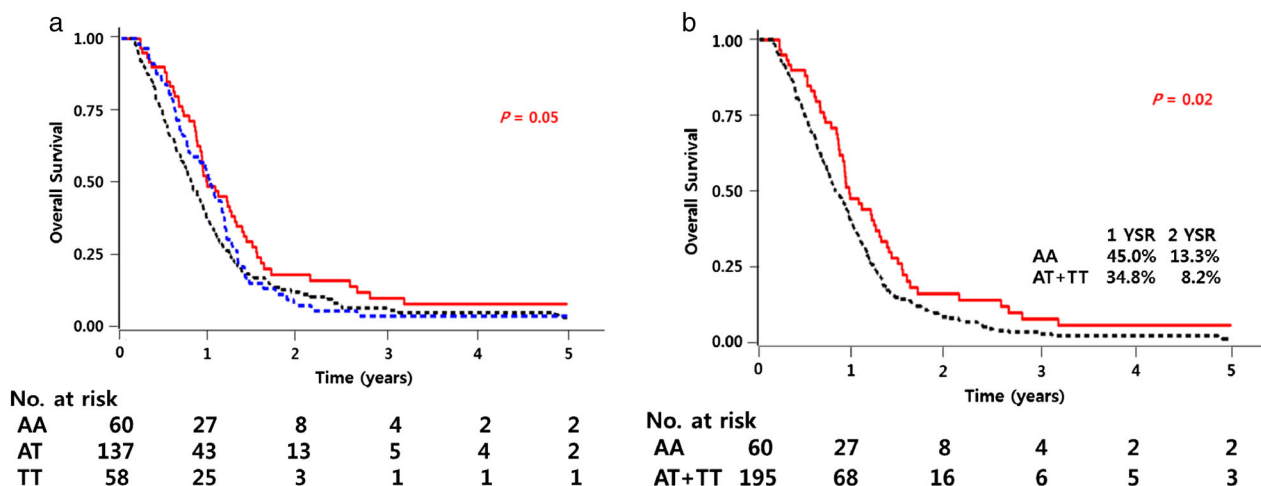


**Figure 1** Overall survival curves according to DDC rs12666409A>T under a codominant model (**a**) and under a dominant model (**b**). *P*, by log-rank test; 1 YSR, one year survival rate; 2 YSR, two year survival rate. (**a**) (——) AA, (·······) AT and (·······) TT. (**b**) (——) AA and (·······) AT + TT

**Table 3** Multivariate analysis of the predictive factors for response to chemotherapy and overall survival

| Variables | Response to chemotherapy | | Overall survival | |
|---|---|---|---|---|
| | OR (95% CI)[†] | *P*[†] | HR (95% CI)[‡] | *P*[‡] |
| rs12666409 (AT+TT/AA) | 0.40 (0.18–0.90) | 0.03 | 1.52 (1.10–2.10) | 0.01 |
| Age | 0.99 (0.96–1.03) | 0.65 | 1.02 (1.00–1.04) | 0.05 |
| Gender (Female/Male) | 1.00 (0.32–3.09) | 1.00 | 0.87 (0.50–1.52) | 0.62 |
| Smoking status (Ever/never) | 0.60 (0.12–3.12) | 0.54 | 0.65 (0.32–1.34) | 0.24 |
| Clinical stage (ED/LD) | 1.43 (0.73–2.78) | 0.30 | 1.59 (1.14–2.21) | 0.01 |
| ECOG PS (2/0–1) | 0.53 (0.25–1.12) | 0.10 | 1.31 (0.91–1.88) | 0.15 |
| Weight loss (yes/no) | 0.74 (0.39–1.41) | 0.36 | 1.08 (0.80–1.45) | 0.61 |
| Chemotherapy regimen (EP/IP) | 0.45 (0.25–0.83) | 0.01 | 1.27 (0.96–1.67) | 0.09 |
| Second-line chemotherapy (no/yes) | | | 1.49 (1.10–2.02) | 0.01 |
| Radiation to tumor (no/yes) | | | 2.24 (1.38–3.61) | 0.001 |

[†]ORs, 95% CI, and their corresponding *P* values were calculated using multivariate regression analysis including rs12666409 genotypes, age, gender, smoking status, stage, ECOG performance status, weight loss, and chemotherapy regimen. [‡]HRs, 95% CI and their corresponding *P* values were calculated using multivariate Cox proportional hazard models including rs12666409 genotypes, age, gender, smoking status, stage, ECOG performance status, weight loss, chemotherapy regimen, 2nd line chemotherapy and radiotherapy. CI, confidence interval; ED, extensive disease; EP, etoposide/cisplatin; HR, hazard ratio; IP, irinotecan/cisplatin; LD, limited disease; *L-R-P*, Log-rank *P*; OR, odds ratio; PS, performance status.

patients who received etoposide/cisplatin under a dominant model (aOR = 0.35, 95% CI = 0.13–0.94, *P* = 0.04; aHR = 1.65, 95% CI = 1.05–2.59, *P* = 0.03, respectively), although not under recessive and codominant models. However, the association was not significant in those who received irinotecan/cisplatin (aOR = 0.52, 95% CI = 0.13–2.05, *P* = 0.35; aHR = 1.30, 95% CI = 0.81–2.09, *P* = 0.28, respectively, under a dominant model) (Table 4).

## Discussion

In this study, we investigated whether genetic polymorphisms of *ASCL1* target genes were associated with clinical outcomes of SCLC patients. Among 103 SNPs in 58 *ASCL1* target genes, 28 SNPs were significantly associated with the response to chemotherapy or survival of patients. Most importantly, *DDC* rs12666409A>T was significantly associated with both worse response to chemotherapy and worse OS. In stratified analyses, the association was significant in patients with ED, and in patients who received an etoposide/cisplatin regimen. These findings suggest that *DDC* rs12666409A>T may be of potential use for predicting clinical outcomes in SCLC patients who receive chemotherapy, especially etoposide/cisplatin as the first-line regimen.

In this study, response to chemotherapy and survival of SCLC patients were significantly different according to the genotypes of *DDC* rs12666409A>T. The *DDC* gene encodes DOPA decarboxylase which is responsible principally for the synthesis of the key neurotransmitters dopamine and serotonin. Many studies have revealed that polymorphisms in the *DDC* gene are associated with various traits or diseases, such as smoking habit and nicotine dependence, alcohol consumption phenotypes, autism and

attention deficit hyperactivity disorder among others.[19–22] In addition to the central nervous system where DDC exerts its biosynthetic function of neurotransmitters, it is expressed in many peripheral organs including liver, kidney, lungs, and gastrointestinal tract, and even in peripheral leukocytes in which its biologic function is yet to be clarified.[23] Evidence has shown that biogenic amines, including dopamine, participate in various biological processes, such as angiogenesis, cell proliferation, differentiation, and apoptosis, implying a potential role of DDC in the pathogenesis of cancer.[24,25] DDC expression has been associated with many cancers. It has been regarded as a marker for neuroendocrine tumors.[26,27] High DDC mRNA expression has been reported in SCLC, neuroblastoma, and pheochromocytoma.[24] More recently, its clinical significance has been demonstrated in other solid tumors.[23,24,28,29] Increased DDC expression was reported in prostate cancer, and higher expression was associated with more aggressive tumors.[28] In contrast, high DDC expression was associated with low grade tumors and better outcomes in colorectal cancer.[24] The association was further complicated as DDC mRNA expression was significantly downregulated in laryngeal cancer compared with nonmalignant tissues, and DDC expression was associated with lower stages and favorable survival outcomes.[23] These conflicting results suggest that DDC expression may be associated with the pathogenesis of diverse cancers in a cell-type specific, context-dependent manner.

There is an increasing body of research suggesting that neuropeptides and neurotransmitters in the tumor microenvironment play an important role in the pathogenesis of cancer.[30] Some of these have been shown to promote tumor growth and affect the chemotherapeutic response of cancer cells.[30] Studies have revealed that dopamine has

**Table 4** Stratified analysis of the association between rs12666409 A>T genotypes and clinical outcomes according to stage and chemotherapy regimen

| | Genotype | Responders (%)† | Nonresponders (%)† | OR (95% CI)‡ | P-value‡ | No. of cases (%)§ | MST (months) | 95% CI | L-R-P | HR (95% CI)¶ | P-value¶ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Limited stage | AA | 11 (68.8) | 5 (31.2) | 1.00 | | 16 (24.6) | 14.3 | 10.6–18.3 | 0.79 | 1.00 | |
| | AT | 24 (72.7) | 9 (27.3) | 1.08 (0.26–4.44) | 0.91 | 33 (50.8) | 12.8 | 8.6–17.8 | | 1.23 (0.60–2.53) | 0.58 |
| | TT | 11 (68.8) | 5 (31.2) | 0.82 (0.16–4.18) | 0.81 | 16 (24.6) | 14.0 | 6.4–19.1 | | 1.15 (0.50–2.61) | 0.74 |
| | Dominant | | | 0.99 (0.26–3.73) | 0.99 | | 12.8 | 8.6–16.7 | 0.57 | 1.20 (0.61–2.35) | 0.60 |
| | Recessive | | | 0.78 (0.20–2.96) | 0.71 | | 14.0 | 6.4–19.1 | 0.58 | 1.01 (0.51–2.01) | 0.97 |
| | Codominant | | | 0.91 (0.40–2.07) | 0.81 | | | | | 1.07 (0.72–1.60) | 0.73 |
| Extensive stage | AA | 40 (90.9) | 4 (9.1) | 1.00 | | 44 (23.2) | 10.9 | 8.2–14.8 | 0.02 | 1.00 | |
| | AT | 70 (67.3) | 34 (32.7) | 0.17 (0.05–0.56) | 0.003 | 104 (54.7) | 8.9 | 7.1–10.3 | | 1.83 (1.22–2.74) | 0.004 |
| | TT | 33 (78.6) | 9 (21.4) | 0.26 (0.07–1.00) | 0.05 | 42 (22.1) | 11.7 | 8.5–13.5 | | 1.47 (0.90–2.39) | 0.12 |
| | Dominant | | | 0.19 (0.06–0.60) | 0.01 | | 9.3 | 8.0–11.0 | 0.01 | 1.73 (1.16–2.56) | 0.01 |
| | Recessive | | | 1.06 (0.44–2.53) | 0.90 | | 11.7 | 8.5–13.5 | 0.81 | 0.94 (0.65–1.37) | 0.76 |
| | Codominant | | | 0.60 (0.35–1.03) | 0.07 | | | | | 1.19 (0.95–1.48) | 0.13 |
| Etoposide-cisplatin | AA | 30 (83.3) | 6 (16.7) | 1.00 | | 36 (27.1) | 12.7 | 10.6–15.3 | 0.01 | 1.00 | |
| | AT | 42 (60.9) | 27 (39.1) | 0.33 (0.12–0.93) | 0.04 | 69 (51.9) | 8.6 | 6.4–11.2 | | 1.83 (1.13–2.96) | 0.01 |
| | TT | 18 (64.3) | 10 (35.7) | 0.38 (0.12–1.26) | 0.11 | 28 (21.1) | 11.7 | 7.2–15.6 | | 1.36 (0.78–2.39) | 0.28 |
| | Dominant | | | 0.35 (0.13–0.94) | 0.04 | | 9.7 | 7.4–12.0 | 0.01 | 1.65 (1.05–2.59) | 0.03 |
| | Recessive | | | 0.83 (0.34–2.04) | 0.69 | | 11.7 | 7.2–15.6 | 0.99 | 0.95 (0.59–1.51) | 0.82 |
| | Codominant | | | 0.64 (0.37–1.11) | 0.11 | | | | | 1.18 (0.91–1.52) | 0.22 |
| Irinotecan-cisplatin | AA | 21 (87.5) | 3 (12.5) | 1.00 | | 24 (19.7) | 10.5 | 6.6–16.3 | 0.87 | 1.00 | |
| | AT | 52 (76.5) | 16 (23.5) | 0.46 (0.12–1.86) | 0.28 | 68 (55.7) | 9.6 | 7.5–11.4 | | 1.35 (0.83–2.21) | 0.23 |
| | TT | 26 (86.7) | 4 (13.3) | 0.77 (0.14–4.13) | 0.76 | 30 (24.6) | 12.0 | 7.9–13.5 | | 1.18 (0.66–2.11) | 0.58 |
| | Dominant | | | 0.52 (0.13–2.05) | 0.35 | | 10.2 | 8.5–11.5 | 0.65 | 1.30 (0.81–2.09) | 0.28 |
| | Recessive | | | 1.41 (0.41–4.82) | 0.58 | | 12.0 | 7.9–13.5 | 0.89 | 0.95 (0.60–1.49) | 0.82 |
| | Codominant | | | 0.92 (0.43–1.94) | 0.82 | | | | | 1.08 (0.82–1.42) | 0.60 |

†Row percentage. ‡ORs, 95% CI, and their corresponding P-values were calculated using multivariate regression analysis, adjusted for age, gender, smoking status, ECOG performance status, weight loss and CTx regimen for the stage-stratified analysis; and adjusted for age, gender, smoking status, stage, ECOG performance status, weight loss for the chemotherapy regimen-stratified analysis. §Column percentage. ¶HRs, 95% CI and their corresponding P-values were calculated using multivariate Cox proportional hazard models, adjusted for age, gender, smoking status, ECOG perfor-mance status, weight loss, chemotherapy regimen, second-line chemotherapy and radiotherapy for the stage-stratified analysis; adjusted for age, gender, smoking status, stage, ECOG performance status, weight loss, second-line chemotherapy and radiotherapy for the chemotherapy regimen-stratified analysis. CI, confidence interval; L-R-P, Log-rank P; HR, hazard ratio; OR, odds ratio.

inhibitory effects on cancer growth by various mechanisms such as inhibiting proliferation and angiogenesis, and inducing cell cycle arrest and apoptosis.[30–34] The administration of dopamine or dopamine agonist bromocriptine has also been linked to growth inhibition mediated by dopamine D2 receptor in various cancer models including SCLC.[35–38] Notably, administration of dopamine increased the efficacy of anticancer drugs.[36,39] Taken together, the above studies suggest that elevated DDC expression and possibly the resulting increased dopamine level may have a potential tumor suppressor function in SCLC. In this study, we reported that *DDC* rs12666409A>T was associated with worse response to chemotherapy and worse survival in SCLC. The DDC has been experimentally identified as a target gene of transcription factor ASCL1.[17] In addition, FuncPred utility for functional SNP prediction in the SNPinfo web server (https://snpinfo.niehs.nih.gov/) predicts that rs12666409 (−1823 bp from transcription start site) resides in putative binding sites for multiple transcription factors. Therefore, based on the potential tumor suppressor function of DDC and dopamine, *DDC* rs12666409A>T may be associated with the decreased expression of DDC and thereby decreased dopamine activity, leading to worse response to chemotherapy and survival of patients. Stratified analyses showed that the effect of the SNP was different in different subgroups according to stage and chemotherapy regimen. Because DDC expression has been associated with the aggressiveness of cancer,[23,24,28] the effect of different DDC expression among rs12666409 genotypes may be more obvious in more aggressive disease. In addition, the effect of altered DDC expression and resulting dopamine level may exert different effects on the clinical outcomes based on the action mechanisms of different chemotherapeutic agents considering that dopamine participates in various biological processes relevant in cancer biology, such as cell proliferation and apoptosis.[24,25] However, future studies are needed to understand the mechanism of association between this SNP and the clinical outcomes of SCLC.

In this study, among 103 SNPs in 58 *ASCL1* target genes, 27 SNPs in 20 genes other than *DDC* rs12666409A>T were significantly associated with either chemotherapy response (nine SNPs in eight genes) or survival of patients (18 SNPs in 16 genes) (Table S1). Evidence suggested that some of those genes, such *SNAP25*, *GRM8*, *FOXA2*, *DLL3*, may play a role in the development and progression of SCLC.[40–44] Therefore, although we focused on the *DDC* rs12666409A>T which was associated with both clinical outcomes, those 27 SNPs are also worth further validation in future studies to investigate the potential clinical implications for predicting chemotherapy response or survival of patients.

There are some limitations in this study. First, because this study included only a Korean population, the results may not be generalizable for other ethnic groups. Second, we could not investigate the survival of patients according to *DDC* expression, and we could not correlate the genotypes of rs12666409A>T with *DDC* expression due to the lack of adequate clinical samples of SCLC because SCLC is rarely resectable. Third, this study did not provide direct evidence that DDC is involved in pathogenesis of SCLC.

In conclusion, we found that genetic polymorphisms in *ASCL1* target genes, especially *DDC* rs12666409A>T, were associated with clinical outcomes in patients with SCLC who receive chemotherapy, especially etoposide/cisplatin as the first-line regimen. *DDC* rs12666409A>T could be of potential use for predicting the clinical outcomes of SCLC patients treated with chemotherapy. To verify *DDC* rs12666409 as a biomarker for predicting clinical outcomes, the results of this study need to be further tested in a larger population with diverse ethnicity and validated in prospective studies including clinical trials. In addition, further studies are warranted to understand the biological function of DDC in the development and progression of SCLC.

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## Disclosure

The authors declare no conflict of interests.

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## Supporting Information

Additional Supporting Informationmay be found in the online version of this article at the publisher's website:

**Table S1** Summary of selected 103 SNPs in ASCL1 target genes and response to chemotherapy and overall survival.

**Table S2** The 27 SNPs and the association with either the response to chemotherapy or overall survival.