Table 3. Multivariate analysis of pretreatment and treatment-related parameters and groups categorized according to worst creatinine grade

Parameter		Odds ratio (95% confidence interval†)	*P*-value
Sex	Male	1	0.082
	Female	2.34 (0.90–6.10)	
Age	10-year increments	1.55 (0.91–2.64)	0.11
Average daily urine volume†	100-mL increments	0.94 (0.88–1.00)	0.073
Body weight loss	1-kg decrements	1.77 (1.08–2.90)	0.024
Total furosemide dose	10-mg increments	1.21 (1.11–1.33)	<0.001

†The average daily urine volume on days 1–5 of chemotherapy.

a simple and useful indicator of the hydration status of these patients.

The current study also showed that the total furosemide dose was associated with the development of renal toxicity. Vigorous fluid infusion and diuresis with mannitol or furosemide have been used widely for the prevention of cisplatin nephrotoxicity.[11,12] These interventions are thought to reduce the cisplatin concentration in the renal tubules and the time during which this drug and the tubular epithelial cells are in contact.[5] However, numerous experimental studies have provided conflicting results regarding the renal protective effects of these diuretics; cisplatin nephrotoxicity was reduced in some studies but was enhanced in others.[5] A randomized trial of cisplatin at a dose of 100 mg/m^2 and hydration with or without mannitol in patients with malignant melanoma showed that this regimen prevented nephrotoxicity during the first treatment course.[13] Another randomized trial of cisplatin hydration with mannitol or furosemide in patients with advanced solid tumors showed that a serum creatinine elevation of more than 2 mg/dL was observed in 28% of the courses in the mannitol-treated group and 19% of the courses in the furosemide-treated group.[14] A third randomized trial of cisplatin at a dose of 75 mg/m^2 and hydration alone, hydration with mannitol, or hydration with furosemide showed that creatinine clearance did not change before or after cisplatin treatment in the hydration alone and the furosemide-treated groups, but decreased in the mannitol-treated group.[15] However, these randomized trials included only small numbers of patients and therefore are not conclusive. Thus, no reports have convincingly shown any advantage of diuretics in preventing cisplatin nephrotoxicity. These studies differed from the current study, in which furosemide was administered only when fluid retention was suspected based on an increased BW or a decreased UV. Although an association between renal toxicity and the total furosemide dose was observed in this study, patients with fluid retention may be more prone to develop renal toxicity. Another explanation is that furosemide may have a direct toxic effect on the kidney. Thus, the administration of furosemide may be inevitable in some cases to prevent fluid overload during aggressive hydration, but its frequent use should be avoided.

Because renal function decreases physiologically with aging,[16] cisplatin use in elderly patients remains controversial. Some authors of clinical studies for patients aged 70 years or older have concluded that the use of cisplatin at moderate doses (60–100 mg/m^2) should be encouraged in these patients, just as it is in younger patients.[17–19] Studies that evaluated risk factors for cisplatin nephrotoxicity in more than 400 patients showed that an older age was a significant risk factor in two studies[7,20] but not in a third study.[8] In the current study, age was not a risk factor for renal toxicity according to a multivariate analysis, probably because 80 mg/m^2 of cisplatin was administered only to selected elderly patients. In our practice, many elderly patients are treated with cisplatin at a dose of 25 mg/m^2 on three consecutive days or weekly; these patients were excluded from the present study.

In the present study women were more likely to suffer from cisplatin nephrotoxicity than men. Another study also showed that women had a twofold increased risk for renal toxicity compared with men.[7] Although the reason for this difference is not definitely known, it may be explained, at least in part, by a 15% lower unbound cisplatin clearance in women than men,[7,21] because pharmacokinetics of unbound cisplatin have been repeatedly shown to be correlated with cisplatin nephrotoxicity.[22–24]

Although intravenous fluid infusion on the day of cisplatin administration is a well established treatment for preventing nephrotoxicity, the use of subsequent fluid infusions has not been reported. Because the present study showed that dehydration progressed on day 5 in many cases and an elevated serum creatinine level appeared thereafter, maintaining the total body water level during days 1–5 of chemotherapy seems to be important for the prophylaxis of cisplatin nephrotoxicity. For this purpose, a BW measurement carried out before breakfast would be a simple and useful indicator; if oral intake is found to be insufficient, vigorous infusion therapy on days 2–5 may be effective.

In conclusion, the maximum BW loss during days 1–5 of chemotherapy and the total furosemide dose were associated with the development of cisplatin renal toxicity. Maintaining total body water levels during this period seems to be important, and measuring BW would be a simple and useful indicator for this purpose.

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Concurrent Chemoradiotherapy for Limited-disease Small Cell Lung Cancer in Elderly Patients Aged 75 Years or Older

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Background: The optimal treatment for limited-disease small cell lung cancer (LD-SCLC) in patients aged 75 years or older remains unknown.
Methods: Elderly patients with LD-SCLC who were treated with chemoradiotherapy were retrospectively reviewed to evaluate their demographic characteristics and the treatment delivery, drug toxicities and antitumor efficacy.
Results: Of the 94 LD-SCLC patients treated with chemotherapy and thoracic radiotherapy at the National Cancer Center Hospital between 1998 and 2003, seven (7.4%) were 75 years of age or older. All of the seven patients were in good general condition, with a performance status of 0 or 1. Five and two patients were treated with early and late concurrent chemoradiotherapy, respectively. While the four cycles of chemotherapy could be completed in only four patients, the full dose of radiotherapy was completed in all of the patients. Grade 4 neutropenia and thrombocytopenia were noted in seven and three patients, respectively. Granulocyte-colony stimulating factor support was used in five patients, red blood cell transfusion was administered in two patients and platelet transfusion was administered in one patient. Grade 3 or more severe esophagitis, pneumonitis and neutropenic fever developed in one, two and three patients, respectively, and one patient died of radiation pneumonitis. Complete response was achieved in six patients and partial response in one patient. The median survival time was 24.7 months, with three disease-free survivors for more than 5 years.
Conclusion: Concurrent chemoradiotherapy promises to provide long-term benefit with acceptable toxicity for selected patients of LD-SCLC aged 75 years or older.

Key words: elderly – small cell lung cancer – chemotherapy – radiotherapy

INTRODUCTION

Small cell lung cancer (SCLC) accounts for approximately 20% of all pulmonary neoplasms and 25–40% of patients with this disease are 70 years of age or older. The number of elderly patients with such disease are expected to increase with the growing geriatric population (1).

Because SCLC is highly sensitive to chemotherapy and radiotherapy, the standard treatment for limited-disease SCLC (LD-SCLC) has been a combination of platinum and etoposide with concurrently administered thoracic radiotherapy, as long as the patients are in good general condition (2, 3). Such elderly patients, however, may show decreased clearance of the anticancer agents commonly used for the treatment of SCLC, including cisplatin and etoposide, because of the decrease of the lean body mass, hepatic blood flow and renal function that are associated with aging. In addition, myelotoxicity is sometimes more severe in this population than in younger populations, because the absolute area of hematopoietic marrow decreases with age (4). Retrospective subset analyses of patients with LD-SCLC treated with concurrent chemotherapy and radiotherapy in phase III trials have shown that the percentage of patients in whom the planned number of chemotherapy cycles can be completed is usually 10% lower in patients

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70 years of age or older as compared with that in younger patients (5). One study reported that myelotoxicity was more severe in elderly patients than in younger patients (5), while another reported no such difference between the patients of the two age groups (6). The delivery of thoracic radiotherapy was not influenced by age in these patients (7). However, 78–85% of patients in these analyses were aged between 70 and 75 years old and a few were over 80 years old. Thus, the most suitable treatment options for elderly patients with LD-SCLC aged 75 years or older still remain unknown.

The objective of this retrospective analysis was to evaluate the patient characteristics and the treatment delivery, toxicity and antitumor efficacy of the administered treatments in LD-SCLC patients 75 years of age or older who were treated with chemotherapy and thoracic radiotherapy.

PATIENTS AND METHODS

We retrospectively reviewed the medical charts, chest X-rays and computed tomography (CT) scans of LD-SCLC patients aged 75 years or older. To evaluate the thoracic irradiation field, the standard initial field was defined as follows: the field including the primary tumor and involved nodes with a short axis length of 1 cm or more on CT scans with a 1.0–1.5 cm margin, and the subclinical ipsilateral hilum and bilateral mediastinal lymph node regions with a 1.0 cm margin. The supraclavicular lymph node regions were included only if there was tumor involvement of these nodes. Toxicity was graded according to the Common Terminology Criteria for Adverse Events, version 3.0, Japanese edition (8). The objective tumor response was evaluated according to the WHO criteria issued in 1979 (9). The overall survival time was measured from day 1 of chemotherapy to the date of death as a result of any cause or the date of the last follow-up.

RESULTS

Of the 94 LD-SCLC patients treated with chemotherapy and thoracic radiotherapy at the National Cancer Center Hospital between 1998 and 2003, seven (7.4%) were 75 years of age or older (Table 1). During this period, we had three other patients with LD-SCLC who were aged 75 years or older. They were treated with chemotherapy alone because of complications in two patients and refusal of intensive therapy in one patient. There were five males and two females, and four patients were between 75 and 79 years of age and three patients were 80 years old or older. Three patients presented with persistent cough, while the remaining four patients complained of no symptoms and were diagnosed based on the detection of an abnormal shadow on a plain chest X-ray obtained during a mass screening or routine health examination program. All the patients were in good general condition. One patient had a history of inferior wall myocardial infarction suffered 9 years prior to this admission. However, echocardiography at this admission revealed normal heart function with an ejection fraction of 73%. One patient had stage I pulmonary emphysema with % FEV_1 predicted of 58%, but no abnormal findings on blood gas analysis. The % FEV_1 predicted in other four patients was within 98% and 116%, and was not measured in the other two patients. A median (range) PaO_2 level at the room air before treatment in the seven patients was 77.4 (66.9–87.2) Torr. A decreased creatinine clearance, 48.8 ml/min at a urine volume of 600 ml/day, was noted in one patient, while the other patients had a creatinine clearance of 78 ml/min or higher. Four and three patients had a performance status of 0 and 1, respectively, and five patients gave no history of loss of body weight. The diagnosis of small cell carcinoma was confirmed cytologically or histologically in all the patients.

The chemotherapy regimens used were cisplatin at 80 mg/m^2 on day 1 combined with etoposide at 100 mg/m^2 on days 1–3 in four patients aged between 75 and 19 years. For patients aged 80 years of older, carboplatin was dosed to a

Table 1. Patient characteristics

n	Age (yr)/ gender	Smoking history	Symptom	Weight loss (%)	Complications	Performance status	TNM stage
1	81/male	6/day × 62 yr	None	0	Type 2 DM	0	T1N2M0
2	81/female	20/day × 62 yr	None	0	OMI (inferior wall), thoracic aortic aneurysm	0	T1N1M0
3	80/female	20/day × 50 yr	Cough	11	Hypertension	1	T4N3M0
4	78/male	20/day × 46 yr	None	0	None	0	T2N2M0
5	77/male	30/day × 50 yr	Cough	7	COPD, Hypertension	1	T4N3M0
6	75/male	10/day × 55 yr	None	0	None	0	T1N2M0
7	75/male	10/day × 55 yr	Cough, Hoarseness	0	None	1	T4N2M0

COPD, Chronic obstructive pulmonary disease; OMI, old myocardial infarction; DM, diabetes mellitus.

target AUC of 5 by Calvert's formula on day 1 combined with etoposide at 80 mg/m^2 on days 1–3 in two patients and cisplatin at 25 mg/m^2 on days 1–3 combined with etoposide at 80 mg/m^2 on days 1–3 in one patient (Table 2). These regimens have been reported to be used in a JCOG phase III trial for elderly patients with extensive SCLC (10). Four cycles of chemotherapy could be completed in four patients, whereas only three cycles could be completed in two patients and only one cycle could be completed in one patient. The reason for discontinuation of the chemotherapy in these patients was prolonged myelosuppression in two patients and patient refusal for continuation of treatment in one patient. The chemotherapy dose was reduced in the subsequent cycles in four patients. The reasons for the dose reduction were grade 4 thrombocytopenia in two patients, grade 4 leukopenia in one patient and both grade 4 thrombocytopenia and leukopenia in one patient. Thoracic radiotherapy was started concurrently with the chemotherapy in five patients (early concurrent chemoradiotherapy). Treatment began with chemotherapy alone in the remaining two patients, because of a mild cytology-negative pleural effusion in one patient and too large an irradiation volume in the other patient. Two cycles of chemotherapy reduced the tumor volume successfully in both the patients and thoracic radiotherapy was then added concurrently with the third and fourth cycles of chemotherapy (late concurrent chemoradiotherapy). Thoracic radiotherapy was delivered using photon beams from a liniac or microtron accelerator with energy between 6 and 20 MV at a single dose of 2 Gy once daily up to a total dose of 50 Gy in four patients aged between 78 years or older and at a single dose of 1.5 Gy twice daily up to a total dose of 45 Gy in three patients aged between 75 and 77 years. This selection of conventional or hyperfractionated radiotherapy was determined arbitrarily. The initial irradiation field was judged as the standard in six patients and reduced in one patient. A multi-leaf collimator and conventional lead blocks were used for shaping of the irradiation field. The median irradiation area was 169 cm^2 (range, 95–278 cm^2). The projected total radiation dose was administered in all the patients, but a treatment delay of 5 days or longer was observed in three patients. The criteria of radiotherapy suspension were white blood cell count $< 1.0 \times 10^9$/L, platelet count $< 20 \times 10^9$/L, esophagitis $\geq$ grade 3, fever ≥ 38°C and performance status ≥ 3. The reason for the delay in the three patients was esophagitis, decreased platelet count and poor performance status.

The hematological toxicities observed in the patients are summarized in Table 3. Grade 4 leukopenia, neutropenia and thrombocytopenia were noted in four, seven and three patients, respectively. Granulocyte-colony stimulating factor support was used in five patients, red blood cell transfusion was administered in two patients and platelet transfusion was administered in one patient. The non-hematological toxicities included grade 3 or more severe esophagitis, pneumonitis and neutropenic fever in one, two and three patients, respectively. One patient died of radiation pneumonitis that developed 4 months after the end of radiotherapy (Case No. 6).

Of the seven patients, complete response was achieved in six patients and partial response in one patient (Table 3). However, prophylactic cranial irradiation was given in only one patient (Case No. 6). Three patients remained alive for

Table 2. Treatment and its delivery

n	Chemotherapy				Thoracic radiotherapy			
	Regimen (mg/m^2 if not specified)	Number of cycles	Dose reduction	Duration of one cycle (days)*	Timing	Total dose (Gy)/fractions	Field size	Delay (days)
1	C (AUC = 5) d1 + E (80) ds1–3	3	Yes	30	Early Co	50/25	S	4
2	P (25) ds1–3 + E (80) ds1–3	1	NA	NA	Early Co	50/25	S	7
3	C (AUC = 5) d1 + E (80) ds1–3	4	Yes	23	Late Co	50/25	S	14
4	P (80) d1 + E (100) ds1–3	4	Yes	26	Late Co	50/25	R	1
5	P (80) d1 + E (100) ds1–3	4	No	28	Early Co	45/30	S	3
6	P (80) d1 + E (100) ds1–3	4	No	27	Early Co	45/30	S	0
7	P (80) d1 + E (100) ds1–3	3	Yes	35	Early Co	45/30	S	7

*Calculated as follows: Duration of one cycle (days) = (Day 1 of the 1st cycle − Day 1 of the last cycle)/(Number of cycles − 1).
C, carboplatin; E, etoposide; NA, not applicable; P, cisplatin; Co, concurrent; S, standard; R, reduced.

Table 3. Toxicity, tumor response and survival

n	Hematological toxicity (grade by CTC-AE v3.0)				Blood transfusion	G-CSF support	Non-hematological toxicity ≥ grade 2 (grade by CTC-AE v3.0)	Tumor response	Survival time (mo)/outcome
	WBC	Neu	Hb	Plt					
1	3	4	1	4	Platelet	None	None	CR	80.3/Alive
2	3	4	1	2	None	Used	Pneumoniti (3), esophagitis (2), anorexia (2)	CR	21.3/Dead
3	4	4	3	4	RBC	Used	Neutropenic fever (3), esophagitis (3)	CR	65.6/Alive
4	4	4	2	1	None	Used	None	CR	97.4/Alive
5	3	4	2	3	None	Used	Neutropenic fever (3), esophagitis (2), anorexia (2)	CR	13.1/Dead
6	4	4	2	1	None	None	Pneumoniti (5), neutropenic fever (3)	CR	6.4/Dead
7	4	4	4	4	RBC	Used	None	PR	24.7/Dead

WBC, white blood cell count; Neu, neutrophil count; Hb, hemoglobin; Plt, platelet count; G-CSF, granulocyte- colony stimulating factor; CTC-AE, Common Terminology Criteria for Adverse Events; CR, complete response; RBC, red blood cell; PR, partial response.

more than 5 years without recurrence. The median survival of the seven patients was 24.7 months.

DISCUSSION

The antitumor effects of the treatment regimens were reasonably good, with six complete responses and one partial response and three long-term disease-free survivors in spite of discontinuation/dose reduction of chemotherapy. This is perhaps mainly attributable to the strict selection of patients in good general condition. Thus, we believe that the standard chemoradiotherapy can be applied to LD-SCLC patients aged 75 years or older as long as they are in good general condition.

The general condition of elderly patients, however, varies widely from patient to patient. Thus, in many elderly patients 75 years of age or older, it may be better to reduce the treatment intensity, although it may be difficult to establish the standard schedule applicable to all elderly patients. There are four possible ways to modify the intensity of therapy: (1) administer chemotherapy alone; (2) change the relative timing of chemotherapy and radiotherapy; (3) decrease the drug doses and number of cycles of chemotherapy, and (4) decrease the dose and intensity of thoracic radiotherapy.

Chemotherapy alone versus chemotherapy and thoracic radiotherapy for LD-SCLC were compared in many randomized trials between the 1970s and 1980s. A meta-analysis of these trials demonstrated survival benefit of radiotherapy added to chemotherapy in younger populations of patients less than 65 years of age, but the benefit is still unclear in older patients (11). Although the findings of this meta-analysis indicated that the standard treatment in elderly patients with LD-SCLC might be chemotherapy alone, the result based on the old trials using cyclophosphamide and doxorubicin-based chemotherapy cannot be applied in the current medical setting, because chemotherapy regimens, irradiation delivery equipment and staging procedures have all evolved greatly over time.

The relative timing of chemotherapy and radiotherapy greatly influences the severity of toxicity. In late concurrent chemoradiotherapy that follows induction chemotherapy, the chemotherapy dose can be adjusted to suit each patient by evaluating the toxicity of the previous chemotherapy. In addition, the irradiation volume can be reduced by modifying the radiation treatment planning in accordance with the extent of tumor shrinkage during the induction phase. In the two patients treated by this approach in this study, the dose of the platinum drug during the concurrent chemoradiotherapy phase was reduced to 66–75% of the initial dose and that of etoposide was reduced to 50–75% of the initial dose. Sequential chemoradiotherapy consists of induction chemotherapy and subsequent radiotherapy. Because the two treatment modalities are administered separately, the treatment dose in each can be optimized for the elderly in this approach. A phase III study of concurrent versus sequential chemoradiotherapy in LD-SCLC patients younger than 75 years old revealed a 5-year survival rate of 24% in the concurrent arm and a 5-year survival rate of 18% with a lower incidence of toxicity in the sequential arm (2). The sequential schedule has not yet been evaluated in LD-SCLC patients 75 years of age or older.

A recent phase III trial showed that etoposide at 80 mg/m^2 on days 1–3 combined with either carboplatin at AUC = 5 by Carvert's formula or cisplatin at 25 mg/m^2 on days 1–3 was feasible and effective in elderly patients with extensive-disease SCLC (10). These regimens may, therefore, be applied for the treatment of LD-SCLC as well. The standard number of chemotherapy cycles administered is four. In many elderly patients, however, all four cycles cannot be completed. In two phase II studies of two cycles

of chemotherapy and concurrent thoracic radiotherapy in elderly patients with LD-SCLC, 13–25% long-term survivors were noted (12,13). Thus, the optimal number of chemotherapy cycles in the elderly should be investigated in future trials.

Thoracic radiotherapy with accelerated hyperfractionation at a total dose of 45 Gy in 30 fractions, the standard schedule for LD-SCLC, was associated with grade 3–4 esophagitis in as high as 32% of the patients and grade 4 leukopenia in 44% of the patients (2,3,5). Thus, the conventional schedule at a total dose of 45–50 Gy in 25 fractions might be preferable in the elderly (3). The severity of esophagitis is also influenced by concomitant chemotherapy, the treatment schedule and the timing of thoracic radiotherapy.

In conclusion, concurrent chemoradiotherapy promises to offer long-term benefit with acceptable toxicity in selected patients of LD-SCLC aged 75 years or older. The optimal schedule and dose of chemotherapy and thoracic radiotherapy still remains to be established in this patient population.

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Conflict of interest statement

None declared.

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Susceptibility to Lung Cancer and Genetic Polymorphisms in the Alcohol Metabolite-related Enzymes Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 in the Japanese Population

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BACKGROUND. It is believed that acetaldehyde plays an important role in alcohol-related carcinogenesis; although current epidemiologic studies have provided inconsistent findings on the association between alcohol consumption and the risk of lung cancer.

METHODS. To clarify the hypothesis that genetic polymorphisms in alcohol-metabolizing enzymes may influence susceptibility to lung cancer, the authors conducted a hospital-based case-control study and examined genetic polymorphisms in the alcohol dehydrogenase 3, aldehyde dehydrogenase 2 ($ALDH_2$), and cytochrome P450 2E1 genes in 505 patients with histologically confirmed lung cancer and in a group of 256 noncancer controls who provided complete cigarette and alcohol consumption histories. Genotyping was conducted by polymerase chain reaction-restriction fragment-length polymorphism assay.

RESULTS. A significant association was noted between alcohol consumption and lung cancer risk. Thus, using the median value for the controls as the cut-off point, the odds ratios (OR) for light and heavy drinkers were 1.76 and 1.95, respectively (P for trend = .012), compared with nondrinkers. In addition, there was a significant trend toward increased risk of lung cancer in drinkers with $ALDH_2$ variant alleles (P for trend <.0001). The adjusted OR for heavy drinkers was 6.15 compared with nondrinkers. Regarding associations between histologic type and genotypes, the $ALDH_2$ variant allele was significantly less common in patients who had adenocarcinoma compared with controls.

CONCLUSIONS. The current observations suggested a positive association between alcohol consumption and the risk of lung cancer: Drinking may increase the risk, especially among individuals who have the variant $ALDH_2$ alleles. ***Cancer* 2007;110:353–62.** © *2007 American Cancer Society.*

KEYWORDS: lung cancer, alcohol consumption, case-control study, genetic polymorphism, alcohol dehydrogenase 3, aldehyde dehydrogenase 2, cytochrome P450 2E1.

Epidemiologic studies have provided inconsistent results regarding the associations between alcohol consumption and the risk of lung cancer. In general, therefore, the involvement of alcohol in lung cancer etiology has been regarded with skepticism, with any indication of an association being attributed in most instances to confounding factors, such as cigarette smoking.[1] It indeed is difficult to separate the effects of alcohol and smoking because, the 2 tend to be

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correlated, but this problem does not automatically exclude the possibility that there is a separate alcohol effect. A panel of experts commissioned by the World Cancer Research Fund and the American Institute for Cancer Research in 1997, after reviewing the epidemiologic evidence, concluded that alcohol intake *possibly* may increase lung cancer risk.[2] Although the mechanism by which alcohol may cause cancer remains obscure, many epidemiologic studies have identified chronic alcohol consumption as a significant risk factor for cancers of the oral cavity, pharynx, larynx, and esophagus in humans.[3] When investigating the role of alcohol-related carcinogenesis, most studies have concentrated on the type of alcoholic beverage consumed and the amount of daily intake, but this does not fully explain the variance in individual susceptibility to alcohol-related cancer.

Recent reports strongly implicate acetaldehyde, the first metabolite of ethanol, rather than alcohol itself, as responsible for the risk of developing alcohol-related cancers. It has been reported that acetaldehyde causes mutations by DNA adduct formation and inhibition of DNA repair. Moreover, drinking or inhaling acetaldehyde has mutagenic and carcinogenic effects and induced nasal and laryngeal carcinomas in experimental animals.[4–8]

Ethanol is primarily (80%) oxidized to acetaldehyde by alcohol dehydrogenase (*ADH*), and most of this acetaldehyde is then eliminated by aldehyde dehydrogenase (*ALDH*). However, ethanol and acetaldehyde also are metabolized through the microsomal ethanol-oxidizing system and the microsomal acetaldehyde-oxidizing system, and cytochrome P450 2E1 (*CYP2E1*) is a major contributor to those systems.[9,10] *CYP2E1* has high oxidation activity and is induced by long-term alcohol intake. These enzymes exhibit wide interindividual variability in their activity, suggesting that the variation may be caused by genetic polymorphisms.

There are several *ADH* subtypes, some of which have genetic variants with altered kinetic properties. ADH_3 is polymorphic, and the enzyme encoded by the ADH_3^1 allele metabolizes ethanol to acetaldehyde 2.5 times faster than that encoded by the ADH_3^2 allele.[11] $ALDH_2$ is a key enzyme in the elimination of acetaldehyde. In individuals with $ALDH_2^2$, a variant allele that is prevalent among East Asians (eg, $\approx$ 50% prevalence in Japan[12]), the activity of this enzyme is extremely low. The *CYP2E1* variant allele, which is detectable by *Rsa*I digestion (termed the c2 variant), corresponds to higher activity ethanol metabolism and is associated with greater alcohol consumption.[13–15] Individuals who have 1 or more ADH_3^1, $ALDH_2^2$, and *CYP2E1* c2 alleles accumulate more acetaldehyde in the blood after drinking ethanol and may be at increased risk for various alcohol-related diseases at similar levels of alcohol intake as individuals who do not carry these alleles. Because the ADH_3 variant allele is common in whites, and the $ALDH_2$ and *CYP2E1* variant alleles are found at high frequency in Asians, research on these genes is most advanced regarding alcohol-related diseases and alcohol metabolism.

The association between genetic polymorphisms in these enzymes and susceptibility to some types of cancer has been reported in case-control studies. The ADH_3^1 and $ALDH_2^2$ alleles are associated closely with alcohol-related cancers in the upper aerodigestive tract,[16–21] and systemic acetaldehydemia has been considered responsible for carcinogenesis in this locality. However, to our knowledge, there are no reports on associations between polymorphisms of *ALDH* and lung cancer risk. In relation to *ADH*, a negative association between genetic variation in ADH_3 and lung cancer has been reported recently.[22] *CYP2E1* is responsible primarily for the bioactivation of many low-molecular-weight, tobacco-specific carcinogens, including certain nitrosamines, such as *N*-nitrosodimethylamine and *N*-nitrosonornicotine. It is possible that the *CYP2E1* c2 variant not only may increase the blood concentration of acetaldehyde but also may activate these carcinogens more strongly. Activated nitrosamines have been linked to the development of numerous cancers. However, results from studies that evaluated the role of *CYP2E1* polymorphisms in relation to lung cancer have been discrepant.[23–28] Because previous investigations did not adjust for alcohol consumption and/or did not have sufficient power to distinguish the risk from alcohol consumption, these inconsistent findings may have been caused by variations in *CYP2E1* enzyme activity induced by ethanol.

We conducted a hospital-based case-control study to evaluate whether ADH_3, $ALDH_2$, or *CYP2E1* polymorphisms are associated with lung carcinogenesis. The primary endpoint of the current study was to clarify the association between each genetic polymorphism and the risk of lung cancer, controlling for the amount of alcohol consumed and smoking habits. Furthermore, associations between alcohol consumption and lung cancer risk in individuals with variant alleles, again controlling for smoking, and associations between these polymorphisms and histologic characteristics were evaluated.

MATERIALS AND METHODS

Participants

This study was approved by the Institutional Review Board and the Ethics Committee of the National

Cancer Center, Japan. The majority of eligible participants in this study were residents of Chiba and East Tokyo, and all were of Japanese nationality. Personal and clinical data from patients who participated in the Lung Cancer Database Project at the National Cancer Center Hospital East (NCCH-E) and the National Cancer Center Research Institute East were used in the current study. The database includes information on demographic factors, physical symptoms, psychological factors, and lifestyle factors (diet, smoking, etc) obtained from self-reported questionnaires and medical information from the patients' medical charts and blood, DNA, and urine specimens. All patients who were enrolled in the current study had primary lung cancer that was newly diagnosed with histologic or cytologic confirmation at the Thoracic Oncology Division of the NCCH-E, Japan, from September 1997 to June 2000. All patients provided their written informed consent prior to enrolment in this project. Unmatched controls were newly recruited individuals from the population with no history of cancer or other tumors who visited the Thoracic Oncology Division of NCCH-E from March 2002 to May 2003 and were confirmed as cancer-free by appropriate examinations (chest computed tomography scans, bronchofibroscopy, video-assisted thoracoscopic biopsy, etc). The major reasons for visiting the hospital were suspicions of lung cancer on chest x-ray or sputum cytology at their annual medical check-up or referral from other hospitals. Epidemiologic data were collected by personal interview. All individuals in the control group completed the same standardized questionnaire that was completed by the Lung Cancer Database Project participants, including detailed demographic information, history of cancer, occupational and residential history, and detailed information regarding alcohol and tobacco consumption. All participants provided their written consent.

Sample Collection and DNA Extraction

Four milliliters of peripheral venous blood were collected into heparinized tubes. Genomic DNA was purified from peripheral blood lymphocytes using a DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and was stored at −80°C.

Polymorphism Analysis

ADH_3 and $ALDH_2$ genotyping was performed by using the polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) method. To prevent the amplification of closely related ADH_1 and ADH_2 genes, samples initially were digested with the *Nla*III restriction enzyme (TOYOBO, Osaka, Japan). A 145-base pair (bp) section of the ADH_3 gene was amplified by PCR using 200 ng of predigested genomic DNA with primers (sense, 5′-GCTTTAAGAGT AAATATTCTGTCCCC-3′; antisense, 5′-AATCTACCTCT zTTCCGAAGC-3′). The PCR product obtained in this manner then was digested directly with restriction enzyme *Ssp*I (TOYOBO). After polyacrylamide gel electrophoresis, ADH_3 alleles were visualized by ethidium bromide and were photographed under ultraviolet light. The ADH_3^1 allele produced fragments of 67 bp, 63 bp, and 15 bp; and the ADH_3^2 allele produced fragments of 131 bp and 15 bp.

A 134-bp fragment of the $ALDH_2$ gene was amplified by PCR according to a slightly modified method of Harada et al.[12] One hundred fifty nanograms of genomic DNA were mixed with 5 pmol of each primer (sense, 5′-CAAATTACAGGGTCAAGGGCT-3′; antisense: 5′-CCACACTCACAGTTTTCTCTT-3′) in a total volume of 50 μL that contained 50 μM deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, and 1 U Taq DNA polymerase; Takara Shuzo, Kyoto, Japan). Thirty-five cycles (denaturation at 94°C for 15 seconds, annealing at 58°C for 1 minute and 30 seconds, and polymerization at 72°C for 30 seconds) were performed using a GeneAmp PCR system 9600 (PerkinElmer, Oak Book, Ill). After purification, each PCR product was digested with *Mbo*II (TOYOBO), electrophoresed on a 20% polyacrylamide gel, stained with ethidium bromide, and photographed. The $ALDH_2^1$ allele produced fragments of 125 bp and 9 bp, and the $ALDH_2^2$ allele produced fragments of 134 bp.

The *CYP2E1* genotypes ascribed to the *Rsa*I site in the 5′-flanking region also were identified as RFLPs by PCR. Genomic DNA (100 ng) was subjected to PCR with each primer (sense, 5′-ATCCACAAGTG ATTTGGCTG-3′; antisense, 5′-CTTCATACAGACCCTC TTCC-3′). PCR was performed for 35 cycles under the following conditions: 1 minute at 95°C for denaturation, 1 minute at 55°C for primer annealing, and 1 minute at 72°C for primer extension. The 412-bp fragment was digested with *Rsa*I (TOYOBO). The products that were yielded were fragments with 360 bp and 50 bp for c1/c1; 360 bp, 50 bp, and 410 bp for c1/c2; and 410 bp for c2/c2 detected by electrophoretic analysis in 5% polyacrylamide gels.

Statistical Analysis

Patient characteristic (see Table 1) were compared with characteristic in the control group by using the Student *t* test or the chi-square test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were obtained by unconditional logistic regression analy-

TABLE 1
Baseline Characteristics of Lung Cancer Cases and Controls

Characteristic	No. (%) Cases (n = 505)	Controls (n = 256)	P for difference
Mean age ± SD, y	64.8 ± 8.3	63.5 ± 10.2	.06*
Sex			
Men	360 (71.3)	126 (49.2)	
Women	145 (28.7)	130 (50.8)	<.0001†
Smoking status			
Never	140 (27.7)	129 (50.4)	
Past	97 (19.2)	64 (25)	
Current	268 (53.1)	63 (24.6)	<.0001†
Smoking amounts, pack-years			
Past			
<27	35 (36.1)	32 (50)	
≥27	62 (63.9)	32 (50)	.08†
Current			
<40	71 (26.5)	30 (47.6)	
≥40	197 (73.5)	33 (52.4)	.001†
Alcohol drinking habit, times/wk			
Seldom	116 (23)	118 (46.1)	
≤2	43 (8.5)	42 (16.4)	
3–6	96 (19)	22 (8.6)	
Daily	250 (49.5)	74 (28.9)	≤.0001†
Alcohol amounts, g/day			
0	120 (23.8)	119 (46.5)	
<31.6	154 (30.5)	65 (25.4)	
≥31.6	231 (45.7)	72 (28.1)	≤.0001†

SD indicates standard deviation.
* Determined using the Student *t* test.
† Determined using the chi-square test.

sis. In our regression models, we adjusted ORs for potential confounding variables, including age, sex, smoking status (never, past, current) or amounts smoked (pack-years) and alcohol consumed (none, light, heavy). Because differences in the amount of alcohol consumed (ethanol, in gram per day) were very large, we divided those who drank into 3 categories: nondrinkers, light drinkers (≤31.6 g per day), and heavy drinkers (>31.6 g per day). The amount of tobacco smoke exposure was calculated as pack-years (usual amount per day/20 × overall duration [years] of use). Participants were considered current smokers if they smoked up to 1 year before the date of diagnosis in the case group or up to the date of the interview for the control group. The average amount of daily ethanol intake was calculated in grams. Calculation of this value was based on an average ethanol content of 4-volume% in beer, 15-volume% in Japanese sake (rice wine), 25-volume% in Japanese spirits (syochu), 12-volume% in wine, and 40-volume% in spirits. Drinking frequency was assessed as 5 categories: less than once a week, 1 or 2 days a week, 3 or 4 days a week, 5 or 6 days a week, and daily. Categorical variables were compared with the chi-square test. ORs and 95% CIs were calculated by using logistic regression analysis adjusting for age, sex, smoking, and drinking. The Mantel extension test was used to evaluate linear trends across categories of alcohol consumption that were divided into 4 categories by quartiles for control. Resulting P values <.05 (2-tailed) were considered statistically significant. All statistical analyses were performed using the SAS statistical software package (SAS Institute Inc., Cary, NC).

RESULTS

In total 510 patients with lung cancer (cases) and 260 healthy controls participated in this study. Because of the lack of DNA samples or information on lifestyle, 9 participants were eliminated. Table 1 summarizes the baseline characteristics of selected variables for the lung cancer cases and controls. Age distribution was similar in both groups (mean, 64.8 years and 63.5 years, respectively); however, the cases were more likely than the controls to be men (71.3% and 49.2%), to be current smokers (53.1% and 24.6%) and heavy smokers, and to consume more alcohol. The proportions of those who consumed >31.6 g per day of ethanol and of daily drinkers were 45.7% and 49.5%, respectively, for cases and 28.1% and 28.9%, respectively, for controls. The median values from the control group for the 2 smoking amount categories were used as the cut-off values. The 3 categories of alcohol consumption were lifetime nondrinker, below the median intake, and above the median intake.

The frequency of *ADH*$_3$, *ALDH*$_2$, and *CYP2E1* genotypes and ORs among lung cancer cases and controls are presented in Table 2. After adjustment for age, sex, smoking amount, and amount of alcohol consumed, the ORs for individuals with the *ADH*$_3$, *ALDH*$_2$, and *CYP2E1* variant alleles, compared with individuals who were homozygous for the common allele, were 1.01, 0.73, and 0.93, respectively. Thus, there were no significant differences in the frequencies of any genotypes between cases and controls. The OR for carriers of the *CYP2E1* c2/c2 genotype, compared with the c1/c1 genotype, was 4.66 (P <.05). This genotype is not in Hardy-Weinberg equilibrium in the control population, the observed frequency is most likely an underestimate, and the finding of an association with lung cancer is most likely a false-positive result.

Without taking these genotypes into consideration, a direct association between alcohol consumption and lung cancer occurrence can be derived, as

TABLE 2
The Frequency of Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 Genotypes and Odds Ratios Among Lung Cancer Cases and Controls

	No. (%)		OR	
Genotype	Cases (n = 505)	Controls (n = 256)	Crude	Adjusted*
ADH_3				
C/C	459 (90.9)	227 (88.7)	1	1
C/V	44 (8.7)	29 (11.3)	0.75 (0.46–1.23)	0.71 (0.40–1.16)
V/V	2 (0.4)	0 (0)	—	—
C/V and V/V	46 (9.1)	29 (11.3)	0.78 (0.48–1.28)	0.74 (0.44–1.24)
$ALDH_2$				
C/C	319 (63.2)	134 (52.3)	1	1
C/V	168 (33.3)	108 (42.2)	0.65 (0.48–0.90)†	0.73 (0.52–1.03)
V/V	18 (3.6)	14 (5.5)	0.54 (0.26–1.12)	0.75 (0.35–1.59)
C/V and V/V	186 (36.8)	122 (47.7)	0.64 (0.47–0.87)†	0.73 (0.53–1.02)
CYP2E1				
C/C	300 (59.4)	147 (57.4)	1	1
C/V	175 (34.7)	106 (41.4)	0.81 (0.59–1.11)	0.83 (0.60–1.15)
V/V	30 (5.9)	3 (1.2)	4.90 (1.47–16.32)†	4.66 (1.36–16.0)†
C/V and V/V	205 (40.6)	109 (42.6)	0.92 (0.68–1.25)	0.93 (0.68–1.29)

OR indicates odds ratios; *ADH_3*, alcohol dehydrogenase 3; C, common allele; V, variant allele; *$ALDH_2$*, aldehyde dehydrogenase 2; *CYP2E1*, cytochrome P450 2E1.
* ORs were adjusted for age, sex, smoking amounts (pack-years), and alcohol amounts (ethanol: mg per day).
† $P < .05$.

shown in Table 3. Drinking was classified as none, light (≤31.6 g per day) or heavy (>31.6 g per day). When adjusted for age, sex, and smoking amounts, drinking imposed a significantly greater risk of lung cancer occurrence. The ORs for the light drinkers and heavy drinkers, compared with nondrinkers, were 1.76 and 1.95, respectively (P for trend = .012). Thus, the risk of lung cancer increases as the amount alcohol consumed increases.

ORs for developing lung cancer in association with the *ADH_3*, *$ALDH_2$*, and *CYP2E1* genotypes also are presented in Table 3. Similar to what was observed in all participants taken together, an increased risk for developing lung cancer also was observed among individuals who were homozygous for the common allele ADH_3^{1-1}. However, because there were too few *ADH_3* variant allele carriers to analyze any association between alcohol consumption and lung cancer risk for this allele, it was inappropriate to compare the ADH_3^2 and ADH_3^{1-1} genotypes.

The adjusted OR for the $ALDH_2^{1-1}$ group was 0.75 (95% CI, 0.39–1.42) in light drinkers and 0.46 (95% CI, 0.20–0.99) in heavy drinkers. In contrast, individuals with the $ALDH_2^2$ allele had a significantly greater risk of lung cancer; light drinkers had a 3.6-fold increased risk, and heavy drinkers had a 6.2-fold increased risk compared with nondrinkers (P for trend < .0001). These results indicate that, in individuals with the *$ALDH_2$* variant allele, continuous alcohol consumption is a strong risk factor for lung cancer.

The OR for the *CYP2E1* c1/c1 genotype was 1.81 (95% CI, 0.97–3.38) for light drinkers and 1.67 (95% CI, 0.86–3.21) for heavy drinkers. For individuals with the *CYP2E1* c2 allele, the OR was 1.74 (95% CI, 0.91–3.35) for light drinkers and 2.56 (95% CI, 1.16–5.65) for heavy drinkers (P for trend = .005). These results may indicate that individuals with the *CYP2E1* variant allele are in a high-risk group for lung cancer in heavy drinkers.

It must be emphasized that, because of differences in distribution according to sex between cases and controls, we analyzed relative risks only in men (Table 4). For baseline characteristics among men, higher consumption of alcohol and more smoking were observed, as expected. Regarding associations between alcohol consumption and lung cancer risk, drinking was associated with an increased risk of developing lung cancer in all participants. The adjusted OR for the light and drinkers, compared with nondrinkers, was 6.54 (95% CI, 3.13–13.7) and 6.58 (95% CI, 3.28–13.2), respectively. However, in individuals with active $ALDH_2^{1-1}$ genotypes, there was no association between alcohol consumption and lung cancer risk. In individuals with the inactive $ALDH_2^2$ alleles, the risk for lung cancer was 6.8-fold (95% CI, 2.72–17.1) for light drinkers and 9.3-fold (95% CI, 3.72–23.4) for heavy drinkers compared with nondrinkers (P for trend <.0001). The risk in men who were heavy drinkers was much greater compared with women and those who carried the active $ALDH_2^{1-1}$ genotype.

In individuals with the c2 allele, the risk of lung cancer for light drinkers (OR, 8.31; 95% CI, 2.67–25.9) and for heavy drinkers (OR, 9.93; 95% CI, 3.39–29.1) was increased compared with individuals who were homozygous for the *CYP2E1* c1 allele and compared with the risks in all men. However, it should be noted that, because of the low incidence of homozygosity for variant allele in the control group, statistical power was limited in this instance. Similar assessments also were made in women, but no significant associations between any genotype and lung cancer risk were observed (data not shown).

Table 5 shows the distribution of the *ADH_3*, *$ALDH_2$*, and *CYP2E1* genotypes according to tumor histology. The frequency of the ADH_3^2 allele for all histologic types was similar to the frequency observed in controls. The frequency of the $ALDH_2^2$ allele for squamous cell carcinomas, small cell carci-

TABLE 3
Odds Ratios of Developing Lung Cancer for Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 Genotypes Stratified by Drinking Amounts

			Drinkers						
	Nondrinkers		≤31.6 g/Day			>31.6 g/Day			
Genotype	No.*	Reference	No.*	OR (95% CI)†	*P*	No.*	OR (95% CI)†	*P*	*P* for trend‡
All	120/119	1	154/65	1.76 (1.12–2.75)	.014	231/72	1.95 (1.19–3.21)	.0085	.012
ADH$_3$									
C/C	112/105	1	141/60	1.59 (0.99–2.55)	.054	206/62	1.88 (1.10–3.21)	.02	.025
C/V and V/V	8/14	1	13/5	4.31 (0.912–20.38)	.065	25/10	3.28 (0.742–14.55)	.12	.17
ALDH$_2$									
C/C	57/41	1	99/39	0.75 (0.39–1.42)	.37	163/54	0.46 (0.2– 0.99)	.049	.03
C/V and V/V	63/78	1	55/26	3.63 (1.76–7.46)	.0005	68/18	6.15 (2.77–13.65)	<.0001	<.0001
CYP2E1									
C/C	72/61	1	95/36	1.81 (0.97–3.38)	.061	133/50	1.67 (0.86–3.21)	.13	.31
C/V and V/V	48/58	1	59/29	1.74 (0.91–3.35)	.097	98/22	2.56 (1.16–5.65)	.02	.005

OR indicates odds ratio; 95% CI, 95% confidence interval; *ADH*$_3$, alcohol dehydrogenase 3; C, common allele; V, variant allele; *ALDH*$_2$, aldehyde dehydrogenase 2; *CYP2E1*, cytochrome P450 2E1.
* The number of cases/number of controls.
† ORs were adjusted for age, sex, and smoking amount (pack-years).
‡ The Mantel extension test.

TABLE 4
Odds Ratios of Developing Lung Cancer for Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 Genotypes Stratified by Drinking Amounts Among Men

			Drinkers						
	Nondrinkers		≤31.6 g/Day			>31.6 g/Day			
Genotype	No.*	Reference	No.*	OR (95% CI)†	*P*	No.*	OR (95% CI)†	*P*	*P* for Trend‡
All	17/31	1	120/36	6.54 (3.13–13.65)	<.0001	223/59	6.58 (3.28–13.22)	≤.0001	<.0001
ADH$_3$									
C/C	15/27	1	110/34	6.14 (2.83–13.29)	<.0001	201/49	7.27 (3.44–15.36)	≤.0001	<.0001
C/V and V/V	2/4	1	10/2	23.31(1.41–286.0)	.028	22/10	5.43 (0.63–47.09)	.12	.47
ALDH$_2$									
C/C	5/2	1	72/16	1.47 (0.25–8.67)	.67	158/42	1.10 (0.20–6.23)	.91	.29
C/V and V/V	12/29	1	48/20	6.82 (2.72–17.13)	<.0001	65/17	9.33 (3.72–23.39)	≤.0001	<.0001
CYP2E1									
C/C	10/14	1	77/24	5.22 (1.95–13.94)	.0003	125/42	4.71 (1.85–12.05)	.0012	.08
C/V and V/V	7/17	1	43/12	8.31 (2.67–25.89)	.0001	98/17	9.93 (3.39–29.09)	≤.0001	<.0001

OR indicates odds ratio; 95% CI, 95% confidence interval; *ADH*$_3$, alcohol dehydrogenase 3; C, common allele; V, variant allele; *ALDH*$_2$, aldehyde dehydrogenase 2; *CYP2E1*, cytochrome P450 2E1.
* Values shown represent the number of cases/number of controls.
† OR were adjusted for age, sex, and smoking history (pack-years).
‡ Mantel extension test.

nomas, and other histologic types was similar to that observed in controls. However, the *ALDH*$_2^2$ allele was significantly less common in patients with adenocarcinomas than in controls (36.1% vs 47.7%; $P = .018$). In contrast, the *CYP2E1* c2/c2 genotype was more common in patients with adenocarcinomas (5.8%) and small cell carcinomas (9.8%) than in controls (1.2%).

In this study, we observed that alcohol consumption was an independent risk factor for lung cancer after adjusting for the influence of smoking (P for trend = .012). Although we assumed that individuals who had the *ADH*$_3^{1-1}$ genotype were at greater risk for lung cancer compared with individuals who had the *ADH*$_3^2$ allele, there was no evidence of an association between lung cancer and the *ADH*$_3$ genotype

TABLE 5
Distribution of Alcohol Dehydrogenase 3, Aldehyde Dehydrogenase 2, and Cytochrome P450 2E1 Genotype According to Histologic Findings

	No. (%)				
		Histologic type			
Genotype	Control group (n = 256)	Adenocarcinoma (n = 330)	Squamous cell (n = 100)	Small cell (n = 51)	Other (n = 24)
ADH_3					
C/C	227 (88.3)	297 (90)	91 (91)	48 (94.1)	23 (95.8)
C/V	29 (11.7)	31 (9.4)	9 (9)	3 (5.9)	1 (4.2)
V/V	0 (0)	2 (0.6)	0 (0)	0 (0)	0 (0)
P for difference*		.35	.52	.25	.28
$ALDH_2$					
C/C	134 (52.3)	211 (63.9)	54 (54)	36 (70.6)	18 (75)
C/V	108 (42.2)	104 (31.5)	45 (45)	13 (25.5)	6 (25)
V/V	14 (5.5)	15 (4.6)	1 (1)	2 (3.9)	0 (0)
P for difference*		.018	.17	.056	.083
CYP2E1					
C/C	147 (57.4)	197 (59.7)	59 (59)	31 (60.8)	13 (54.2)
C/V	106 (41.4)	114 (34.6)	37 (37)	15 (29.4)	9 (37.5)
V/V	3 (1.2)	19 (5.8)	4 (4)	5 (9.8)	2 (8.3)
P for difference*		.0067	.19	.001	.04

ADH_3 indicates alcohol dehydrogenase 3; C, common allele; V, variant allele; *$ALDH_2$*, aldehyde dehydrogenase 2; *CYP2E1C*, cytochrome P450 2E1.
* Chi-square test for comparison with controls.

in any analysis. Because the enzyme activity of $ALDH_2^2$ is extremely low, acetaldehyde accumulates after alcohol intake. We could not demonstrate any association of *$ALDH_2$* genotypes with the risk of lung cancer after adjusting for smoking and the amount of alcohol consumed. However, we observed that individuals who had the $ALDH_2^2$ allele were at a significantly greater risk of lung cancer because of alcohol consumption, although there was a significant trend for lower levels of alcohol consumption in individuals who had the $ALDH_2^{1-1}$ genotype (*P* for trend = .03). We hypothesized that not only the differences in blood acetaldehyde concentrations but also the differences in enzyme activity on tobacco-specific carcinogens contribute to carcinogenesis. However, we produced no evidence that lung cancer risk is related to possession of the *CYP2E1* c2/c2 genotype or that the *CYP2E1* genotype modifies lung cancer susceptibility related to alcohol intake.

DISCUSSION

The control population for this study was recruited from the visitors to the NCCH-E. The majority of patients had false-positive chest x-rays at their annual check-up and had normal chest computed tomography scans, and they were not suffering from any respiratory illness. Furthermore, their family medical histories were similar to those expected in the ordinary Japanese population, although the number of current smokers among both men (42.9%) and women (6.9%) may have been somewhat lower than the average (46.8% and 11.1%, respectively, for 2003 according to the Announcement of the Ministry of Health, Labor, and Welfare). For these reasons, we believe that our control group was not at greater risk of cancer occurrence compared with the regular Japanese population. Moreover, it was not necessary to take into account any biases stemming from the selective inclusion only of consenting participants, because the great majority of both patients and controls agreed to participate in the study.

The data from the control group showed that individuals who had the *$ALDH_2$* wild-type genotype consumed more alcohol than individuals who had the variant genotype. This may suggest that genetic polymorphisms of alcohol-metabolizing enzymes influence drinking habits, because consumption may be limited by the unpleasant reactions caused by the accumulation of acetaldehyde in individuals with *$ALDH_2$* variant genotypes. Nonetheless, habitual drinking can increase consumption because of increased microsomal acetaldehyde-oxidizing system activation, further promoting the oxidation of acetaldehyde. The association between drinking habit and *ADH_3* and *CYP2E1* genotypes remains uncertain.

Regarding correlations between smoking and drinking habits, the coexistence of smoking and

drinking increased the risk of lung cancer compared with nondrinkers who never smoked, particularly the OR for heavy smokers (>37 pack-years) and drinkers, which was 8.4 (95% CI, 2.3–30.2; $P = .0012$) in the light drinkers and 7.0 (95% CI, 2.1–23.4) in the heavy drinkers (data not shown).

The involvement of alcohol in lung cancer etiology has been controversial, although many epidemiologic studies have suggested positive associations between different parameters of alcohol consumption and lung cancer risk. In the current study, we have demonstrated that drinking is a strong risk factor for lung cancer that is dose-dependent and is stronger in men than in women. This same tendency was observed even in the genotype analysis, but none of the results indicated a significant association between lung cancer and drinking in women. Furthermore, no associations were observed between peripheral lung adenocarcinoma, drinking, and genotypes of alcohol metabolite-related enzymes in women.

The question of ethnicity in the distribution of the polymorphisms of these alcohol metabolite-related enzyme genes always must be considered. The ADH_3^2 allele is present in almost 60% of whites but is far more rare (5–10%) in Japanese. In contrast, the $ALDH_2^2$ allele is found only in Asians. The *CYP2E1* c2 allele is present in 35% to 56% of Japanese and Chinese, and in 2% to 5% of whites. In the current study, the frequency of variant alleles of each polymorphism was 9.9% for ADH_3, 40.5% for $ALDH_2$, and 41.3% for *CYP2E1*. This is consistent with previous studies in Japanese and other Asians.

We observed that the risk for lung cancer was increased significantly by alcohol consumption in a dose-dependent fashion in individuals with the $ALDH_2^2$ alleles. Previously, some Japanese studies also showed a strong genetic and environmental interaction between $ALDH_2^2$ and alcohol intake for the risk of developing esophageal and upper aerodigestive tract cancer.[18–21] In contrast, for individuals with the $ALDH_2^{1-1}$ genotype, there was an inverse association between alcohol consumption and the risk of lung cancer. These results suggest that increased acetaldehyde concentrations from a reduction in acetaldehyde oxidation caused by the presence of the $ALDH_2^2$ allele contribute to the development of lung cancer. Significantly higher blood acetaldehyde concentrations after drinking in individuals with the ADH_3^1 or $ALDH_2^2$ allele have been reported compared with the concentrations in individuals who lacked these alleles,[11,29] and it has been demonstrated that breath acetaldehyde levels are proportional to blood acetaldehyde levels. Indeed, Muto et al.[30] and Jones[31] observed significantly higher acetaldehyde levels in the breath from individuals with the $ALDH_2^2$ allele than in those without that allele. Therefore, exposure to higher concentrations of acetaldehyde in the lower respiratory tract may play a critical role in alcohol-related carcinogenesis. Regarding the influence of smoking, when adjusted for age, sex, and amount of alcohol consumed, the risks for developing lung cancer in current smokers were 1.5-fold greater for those with the inactive $ALDH_2$ genotype (data not shown) compared with nonsmokers. The lung cancer risk for individuals with the $ALDH_2^2$ allele was not increased further by smoking.

Although there have been some reports of a significant association between the ADH_3^1 allele and some types of upper aerodigestive tract cancer, this association has been controversial.[16,17,32–34] We failed to observe an association between ADH_3 gene polymorphisms and the development of lung cancer, most likely because of the limited statistical power from the low frequency of the variant allele in the Japanese population.

Several investigations[24,31,35,36] have indicated that the *CYP2E1* c2 allele is associated with susceptibility to some types of cancer. However, other investigators reported that carriers of the c2 allele had decreased susceptibility to a number of cancers[25–27,37] and reported no association between *CYP2E1* genotypes and cancer.[23,28,38] Discrepancies among these results may be caused by several factors, including differences in study design, sample size, and the populations' ethnicity. Statistical power usually is very limited in studies of the white population because of the extreme rarity of variant genotypes. Although *CYP2E1* enzyme activity is induced by certain chemicals, such as ethanol, large interindividual variation has been observed in its constitutive activity as well as after induction. Watanabe et al.[39] and Hayashi et al.[15] reported that the *RsaI* variant c2 allele produced higher enzyme activity than the c1/c1 genotype in Japanese individuals, although this finding is itself controversial.[40–42] Highly activated *CYP2E1* induced by alcohol may play a more important role in the metabolic activation of several tobacco-specific procarcinogens, including various nitrosamines. It has been suggested that these low-molecular-weight carcinogens are associated with the development of peripheral adenocarcinoma. This finding is consistent with the results from our analysis of *CYP2E1* presented in Table 5. However, the *CYP2E1* c2/c2 genotype is not in Hardy-Weinberg equilibrium in the control population, the observed frequencies most likely are underestimates, and these findings of

an association with histologic type most likely are false-positive results. In our analysis of $ALDH_2$, the incidence of adenocarcinoma was high among individuals who had the wild-type genotype. Although a high incidence of squamous cell carcinoma was not observed, this result may imply that carcinogenesis caused by acetaldehyde occurs more in cancers other than adenocarcinoma as well as in esophageal and upper aerodigestive tract cancers.

A previous hospital-based study that was conducted in Japan failed to identify any association between the *RsaI* polymorphism and lung cancer, even when the analysis was stratified according to different histologic type.[28] A more recent study indicated that there was a significant decrease in overall lung cancer risk associated with the possession of at least 1 copy of the *CYP2E1 RsaI* variant allele, whereas there was no association between the *CYP2E1 RsaI* polymorphism and the histologic type of lung cancer.[27] However, none of the previous studies had adjusted for risk according to alcohol consumption levels, which strongly influence the activity of this enzyme. In the current study, we demonstrated that there is a difference between individuals who have the *CYP2E1 RsaI* c2/c2 genotype compared with individuals who have the common c1/c1 genotype, with an adjusted OR of 4.66 (95% CI, 1.36–16.0) for the former group. Because of the low incidence of homozygosity in controls, the genotype distribution was not in Hardy-Weinberg equilibrium in our control population. The increased lung cancer risk among individuals with the *CYP2E1* c2/c2 genotype likely was a false-positive result.

A correlation between the amount of alcohol consumed, genetic polymorphisms in the alcohol metabolite-related enzymes, and the stage of lung cancer was not observed in the current study, and we could not confirm that these factors were related to the aggressiveness of lung cancer. Furthermore, no associations were identified between the location of the primary cancer, the amount of alcohol consumed, and the genotype of these enzymes or between the risk for lung cancer and the type of alcoholic beverage consumed.

In summary, we report a significant association between amounts of alcohol consumed and susceptibility to lung cancer and that the risk of lung cancer in individuals with $ALDH_2$ variant alleles, but not with ADH_3 or *CYP2E1* variant alleles, apparently was enhanced more by alcohol intake than in individuals with common genotypes. Moreover, to our knowledge, this is the first report documenting an association between lung cancer and genetic polymorphisms of alcohol metabolite-related enzymes. Because the sample size was relatively small for the investigation of effects stratified by each genotype, the current findings should be confirmed in large-scale studies with greater statistical power.

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Detection of unsuspected distant metastases and/or regional nodes by FDG-PET in LD-SCLC scan in apparent limited-disease small-cell lung cancer

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Summary We retrospectively investigated the clinical usefulness of fluorodeoxyglucose positron emission tomography (FDG-PET) for evaluation of patients with limited-disease small-cell lung cancer (LD-SCLC) diagnosed by conventional staging procedures. Sixty-three patients received whole body FDG-PET scans after routine initial staging procedures. The findings of FDG-PET scans suggesting extensive-stage disease were confirmed by other imaging tests or by the patient's clinical course. FDG-PET scan findings indicated distant metastases in 6 of 63 patients. Metastatic disease was confirmed in five of these six patients (8%, 95% confidence interval: 3–18%). FDG-PET scan also detected regional lymph node metastases even in nine patients (14%) in whom computed tomography images had been negative, including contralateral lymph node metastasis in three patients. FDG-PET scan detected additional lesions in patients diagnosed as having LD-SCLC by conventional staging procedures. The therapeutic strategies were changed in 8% of patients based on the results of FDG-PET. FDG-PET scan is recommended as an initial staging tool for patients with this disease.

1. Introduction

Small-cell lung cancer (SCLC) accounts for 15–20% of all lung cancers. SCLC shows more aggressive biological behaviour than non-small cell lung cancer (NSCLC). A clinical two-stage system proposed by the Veterans Administration Lung

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Study Group (VALSG) distinguishes limited-disease (LD) and extensive-disease (ED) in SCLC [1]. LD is defined as limited to one hemithorax, including mediastinal, contralateral hilar and ipsilateral supraclavicular lymph nodes, while ED represents tumour spread beyond these regions. Approximately two-thirds of patients with SCLC are diagnosed as having ED at the initial staging. The current standard care for LD-SCLC is a combination of chemotherapy and chest irradiation. With current treatment, patients with LD have a median survival of 23–27 months [2,3], compared to 10–12 months for those with ED [4]. Therefore, accurate pretreatment staging is important for patients with SCLC in order to determine the appropriate therapy.

Conventional staging procedures for lung cancer consist of computed tomography (CT) of the chest and upper abdomen, bone scan, and CT scan or magnetic resonance imaging (MRI) of the brain. Recently, fluorodeoxyglucose positron emission tomography (FDG-PET) was introduced as a staging tool for NSCLC. According to the guidelines of the American Society of Clinical Oncology, PET scan is recommended for survey occult locoregional lesions and distant metastases in patients with NSCLC [5]. Two separate prospective studies demonstrated that FDG-PET detected unsuspected distant metastases in 24% of patients with apparent stage III NSCLC [6,7]. Another study showed that FDG-PET changed or influenced management decisions in 67% of patients with NSCLC. PET plays an important role in staging of NSCLC [8]. However, previous PET studies of SCLC involved only a relatively small number of patients [9–17]. In a prospective study, FDG-PET was performed for 24 patients diagnosed as having LD-SCLC by conventional staging procedures [9]. Based on FDG-PET findings, two of these 24 patients were upstaged to ED. Bone metastases were found in one patient, and contralateral supraclavicular lymph node metastasis in another. Larger studies are required to confirm the role of FDG-PET in the staging of LD-SCLC. In this study, we retrospectively investigated the usefulness of FDG-PET to detect distant metastases or unsuspected regional nodal metastases in patients with LD-SCLC diagnosed by conventional staging procedures.

2. Patients and methods

2.1. Patients

Seventy patients were newly diagnosed as having LD-SCLC by conventional staging procedures at the National Cancer Center Hospital East between July 2003 and December 2006. Conventional staging procedures included history and physical examination, chest radiography, CT scan of the chest, CT scan or ultrasound (US) of the abdomen, bone scan, and CT scan or MRI of the brain. CT scan and MR images were enhanced with contrast media. LD is defined in this study as disease limited to one hemithorax, including mediastinal, contralateral hilar and supraclavicular lymph nodes, ipsilateral pleural effusion, and pericardial effusion, while ED represents tumour spread beyond these manifestations [18]. This study included 63 patients who received whole body FDG-PET scan after the routine initial staging procedures. Fifty-seven were male and the remaining 6 were female. Median age was 64 years, range 48–80 years. Forty-two patients received FDG-PET before commencement of chemotherapy. The remaining 21 patients received FDG-PET 1 to 11 days (median: 4 days) after commencement of chemotherapy. Forty-four and 19 patients received CT scan and US of the abdomen, respectively.

2.2. FDG-PET scan

FDG-PET scans were performed before March 2005 (patients No. 1–25), and FDG-PET/CT scans were performed after April 2005 (patients No. 26–63). Three hundred MBq of F-18 FDG were intravenously injected after at least 6 h of fasting. Acquisition was initiated 60 min after the injection. FDG-PET imaging was performed using a GE Advance Scanner (General Electric Medical System, Milwaukee, WI), whose axial field of view was 15.2 cm and spatial resolution 4.9 mm of full-width-half-maximum. Scans were performed using two-dimensional acquisition mode from the thigh to the skull base with seven bed positions. Each bed position was composed of 1 min of transmission scanning and 5 min of emission scanning.

FDG-PET/CT imaging was performed using a GE Discovery LS Scanner (General Electric Medical System, Milwaukee, WI) or a GE Discovery ST Scanner (the same manufacturer). The PET component of the GE Discovery LS Scanner was the same as that of the GE Advance Scanner. For the PET component of the GE Discovery ST Scanner, the axial field of view was 15.7 cm and the spatial resolution was 6.2 mm of full-width-half-maximum. PET scans were performed with both scanners using 2-dimensional acquisition mode from the thigh to the skull base with 7 bed positions. Each bed position was composed of 4 min of emission scanning. The CT component of both PET/CT scanners was a 16-row multidetector CT scanner and CT images were acquired with a tube voltage of 140 kV, and the tube current was automatically set using the auto-exposure control function so that the number of standard deviations of noise was limited to 10. Attenuation correction of PET images was performed using the data from CT images.

Image reconstruction was performed using an ordered subsets expectation maximization (OSEM) algorithm with subset and iteration values of 14 and 2, respectively.

2.3. Image interpretation

All PET and CT images were interpreted by experienced radiologists and physicians. The 4.25 mm-thick images of axial, coronal and sagittal planes on hard copy films were reviewed. Uptake stronger than mediastinal blood pool activity was diagnosed as malignancy by the visual estimation. Symmetrical activities observed in both hilar regions were considered to be benign reactive changes. Any discrepancies between the radiologist and physician were resolved by discussion. The findings detected by FDG-PET were confirmed by other image tests or observation of the clinical course. FDG-PET was conducted after conventional staging procedures. CT, US and bone scans were interpreted without the FDG-PET findings. However, FDG-PET scan was interpreted in comparison with CT findings, while PET/CT findings were interpreted independently.

Table 1 Discrepancy between FDG-PET and conventional staging procedures (distant metastases)

Patient no.	Age (years)	Gender	CT N	PET N	PET M	Interval between conventional staging procedures and FDG-PET (days)	Comments
2	61	Male	2	2	1	20	Multiple bone metastases (PET)
6	68	Male	2	2	1	7	Lymph* node metastasis around the cardia (PET)
47	61	Male	3	3	1	28	Multiple bone metastases (PET)
55	68	Male	2	2	1	20 (CT) and 14 (bone scan)	Liver, axillary lymph node, and iliac bone metastases (PET)
59	52	Male	3	3	1	13	Adrenal, cervical and mandibular lymph node metastases (PET)
63	59	Male	3	3	1	18 (CT) and 11 (bone scan)	Multiple bone and liver metastases (PET)

FDG, fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; N, node; M, metastasis.
* Diagnosis of lymph node metastasis was not confirmed by other imaging modalities or observation of the clinical course.

3. Results

3.1. Detection of distant metastasis

FDG-PET showed results different from those of conventional staging procedures in 17 of 63 patients. PET scan demonstrated findings suggesting distant metastases in 6 of 63 patients (Table 1). The median interval between conventional staging procedures and FDG-PET was 16 days (range: 7–28). Abnormal uptake was observed around the cardia in one of these six patients (No.6). A repeat FDG-PET study demonstrated a longer uptake stripe indicating radiation-induced oesophagitis and the diagnosis could not be established, as there was a remaining possibility of physiological uptake in the oesophagus. The diagnosis of metastatic disease was confirmed in the remaining five patients (8%, 95% confidence interval (CI): 3–18%). Among these five patients, four had bone metastases, two had liver metastases, one had adrenal metastasis, and two had lymph node metastases in the cervical or axillary region. The therapeutic strategy for these five patients was changed and they received only chemotherapy without thoracic radiotherapy. One patient (No. 47) had shown negative findings on bone scintigraphy four weeks before the FDG-PET study, but PET scan demonstrated increased FDG uptake in bones throughout the body. MRI of the spine confirmed the diagnosis of multiple bone metastases (Fig. 1). A repeat bone scan after three months detected obvious multiple bone metastases in No. 2 patient. Two hepatic lesions, as well as the primary tumour, mediastinal and hilar lymph nodes, had all increased in size after two cycles of chemotherapy in patient No. 55. A hepatic lesion, as well as the primary tumour, had decreased in size after two cycles of chemotherapy in patient No. 63. These hepatic lesions were compatible with liver metastases. Abnormal uptake by the right adrenal gland disappeared on repeat PET/CT after four cycles of chemotherapy in patient No. 59. Abnormal uptake in primary and mediastinal lesions was extremely decreased in this patient. The right adrenal gland lesion was compatible with metastasis.

FDG-PET detected liver metastasis in one of 44 patients staged by CT scan of the abdomen (No. 55), and liver or adrenal metastasis in two of 19 patients staged by US (Nos. 59 and 63). Liver and adrenal metastases not detected by US were small, such that the CT part of PET/CT could not detect them as metastases. Ratios of upstaging by FDG-PET between initial CT scan and US of the abdomen were not statistically significant (1/44 versus 2/19, $P=0.214$).

3.2. Detection of regional lymph node metastases

FDG-PET scans detected regional lymph node metastases that had been negative on CT scans in nine patients (14%) (Table 2). The median interval between CT of the chest and FDG-PET was 19 days (range: 7–34). FDG-PET scans newly detected ipsilateral supraclavicular lymph node metastasis in four patients, contralateral lymph node metastasis in three, and mediastinal lymph node metastasis in two. These nine patients all underwent curative chemoradiotherapy, and abnormal FDG uptake in mediastinal and/or supraclavicular lymph nodes disappeared or decreased on repeat PET scans after chemoradiotherapy. These lymph nodes were considered positive for metastasis.

CT scan detected swollen mediastinal lymph nodes without abnormal FDG uptake in two patients. One patient had a past history of pulmonary tuberculosis complicated by pulmonary fibrosis. The swollen pretracheal lymph node was considered negative for metastasis because the node size remained unchanged after four cycles of chemotherapy although the primary tumour shrank. This case showed false positive findings on CT whereas FDG-PET correctly diagnosed the extent of disease (No. 43). The other patient had atelectasis of the right middle lobe due to the primary tumour. Superior mediastinal and subcarinal lymph nodes were considered to be metastatic on CT, but abnormal FDG uptake was absent. After three cycles of chemotherapy the