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LUNG CANCER

SHORT COMMUNICATION

# Sequential occurrence of non-small cell and small cell lung cancer with the same *EGFR* mutation

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**KEYWORDS**
EGFR mutation;
Non-small cell lung cancer;
Small cell lung cancer

**Summary** We report a case of small cell lung cancer (SCLC) developing after prolonged treatment (more than 2 years) for primary adenocarcinoma of the lung, and we show that both the SCLC and non-small cell lung cancer (NSCLC) tissues obtained from the same site share the same deletion in exon 19 of *EGFR*. This case suggests that the activating *EGFR* mutations may confer the pathogenesis of a subset of SCLC.


## 1. Introduction

The identification of somatic mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) in patients with NSCLC and the association of such mutations with the clinical response to EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib have had a substantial impact on the treatment of this disease [1,2]. To date, however, only a few *EGFR* mutations have been detected in other solid tumors including SCLC.

## 2. Case report

A 46-year-old Japanese woman with no smoking history was diagnosed in July 2003 with stage IIIB adenocarcinoma (acinar type) of the lung, with a primary tumor in the left lower lobe and pleural disseminations. A computed tomography (CT) scan showing the tumor (arrow) and hematoxylin–eosin (HE) staining of a tumor biopsy specimen are shown (Fig. 1A). The patient received first-line treatment with cisplatin and vinorelbine and showed a brief partial response. She

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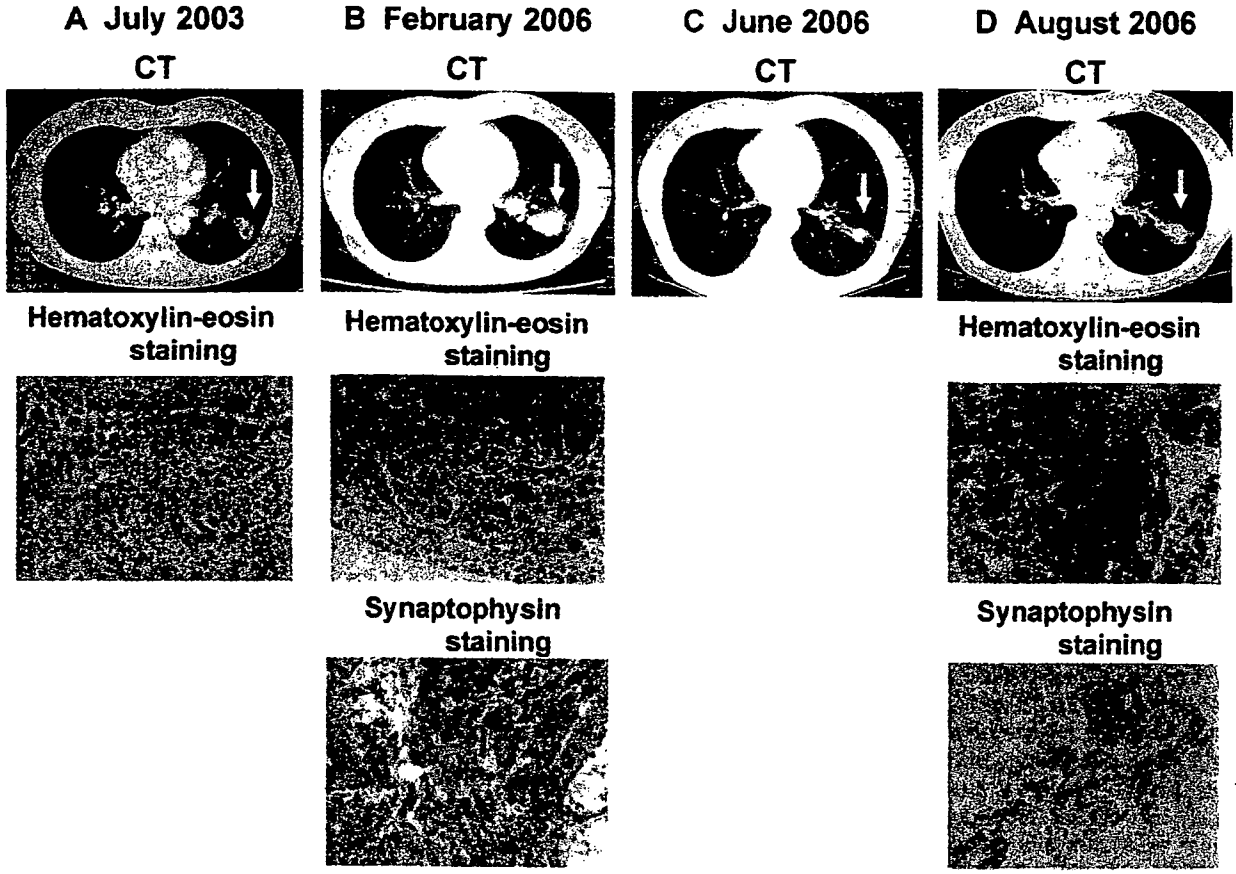


Fig. 1 Chest CT scan: (A) before treatment and HE staining of a tumor biopsy specimen; (B) before second lung biopsy and HE and synaptophysin stainings of a tumor biopsy specimen; (C) after four cycles of cisplatin and irrinotecan; (D) before third lung biopsy and HE and synaptophysin stainings of a tumor biopsy specimen.

subsequently underwent combination chemotherapy with gemcitabine and paclitaxel, manifesting a minor response on radiographic examination. In September 2004, the mass in the left lower lobe had progressed and treatment with gefitinib (250 mg daily) was initiated. After 10 months of treatment with gefitinib alone and transient disease stabilization, a repeat evaluation in July 2005 showed progression of the primary lung tumor. Gefitinib was discontinued, and the patient was enrolled in a phase I clinical trial of new agents. The primary tumor showed no evidence of regression on radiological examination. A magnetic resonance imaging (MRI) scan in December 2005 revealed multiple brain metastases in both hemispheres, which were accompanied by symptoms including headache, nausea, and visual disturbances. After surgical resection of the largest tumor in the right parietal lobe, the patient was exposed to 10 fractions of 3 Gy whole-brain radiotherapy. Her symptoms improved markedly, and MRI scans after radiotherapy revealed almost complete regression of the brain metastases. Histological examination of the resected brain tumor revealed a synaptophysin-positive small cell cancer. The patient provided informed consent to repeated lung biopsies for histological examination. A biopsy specimen of the progressive mass in the left lower lobe in February 2006 revealed SCLC by HE staining and was positive for synaptophysin by immunohistochemical analysis (Fig. 1B). A second lung biopsy specimen was microdissected for extraction of genomic DNA and analysis of *EGFR* mutations. A heterozygous in-frame 15-bp deletion in exon 19 of *EGFR* was detected with the use of the amplification refractory mutation system (ARMS); the genomic DNA of the patient was thus subjected to amplification by the polymerase chain reaction with primers specific for the wild-type (Fig. 2A, left panel) or mutant (Fig. 2A, right panel) versions of exon 19. The deletion was confirmed to be delE746–A750 by nucleotide sequencing. On the basis of the histological diagnosis of SCLC, the patient was treated with four cycles of cisplatin and irrinotecan, and she achieved a partial response (Fig. 1C). A repeat chest CT evaluation in August 2006 showed progression of the primary lung tumor (Fig. 1D). A new lung biopsy specimen revealed nests of adenocarcinoma cells forming small tubular structures, the same subtype of the adenocarcinoma at initial diagnosis on July 2003, and was negative for synaptophysin staining (Fig. 1D). In addition, ARMS analysis of the adenocarcinoma specimen detected the same in-frame 15-bp deletion in exon 19 of *EGFR* that had been identified in the previous SCLC specimen (Fig. 2B).

## 3. Discussion

*EGFR* mutations are more frequent in women, Asians, individuals with adenocarcinoma, or those who have never

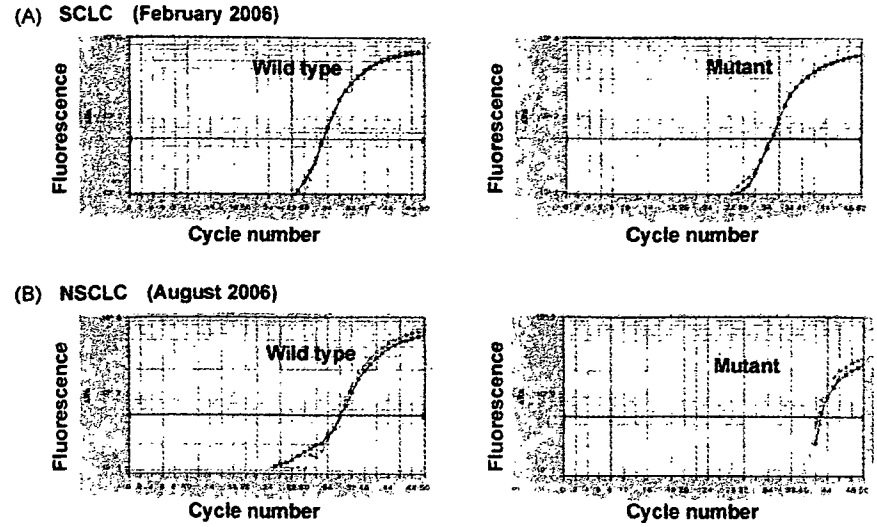


**Fig. 2** Results of ARMS analysis of (A) the SCLC. Ascending curves, performed in duplicate (green and red), indicate that wild type (left panel) and deletion mutation in exon 19 (right panel) were detected; (B) the adenocarcinoma. Ascending curves, performed in duplicate (green and red), indicate that wild type (left panel) and deletion mutation in exon 19 (right panel) were detected.

smoked [3–5]. However, EGFR expression has been shown to be low or undetectable in SCLC, and screening of SCLC for *EGFR* mutations has yielded negative results [5]. We previously described the first case of SCLC with a deletion in exon 19 of *EGFR* in a nonsmoking Japanese woman [6]. Another case of SCLC with an 18-bp deletion in exon 19 of *EGFR* in a nonsmoking woman was also recently reported [7]. All reported cases of SCLC with *EGFR* mutations, including the present case, have thus been in women who have never smoked, even though SCLC occurs almost exclusively in smokers. Furthermore, all three of these SCLC cases were initially diagnosed as adenocarcinoma. In the present case, SCLC developed after prolonged treatment (>2 years) for primary adenocarcinoma, and both SCLC and NSCLC (adenocarcinoma) tissues obtained from the same site shared the same *EGFR* mutation. Small cell carcinoma of the prostate, which shares histological similarities with SCLC, has been shown to arise during the course of treatment for prostatic adenocarcinoma, suggesting that prostatic small cell carcinoma may originate from multipotent stem cells of the prostate that have the ability to differentiate into either epithelial or neuroendocrine type carcinoma [8–10]. It remains unclear whether the primary tumor of the present patient originally had a minor SCLC component or whether SCLC arose from transdifferentiation of the adenocarcinoma. Our finding that SCLC and NSCLC developed at the same site in the lung and shared the same somatic *EGFR* mutation suggests, however, that different types of lung cancer may arise from a common stem cell with multiple potential pathways of differentiation.

## Conflict of interest

We, all authors, indicate no potential conflicts of interest.

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# Skeletal metastases in non-small cell lung cancer: A retrospective study

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**KEYWORDS**
Skeletal metastasis;
Skeletal-related event;
Retrospective study;
Median survival;
Non-small cell lung cancer;
Bisphosphonate

Summary
*Background:* The skeleton is one of the most common sites of metastasis in patients with advanced cancer. Bone metastases often cause SREs (skeletal-related events). Despite advances in the treatment of primary lung cancer, SREs still affect many patients. Therefore, we planned a retrospective study to investigate the clinical impact of SREs, and to compare differences in the therapeutic outcome between patients with and without skeletal metastases or SRE.
*Patients and methods:* We retrospectively investigated the charts of all 259 patients with non-small cell lung cancer (NSCLC) who consulted the Department of Medical Oncology at Kinki University School of Medicine between February 2002 and January 2005. We assessed their TNM stage, presence of skeletal metastases (on bone scintigraphy, MRI, and plain X-ray films), and outcome parameters such as SREs, analgesic use, and survival.
*Results:* A total of 70 patients (30.4%) were found to have skeletal metastases during their clinical course and 35 patients (50%) out of all 70 patients had SREs. Among 135 stage IV patients, a total of 56 (41%) had skeletal metastases, and 25 of these 56 patients (45%) had SREs. The most common SREs were the need for radiotherapy (34.3%) and hypercalcemia (20%). Patients with SREs tended to have worse survival, while no significant difference of survival was observed between patients with and without skeletal metastases.
*Conclusion:* It seems to be important to prevent SREs during the treatment of NSCLC, so further studies evaluating bisphosphonates in combination with chemotherapy are warranted.


## 1. Introduction

Most patients with advanced cancer develop skeletal metastases during the course of their disease, and these are often associated with significant morbidity [1]. The major-

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ity of bone metastases arise from primary tumors of the breast, prostate, thyroid, or lung among others. In Western countries, it has been reported that the incidence of bone metastases in lung cancer patients is approximately 30–40%, and the median survival time (MST) of patients with such metastases is 7 months [2]. A more recent retrospective review of 435 patients with non-small cell lung cancer (NSCLC) revealed an incidence of 24% for skeletal metastases. In this review, the majority of skeletal metastases (66%) were detected at the time of initial staging [3]. Bone is a common site of cancer spread, ranking only behind the liver and the lungs in frequency.

Despite advances in the treatment of primary lung cancer, skeletal-related events (SREs) still affect many patients during their clinical course. Common complications of skeletal metastasis include bone pain, symptomatic pathologic fracture, spinal cord compression, and hypercalcemia of malignancy (HCM). These complications often require surgery to correct fractures or spinal deformities and/or radiation therapy to control the severe pain that is a hallmark of bone metastases. Pain due to bone metastases is the most frequent form of pain reported by cancer patients [4]. Thus, SREs have a negative impact on the quality of life, performance status, and function of cancer patients.

Although skeletal metastases due to lung cancer have already attracted attention in Western countries, little is known about the incidence of bone metastases arising from lung cancer in Japan. Therefore, we planned a retrospective study to investigate the clinical impact of SREs and to explore the therapeutic outcome of patients with or without skeletal metastases and/or SREs.

## 2. Patients and methods

### 2.1. Study population

We retrospectively investigated 259 patients with NSCLC who consulted the Department of Medical Oncology at Kinki University School of Medicine between February 2002 and January 2005.

The TNM stage, the presence of skeletal metastases (on bone scintigraphy, MRI, and plain X-ray films), and outcome parameters such as SREs, analgesic use, and survival were investigated.

In this study, SREs were defined as pathologic fracture, spinal cord compression, hypercalcemia, bone radiation therapy (palliative therapy for pain, or treatment/prevention of pathologic fractures and spinal cord compression), and bone surgery (stabilization or decompression).

### 2.2. Statistical analysis

The characteristics of stages III and IV patients were compared using the $\chi^2$-test. Survival curves were calculated and drawn by using the Kaplan–Meier method, and differences between stage IV patients with or without SREs were assessed by the log–rank test. All analyses were two-sided. Statistical software (Statistical Package SAS Software release 8.2) was used for statistical analysis, and $p < 0.05$ was considered statistically significant.

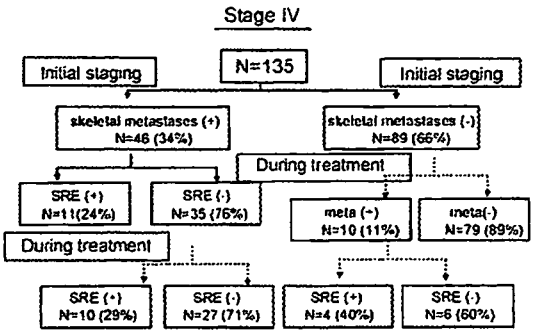


**Fig. 1** Incidence of skeletal metastases and SREs in patients presenting with stage IV disease.

## 3. Results

### 3.1. Patients

We retrospectively investigated 259 NSCLC patients who visited and consulted the department of Medical Oncology, Kinki University School of Medicine, between February 2002 and January 2005. A total of 29 patients were excluded because of early stage disease, so the total number of patients assessed was 230. Among them, 156 patients (68%) were men. The pathologic diagnosis was adenocarcinoma in 140 patients (61%) and most patients had a good performance states (PS 0/1 in 193 patients, or 84%). The median age was 65 years. There were no obvious difference of these characteristics between patients in stage III and stage IV, although statistical analysis was not done.

### 3.2. Incidence of skeletal metastases

A total of 70 patients (30.4%) were found to have skeletal metastases during their clinical course. Among them, 46 patients (65.7%) had skeletal metastases at the time of initial diagnosis. Thirty-five (50%) of the 70 patients suffered from SREs. Eleven (31%) of the 35 patients had SREs at the time of initial staging, and 24 (69%) of the 35 patients developed SREs due to recurrence of their disease after treatment.

Of the 135 patients who were initially in stage IV, 56 patients (41%) had skeletal metastases, and 25 (45%) of these 56 patients suffered from SREs. Among the 56 patients with skeletal metastases, 46 patients (82%) had these metastases at the time of initial staging, while 11 (44%) of the 25 patients with SREs already had them at initial staging (Fig. 1).

A total of 95 patients were initially in stage III. After treatment of their cancer, 14 patients developed skeletal metastases and 10 (71%) of them suffered from SREs (Fig. 2).

### 3.3. Sites of skeletal metastasis

Table 1 shows the sites of skeletal metastasis. The spine was the most common site (50%), followed by the ribs (27.1%).

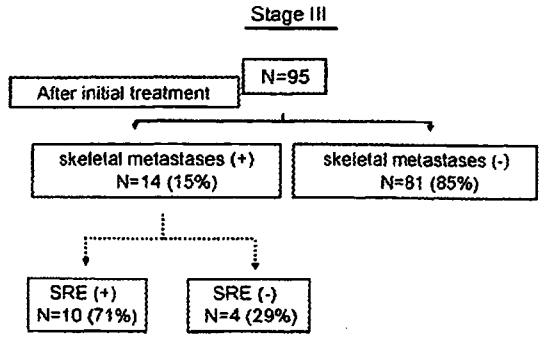


Fig. 2 Incidence of skeletal metastases and SREs in patients presenting with stage III disease.

Table 1 Sites of skeletal metastasis ($n=70$)

| | |
|---|---|
| Spine | 35 (50.0%) |
| Ribs | 19 (27.1%) |
| Ilium | 7 (10.0%) |
| Sacrum | 5 (7.1%) |
| Femur | 4 (5.7%) |
| Skull | 4 (5.7%) |
| Humerus | 2 (2.9%) |
| Scapula | 2 (2.9%) |
| Stermun | 2 (2.9%) |

### 3.4. Complications of skeletal metastasis

Table 2 shows the details of SREs. Among 70 patients with skeletal metastases, a total of 35 (50%) developed SREs. The most common SRE was bone radiation therapy in 24 patients (34.3%), followed by hypercalcemia in 14 patients (20%). Spinal cord compression occurred in 11 patients (15.7%).

### 3.5. Analgesic use

Among the 70 patients with skeletal metastases, 55 (78.6%) had localized bone pain, and 39 (70%) of the 55 patients required NSAIDs for pain relief, while 40 (73%) of them required opioids.

### 3.6. Survival

The MST was 679 days for stage III patients and 447 days for those with stage IV disease.

Fig. 3 shows survival of the patients who had stage IV disease with or without skeletal metastases. For patients with skeletal metastases, MST was 237 days, while it increased to 268 days for those without such metastases. However, there was no statistically significant difference of survival between patients with and without skeletal metastases ($p=0.733$). Interestingly, the MST was 187 days for stage IV patients with SREs, while it was 366 days for patients without SREs (Table 3), but this difference also failed to reach statistical significance. The median time to the first SRE was 182 days for patients presenting with stage III disease and 93 days for those presenting with stage IV disease, while the MST from the date of the first SRE was 40 days for stage III patients and 138 days for stage IV patients. The MST from the date of the first SRE was 92 days for all patients with SRE.

Table 2 Individual SREs in 35 out of 70 patients with skeletal metastases

| | | |
|---|---|---|
| 1 | Radiation to bone | 24 cases: 34.3% |
| 2 | Hypercalcemia | 14 cases: 20.0% |
| 3 | Spinal cord compression | 11 cases: 15.7% |
| 4 | Pathologic fracture | 5 cases: 7.1% |
| 5 | Surgical stabilization/decompression | 0 cases: 0% |
| | Localized bone pain | 55 cases: 78.6% |

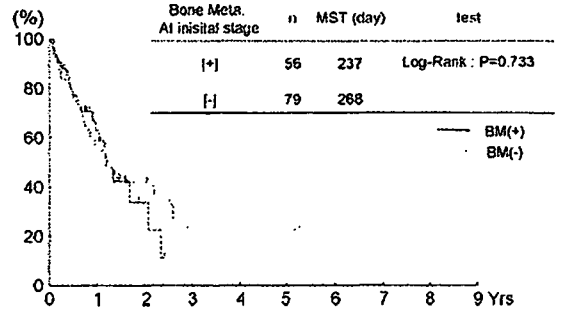


Fig. 3 Overall survival of stage IV patients with and without skeletal metastases.

Table 3 MST of patients presenting in stages III or IV

| | N | MST (days) |
|---|---|---|
| Stage IV | | |
| BM (−) | 79 | 268 |
| BM (+) | 56 | 237 |
| BM (+) | | |
| SRE (+) | 25 | 187 |
| SRE (−) | 31 | 366 |
| Stage III | | |
| BM (−) | 81 | 314 |
| BM (+)[a] | 14 | 298 |
| BM (+) | | |
| SRE (+) | 10 | 240 |
| SRE (−) | 4 | 255 |

[a] Following initial treatment.

## 4. Discussion

As far as we know, this is the first report about the frequency and influence on survival of skeletal metastases and SREs in Japanese lung cancer patients. Our results were similar to 1997 data from the USA [2]. A total of 70 patients (30.4%) were noted to have skeletal metastases during their clinical course, as did 56 patients (41%) with stage IV disease at the time of diagnosis.

Our study also showed that the most common SRE was radiation therapy for bone metastases (34.3%), followed by hypercalcemia (20%), and spinal cord compression (15.7%).

On the other hand, the major SREs in western countries were radiation therapy for bone metastases in 49.9%, hypercalcemia in 8.0%, cord compression in 5.8%, and bone surgery in 12.6%. Compared with Japanese data, the frequency of surgery seems to be higher.

In our analysis, the MST of patients with skeletal metastases was 7.9 months and there was no significant difference of survival between the patients with and without skeletal metastases ($p = 0.733$). A total of 50% of the patients with skeletal metastases had SREs according to this retrospective study, which is similar to the results from Western countries [5–7].

Interestingly, the MST of our lung cancer patients with SREs was only half of that for patients without SREs (6.2 months for patients with SREs versus 12.2 months for patients without SREs). The lack of a significant difference may have been attributable to our small sample size. There seem to be two main reasons why the prognosis of lung cancer patients with SREs is poor. One is that they are unable to receive appropriate or sufficiently aggressive treatment because of deterioration of PS due to SREs. The other is that tumors associated with SREs may be chemoresistant because these lesions have progressed more, even if it is possible to treat them. Therefore, it is more important to prevent SREs than to treat them. In this study, the median time to the first SREs was 3.1 months in patients with stage IV disease. If this period could be extended, more patients would be able to receive second-line and third-line treatment for lung cancer. It is a well-known fact that bisphosphonates is effective in the treatment for hypercalcemia which is one of SREs [8,9]. Recently, bisphosphonates have been shown to be effective for the prevention of SREs in patients with various tumors, particularly breast cancer, prostate cancer, and multiple myeloma [10–13]. Saad et al. conducted a placebo-controlled trial of a new bisphosphonate (zoledronic acid) in patients with hormone-refractory metastatic prostate carcinoma and reported that it significantly reduced SREs and also significantly increased the time to the first SRE [14]. With regard to NSCLC, there has only been one study that evaluated the influence of bisphosphonates on SREs and the time to the first SRE [6]. However, as half of the subjects did not have NSCLC in that study and it was the only trial done previously, no consensus about the value of bisphosphonates for skeletal metastases of NSCLC has been obtained.

The present retrospective study revealed that the prognosis of NSCLC patients with SREs was worse than that of patients without SREs, while there was no survival difference between patients with and without skeletal metastases. Although it is unclear whether it is important to delay the time to the first SRE in NSCLC patients because of their poor prognosis, our data may support the clinical development of bisphosphonates. These drugs have also demonstrated antitumor activity in preclinical models through inhibition of angiogenesis and by the inhibition of cell proliferation and cell adhesion [15]. Therefore, the West Japan Thoracic Oncology Group is planning a randomized clinical trial to compare the combination of chemotherapy and zoledronic acid with chemotherapy alone for NSCLC patients with skeletal metastases. The aim is to examine whether this combination has a survival benefit through delaying SREs and through the antitumor activity of zoledronic acid.

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# Sublobar Resection for Patients With Peripheral Small Adenocarcinomas of the Lung: Surgical Outcome is Associated With Features on Computed Tomographic Imaging

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*Background.* Sublobar resection for peripheral small adenocarcinomas of the lung remains controversial. We studied the feasibility of deciding whether to perform limited pulmonary resection on the basis of preoperative images obtained by high-resolution computed tomography.

*Methods.* A total of 123 patients with adenocarcinoma of the lung underwent sublobar resection of clinical T1N0M0 tumors measuring 2 cm or less in diameter on high-resolution computed tomography. Patients with multiple lung cancers or a history of lung cancer or other malignancies were excluded. The remaining 63 patients were studied. All tumors were classified as "air-containing type" or "solid-density type" according to the tumor shadow disappearance rate on high-resolution computed tomography. We evaluated the surgical outcomes of sublobar resection with respect to findings on high-resolution computed tomography images.

*Results.* Forty-six patients had air-containing type tumors (tumor shadow disappearance rate ≥50%), and 17 had solid-density type tumors (tumor shadow disappearance rate <50%). Forty-nine wedge resections and 14 segmentectomies were performed. Wedge resection was the most common procedure in patients with air-containing type tumors. Pathologically, air-containing type tumors comprised 38 bronchioloalveolar carcinomas and 8 nonbronchioloalveolar carcinomas. No patient with air-containing type tumors had recurrence after a median follow-up of 70 months (range, 21 to 133 months). Overall and relapse-free survival rates at 5 years were 95% and 100%, respectively, in patients with air-containing type tumors, as compared with 69% and 57%, respectively, in those with solid-density type tumors.

*Conclusions.* Sublobar resection might be an acceptable procedure for the treatment of small air-containing type adenocarcinomas of the lung on preoperative high-resolution computed tomography. However, our findings must be confirmed in larger, multicenter studies.

(Ann Thorac Surg 2007;84:1675–9)



Lobectomy has been established as the procedure of choice for most peripheral, clinical T1 N0 M0 lung cancers. This recommendation was based on the results of a randomized trial performed by the Lung Cancer Study Group, showing that sublobar resections, ie, segmental or wedge resections, had a significantly higher risk of locoregional recurrence [1]. However, increased use of computed tomography (CT) and improved scanning techniques after the 1980s, when the Lung Cancer Study Group trial was performed, have enhanced the detection rate of small cancers, leading thoracic surgeons to reassess the potential benefits of sublobar resection for small peripheral lung cancers [2–4]. Important advances have also been made in pathologic and CT evaluations of adenocarcinoma of the lung, especially bronchioloalveolar carcinoma (BAC) [5, 6].

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We previously reported that the tumor shadow disappearance rate (TDR) on high-resolution CT (HRCT), defined as the ratio of the tumor area of the mediastinal window to that of the lung window, closely reflected the biologic characteristics of small peripheral adenocarcinomas of the lung. Tumors with a TDR of 50% or higher showed no lymph node involvement and rarely had microscopic invasion. Such tumors might therefore be appropriate candidates for limited pulmonary resection [7, 8]. In this study, we analyzed follow-up data in patients in whom sublobar resection was performed on the basis of HRCT findings. We focused on the outcomes of limited pulmonary resection in patients with early adenocarcinomas of the lung on radiologic evaluation.

## Patients and Methods

Our ethics committee was informed of this retrospective study and gave their approval for publication. Individual patient consent was waived by the chairman

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*Fig 1. High-resolution computed tomographic image of air-containing type adenocarcinoma (tumor shadow disappearance rate ≥50%).*

of the ethics committee. Between July 1992 and October 2004, 329 patients with adenocarcinoma of the lung underwent complete resection of clinical T1 N0 M0 tumors measuring 2 cm or less in diameter on HRCT at our hospital. Preoperative evaluation included a detailed history and physical examination, chest radiography, CT of the chest and upper abdomen, and bone scintigraphy for staging and assessment of resectability.

Chest images were acquired with a model TCT 900S Super HELIX or X-Vigor/Real CT scanner (Toshiba Medical Systems, Tokyo, Japan). High-resolution images targeted to the tumor were obtained continuously at 120 kVp and 200 mAs, with 2-mm section thickness, pitch 1, 1- to 2-mm section spacing, 512 × 512 pixel resolution, 1-second scanning time, and a high spatial reconstruction algorithm with a 20-cm field of view. Images were photographed onto each sheet of film using the mediastinal (level, 40 HU; width, 400 HU) and lung (level, −600 HU; width, 1,600 HU) window settings. As previously reported [7, 8], we classified tumors into two types according to the TDR. Tumor shadow disappearance rate was defined as follows:

$$\text{TDR} = 1 - (\text{tumor area of the mediastinal windows} / \text{tumor area of the lung windows})$$

*Fig 2. High-resolution computed tomographic image of solid-density type adenocarcinoma (tumor shadow disappearance rate <50%).*

*Table 1. Clinical and Pathologic Characteristics of Patients*

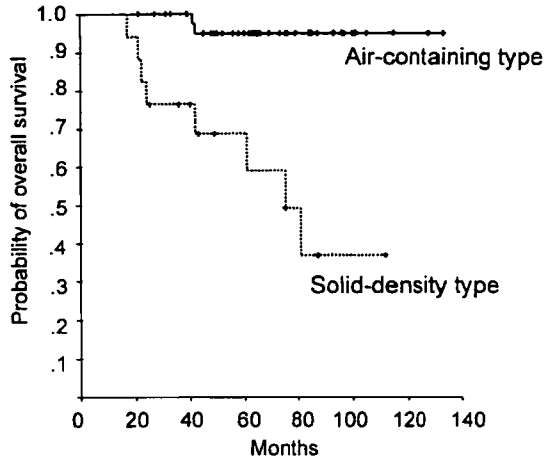
| Characteristic | Type of Lesion on HRCT: Air-Containing Type (n = 46) | Type of Lesion on HRCT: Solid-Density Type (n = 17) | *p* Value |
|---|---|---|---|
| Age | | | 0.7985 |
| Range (years) | 43–84 | 46–79 | |
| Mean (years) | 63 | 64 | |
| Sex | | | 0.5896 |
| Male | 19 | 9 | |
| Female | 27 | 8 | |
| Operative mode | | | 0.2397 |
| Wedge resection | 38 | 11 | |
| Segmentectomy | 8 | 6 | |
| Size (mm)[a] | | | <0.0001 |
| Range | 3–20 | 7–20 | |
| Mean | 10.5 | 15.6 | |
| Pathologic stage | | | 0.0243 |
| T1 | 46 | 14 | |
| T2[b] | 0 | 3 | |
| Histologic subtype | | | <0.0001 |
| BAC | 38 | 0 | |
| Non-BAC | 8 | 17 | |
| Pleural involvement | | | 0.0001 |
| p0/p1/p2 | 46/0/0 | 11/3/3 | |
| Blood vessel invasion | | | <0.0001 |
| Present/absent | 0/46 | 7/10 | |
| Lymphatic invasion | | | 0.0048 |
| Present/absent | 0/46 | 4/13 | |

[a] Size of resected specimen was measured grossly. [b] Visceral pleural invasion discovered.

BAC = bronchioloalveolar carcinoma; HRCT = high-resolution computed tomography.

A TDR of 50% or greater was defined as "air-containing type" (Fig 1), and a TDR of less than 50% was defined as "solid-density type" (Fig 2).

Among the 329 patients who underwent complete resection of small adenocarcinomas, lobectomy was performed in 206 (92 with air-containing type, 114 with solid-density type), and sublobar resection was performed in 123 (78 with air-containing type, 45 with solid-density type). Fifty-one patients who underwent sublobar resection were considered unsuitable candidates for lobectomy because of limited pulmonary function or other comorbidities. These patients were considered to have undergone compromised resections. Based on the results of lobectomy with lymph node dissection for the air-containing type tumors, which showed no lymph node involvement and rarely had microscopic invasion [7, 8], we started to perform intentional sublobar resection in patients with air-containing type tumors in 1995 after obtaining complete, written informed consent from each patient. Intentional sublobar resection was defined as wedge resection or segmentectomy for patients who were considered suitable candidates for lobectomy. Intentional sublobar resections were performed for




Patients at risk

| Months | 0 | 12 | 24 | 36 | 48 | 60 |
|---|---|---|---|---|---|---|
| Air-containing type | 46 | 46 | 45 | 42 | 36 | 30 |
| Solid-density type | 17 | 17 | 14 | 12 | 8 | 7 |

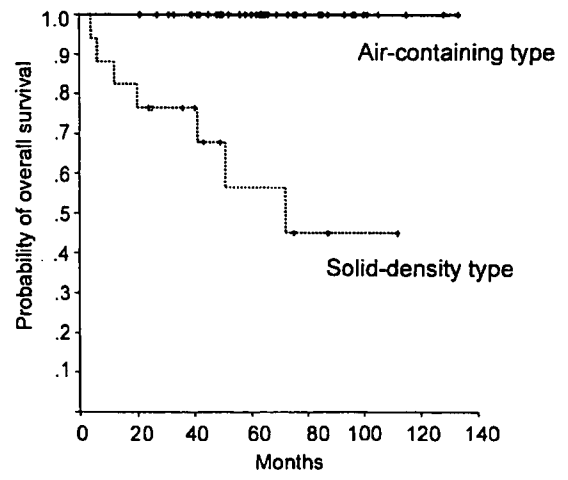
*Fig 3. Overall survival curve for patients with air-containing type (solid line; n = 46) and solid-density type (dotted line; n = 17) tumors.*

air-containing type tumor on CT images, provided they were located in the outer third of the lung parenchyma. Wedge or segmental resection was performed on the basis of only lesion location and size, and the procedure was selected to achieve adequate resection margins. Sixty-eight patients underwent intentional sublobar resection. The other 4 patients with solid-density type tumors on HRCT underwent sublobar resection because metastatic lung cancer or low-grade malignant tumors were diagnosed on frozen section analysis during operation. Among 123 patients who underwent sublobar resection, a total of 60 patients were excluded because they had a history of previously treated cancer or malignancy of other organs (21 patients), multiple lung cancers (27 patients), or second lung cancers detected during follow-up after resection of their primary lung cancers (12 patients). The remaining 63 patients were studied retrospectively.

Tumor types on HRCT were compared with respect to pathologic findings and surgical outcomes. Pathologic findings included pathologic TNM stage, histologic type of adenocarcinoma, pleural involvement, vessel invasion, and lymphatic invasion. The histologic type of adenocarcinoma and TNM stage were determined according to the World Health Organization classification [9] and UICC staging system [10]. Pleural involvement was defined according to the Japanese Lung Cancer Society classification [11]: p0, visceral pleura is not involved by tumor; p1, tumor has reached but not invaded the visceral pleura; and p2, tumor has invaded the visceral pleura but does not involve the parietal pleura. Briefly, p0 and p1 are classified as T1 disease, and p2 as T2 disease. Survival was calculated by the Kaplan–Meier method, and differences in survival were determined by the log-rank test. Unpaired two-tailed Student's *t* tests were used to compare mean values. The $\chi^2$ test was used to compare observed percentages. Differences with probability values of less than 0.05 were considered statistically significant.

## Results

The 63 patients ranged in age from 43 to 84 years (median, 67 years) and comprised 28 men and 35 women. The clinical and pathologic findings of the patients according to tumor type on HRCT are summarized in Table 1. Eleven air-containing type tumors showed pure ground-glass opacity (GGO), defined as a hazy increase in lung attenuation without obscuring the underlying vascular markings on HRCT. Among the patients with air-containing type tumors, intentional sublobar resection was performed in 39 (85%) and compromised resection in 7. On the other hand, 15 (88%) of the 17 patients with solid-density type tumors underwent compromised sublobar resection; in the other 2 patients low-grade malignant tumors were diagnosed on frozen section analysis during operation. As for the type of surgical resection, 49 wedge resections (78%) and 14 segmentectomies (22%) were performed. Most (83%) of the patients with air-containing type tumors underwent wedge resections. Because most of the sublobar resections in the patients with solid-density type tumors were compromised procedures, wedge resection was more common than segmental resection in these patients. Lymph-node sampling was done in 8 patients with air-containing type tumors. As for the patients with solid-density type tumors, lymph-node sampling was done in 6 patients and dissection in 2. The other 9 patients (53%) with solid-




Patients at risk

| Months | 0 | 12 | 24 | 36 | 48 | 60 |
|---|---|---|---|---|---|---|
| Air-containing type | 46 | 46 | 45 | 42 | 36 | 30 |
| Solid-density type | 17 | 15 | 13 | 11 | 7 | 5 |

*Fig 4. Relapse-free survival curve for patients with air-containing type (solid line; n = 46) and solid-density type (dotted line; n = 17) tumors.*

density type tumors had limited cardiopulmonary reserve; lymph node exploration was considered not to be beneficial mainly because of calcified nodes, dense pleural adhesion, and hypoxemia during operation.

Histopathologically, none of the patients with air-containing type tumors had vascular invasion or pleural involvement; however, 8 (17%) were given a diagnosis of not pure BAC (adenocarcinoma with mixed subtypes) because of minimal stromal invasion. In contrast, all solid-density type tumors were non-BACs associated with high rates (47% of all solid-density type tumors) of pleural involvement or vascular invasion.

There were no serious complications after operation and no surgical mortality. Two patients with air-containing type tumors and 1 with a solid-density type tumor died of other diseases, with no evidence of recurrence. Seven patients with solid-density type tumors died of recurrent lung cancer; the first site of recurrence was locoregional in 5 patients (3 wedge resections, 2 segmentectomies) and liver metastasis in 2 (1 wedge resection, 1 segmentectomy). The sites of locoregional recurrence were pleural dissemination in 2 patients (1 wedge resection, 1 segmentectomy), pulmonary metastasis in 2 (1 wedge resection, 1 segmentectomy), and mediastinal lymph node in 1 (wedge resection). No patient with air-containing type tumors had recurrence. Overall and relapse-free survival curves are shown in Figures 3 and 4. Median follow-up of the survivors was 70 months (range, 21 to 133 months) in patients with air-containing type tumors and 49 months (range, 25 to 112 months) in those with solid-density type tumors. Overall and relapse-free survival rates at 5 years were 95% and 100%, respectively, in patients with air-containing type tumors, as compared with 69% and 57%, respectively, in those with solid-density type tumors. Both survival rates were significantly better in patients with air-containing type tumors than in those with solid-density type tumors ($p < 0.0001$).

## Comment

Lobectomy remains the standard operation for small non–small-cell lung cancers. The role of intentional sublobar resection for these tumors should be evaluated in well-designed clinical trials. At present, there are two possible indications for intentional sublobar resection. The first is for clinically diagnosed very early lung cancers, usually defined as clinical T1 N0 M0 tumors 2 cm or less in diameter that are located in the periphery of the lung. Because lymph node metastasis accompanies approximately 15% of adenocarcinomas of this size [12], N0 status must be confirmed at operation. In most series of patients undergoing sublobar resection for clinically very early lung cancers, segmental resection is the procedure of choice to gain access to the hilar lymph nodes [2–4]. In addition, segmentectomy for confirming N0 status should be selected in the patients with adenocarcinomas of solid-density type on HRCT because nodal involvement was found in 12% of these patients [7].

The other possible indication for intentional sublobar resection is pathologically confirmed noninvasive adenocarcinoma of the lung. The histologic classification of the World Health Organization defines BAC as noninvasive adenocarcinoma showing pure lepidic growth without vascular, stromal, or pleural invasion [9]. Lymph node metastasis has not been found in patients with BAC, and cure is likely after complete resection [5]. An improved understanding of the pathologic characteristics of peripheral lung adenocarcinoma and increased use of CT scanning has led interest to focus on the correlation of CT images with the histologic features of BAC. Attempts have been made to identify CT findings that could serve as landmarks for sublobar resection [6–8, 13–18]. Bronchioloalveolar carcinoma components showing lepidic growth along alveoli, without areas of invasion, present as areas of GGO on HRCT [14]. The proportion of GGO is directly related to tumor histology and behavior. Patients with adenocarcinomas measuring 2 cm or less in which the proportion of GGO to the whole tumor area on HRCT was 50% or greater have no lymph node involvement and survive without any recurrence after resection [13, 15]. The TDR on HRCT images is also a simple and useful index for identifying early adenocarcinoma of the lung [7, 8, 16]. Visual evaluation of the GGO ratio is subject to considerable variability among examiners [18]. In contrast, evaluation of the TDR on HRCT has the advantage of simplicity and does not require the use of complex instrumentation: tumor opacity on lung window images is simply compared with that on mediastinal images [7]. Okada and colleagues [16] reported that the extent of both TDR and GGO correlate well with that of the BAC growth of adenocarcinomas; however, the TDR more strongly correlates with the BAC proportion than does the GGO ratio. Previously, we also reported that adenocarcinomas measuring 2 cm or less in diameter in which the TDR on HRCT was 50% or greater have no lymph node involvement and rarely show microscopic invasion. These findings suggested that sublobar resection might be an appropriate approach for the management of such tumors [7, 8]. Few studies have evaluated the outcomes of sublobar resections performed on the basis of HRCT characteristics other than pure GGO. We therefore retrospectively investigated surgical outcomes in patients who underwent sublobar resections according to findings on preoperative HRCT images.

The outcomes of sublobar resection for these possibly indolent tumors should be assessed on the basis of long-term disease-free survival and recurrence patterns. We therefore excluded patients who had a history of previous primary lung cancers or other malignancies, as well as those with multiple lung cancers. About half of the initially screened patients with these small-sized adenocarcinomas of the lung who underwent sublobar resection were excluded. In our series, all patients with air-containing type tumors, excluding 2 who died of other diseases, survived with no evidence of recurrence, despite incomplete lymph node exploration. Sublobar resections for these air-containing type tumors did not require lymph node sampling or dissection, and the

extent of resection depended on only lesion size or location. In addition, not all of the air-containing type lesions were diagnosed as BAC, whereas 8 (17%) were diagnosed as mixed adenocarcinomas with minimal stromal invasion. Those patients who had BAC with focal or minimal invasion survived with no relapse for 41 to 82 months (median, 51 months) after operation. This result suggested that some patients with radiologic evidence of early lung adenocarcinoma on the basis of TDR show minimal invasion on pathologic examination and might be cured by sublobar resection. However, this point remains controversial, and further studies are needed.

In conclusion, our results suggest that the TDR on HRCT images is a simple and useful variable for identifying small adenocarcinomas indicated for limited pulmonary resection. The outcome of sublobar resection for air-containing type lesions may be favorable in patients with curable disease. Larger, multicenter trials are needed to identify HRCT images that more precisely reflect the biologic behavior of these tumors and to assess the surgical outcomes of sublobar resection indicated on the basis of these HRCT images.

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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, 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; *CYP2E1*C, 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 *Rsa*I 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