A case of secondary pneumothorax due to pulmonary metastatic tumor from angiosarcoma of the scalp

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An 80-year-old man was diagnosed with angiosarcoma of the scalp. Treatment with surgery, radiotherapy, and chemotherapy achieved a complete response. However, 20 months after surgery, the patient was diagnosed with lung metastasis with thin-walled cavity formation. A few days later, he developed left pneumothorax. Because air leakage persisted without improvement, left upper lobectomy was performed. Pathologically, tumor cells showed positive immunostaining for CD 31, which proved to be a pulmonary metastatic tumor from angiosarcoma of the scalp. Pneumothorax occurred again within a month. Because of prolonged air leakage, surgical resection was conducted. Many tiny cysts were observed, and some of them caused pneumothorax. Therefore, wedge resection was performed. The pathological diagnosis of the tiny cysts was pulmonary metastatic tumor from angiosarcoma. Although the pneumothorax was resolved, he died from dyspnea 36 days after the second operation.

症 例

後縦隔ミュラー管嚢胞の1例

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要 旨

症例は51歳，女性．不正性器出血を主訴に近医を受診した．CTで骨盤内腫瘍を指摘されたため当院産婦人科を紹介．その際，胸部CTで後縦隔腫瘍を指摘され当科へ紹介．骨盤内腫瘍は成熟奇形腫，後縦隔腫瘍は嚢胞性の良性腫瘍と診断し，同時に摘出術を行った．まず腹腔鏡下に両側付属器切除術を施行し，右半側臥位に体位変換，胸腔鏡下後縦隔腫瘍摘出術を行った．後縦隔腫瘍は薄い被膜に覆われた単房性嚢胞で，慎重に破らず摘出した．周囲との連続性は無かった．病理組織検査で，骨盤内腫瘍は成熟奇形腫瘍，後縦隔腫瘍は，腫瘍内腔は線毛円柱上皮で被覆され，免疫染色で被覆上皮はエストロゲンレセプター，プロゲステロンレセプター陽性でありミュラー管嚢胞と診断された．ミュラー管嚢胞は閉経前後の女性に認める縦隔嚢胞性疾患の新しいカテゴリーである．

索引用語：ミュラー管嚢胞，後縦隔嚢胞
Mullerian cyst, Posterior mediastinal cyst

はじめに

縦隔ミュラー管嚢胞は2005年にHattoriらが報告した縦隔嚢胞性疾患の新しいカテゴリーである．傍～前胸椎体に認められる線毛上皮に裏打ちされた単房性嚢胞で，閉経前後の女性のみに認められ，肥満や婦人科疾患の既往などを併存することが多く，何らかのホルモン環境が関与していると考えられる．その発生部位より神経原性腫瘍，組織所見より気管支原性嚢胞との鑑別が問題になるが，最も重要なマーカーは免疫組織化学検査で嚢胞壁のエストロゲンレセプター，プロゲステロンレセプター陽性の所見である．

今回，卵巣成熟奇形腫の手術前の精査時に偶然発見された後縦隔ミュラー管嚢胞を経験したので報告する．

症 例

患 者：51歳，女性．

主 訴：不正性器出血．

家族歴：特記事項なし．

既往歴：30歳，33歳時帝王切開．

現病歴：不正性器出血を主訴に近医を受診．CTで骨盤内腫瘍を指摘され当院産婦人科を紹介された．その際胸部異常陰影も指摘されたため当科に紹介となった．

入院時現症：身長148 cm 体重56 Kg BMI 25.5と軽度の肥満を認めた．

入院時検査所見：

血液生化学検査所見：CA125：7.5 U/ml，SCC：0.7 ng/ml，CA19-9：16.3 U/mlと測定した腫瘍マーカーに上昇は認めなかった．

胸部X線所見：下行大動脈とシルエットサイン陰性の境界明瞭な腫瘤影を認めた．

胸部造影CT：Th5，6椎体左側に23×19×40 mm大の境界明瞭な腫瘤を認める．造影効果を呈する充実部は認めない（Fig. 1）．

骨盤造影CT：左卵巣領域に62×47 mm大の辺縁平滑な腫瘤を認める．腫瘍内に脂肪や石灰化を有し成熟奇形腫が疑われた（Fig. 2）．

胸部MRI：胸部下行大動脈の背側に境界明瞭な腫瘤

大分大学呼吸器・乳腺外科
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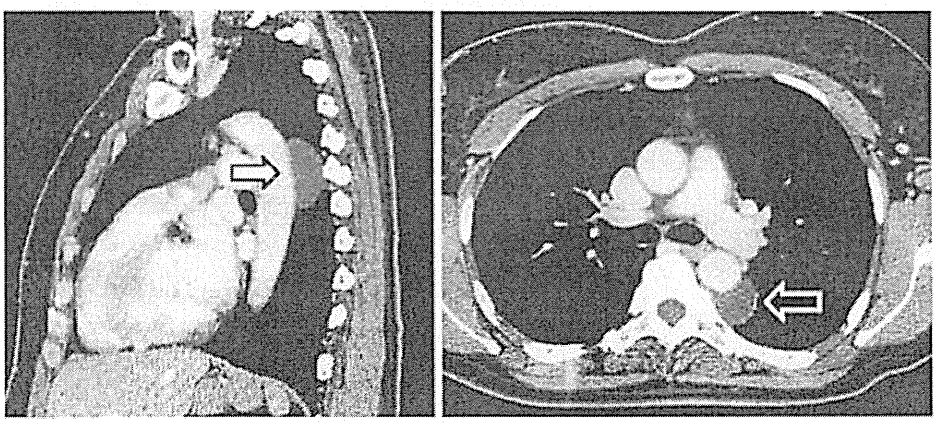

Fig. 1 Enhanced chest CT showing a unilocular, unenhanced cystic mass, 4 cm in diameter, behind Th4, 5, and the descending aorta (arrow).

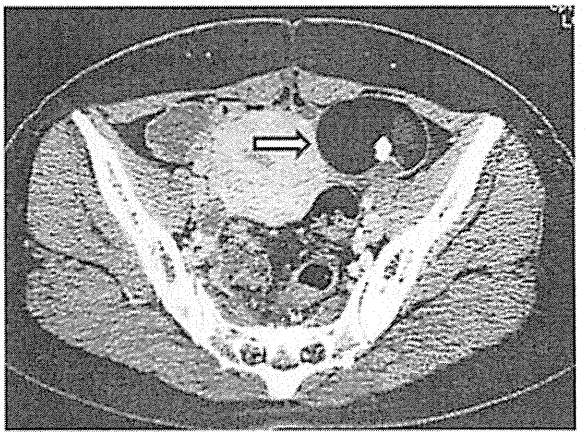

Fig. 2 Enhanced abdominal CT showing a calcification and fatty tissue in the pelvic tumor, suggesting a typical mature cystic teratoma (arrow).

を認め，T1 強調で淡い低信号，T2 強調で均一な高信号を示し，嚢胞性腫瘤が示唆される．嚢胞変性した神経鞘腫，気管支嚢胞などが考えられた（Fig. 3）．

経　過：骨盤内腫瘍は成熟奇形腫疑い，後縦隔腫瘍は発生部位から神経原性腫瘍を疑い，同時に摘出術を行う方針になった．

手術所見：まず産婦人科医により腹腔鏡下両側付属器切除を施行．引き続き，右半側臥位に体位変換し胸腔鏡下後縦隔腫瘍摘出術を行った．後縦隔腫瘍は薄い被膜をもつ嚢胞で，下行大動脈から椎体，交感神経幹に接していたが周囲との癒着は粗であった．被膜を破らぬよう注意しながらベッセルシーリングシステムを用いて周囲から剥離，摘出した．交感神経と連続性はなかった（Fig. 4）．

病理組織検査結果：卵巣腫瘍は成熟奇形腫で矛盾しない所見であった．

後縦隔腫瘍は，単房性の嚢胞で，内腔は線毛円柱上皮，立方上皮により被覆されている．局所的には乳頭状構造も認めるが異型はなく，嚢胞壁は薄く，膠原線維により構成され，平滑筋も認める．免疫染色では被覆上皮は ER，PgR 陽性，Calretinin 陰性であり，ミュラー管嚢胞と診断した（Fig. 5）．

術後経過：経過良好で術後 7 日目に退院となった．

考　察

ミュラー管嚢胞はミュラー管由来の上皮に覆われた嚢胞である．ミュラー管とは，胎生第 5～6 週に至りウォルフ管の両側に生ずる一対の管で，これはウォフル管の外方に沿って下り，下方においてその前を横切って左右接近し，ウォルフ管の内側で尿生殖洞に開口する．胎生 8 週になると左右のミュラー管は下方から癒合し始め，第 12 週頃までに癒合が完成し，癒合した部分から子宮，膣が形成され，癒合しない部分は卵管となる．男性の場合は胎生精索より分泌される抗ミュラー管ホルモンによって退化し，頭側では精巣垂，尾側では前立腺小室として遺残する．通常，この前立腺小室が異常に拡張したのがミュラー管嚢胞と呼ばれ，男性骨盤内に好発し，剖検例では 0.7～1% に認められる[1]．

金子らは後腹膜原発のミュラー管嚢胞について他験例を合わせた 15 例を報告しているが[2]，全て女性であった．その発生機序として，1. 胎生期のミュラー管の痕跡が異

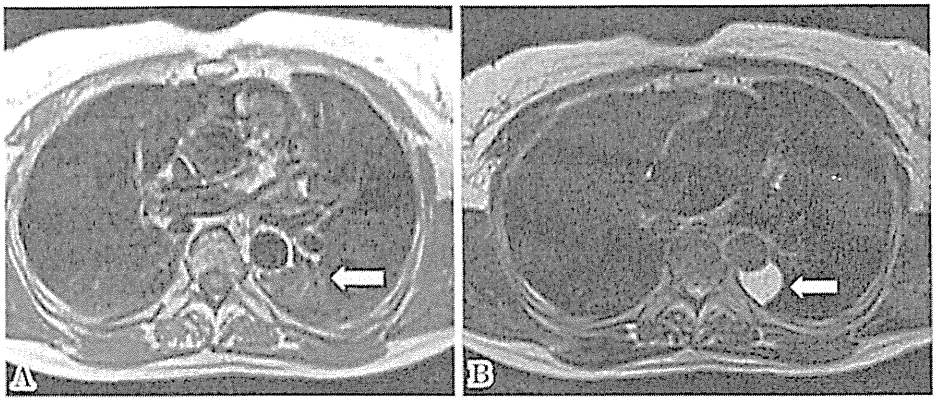

Fig. 3 Chest MRI showing the cystic mass consisting of a thin wall and homogeneous content with a low signal intensity on T1-weighted imaging (A) and a high signal intensity on T2-weighted imaging (B) (arrow).

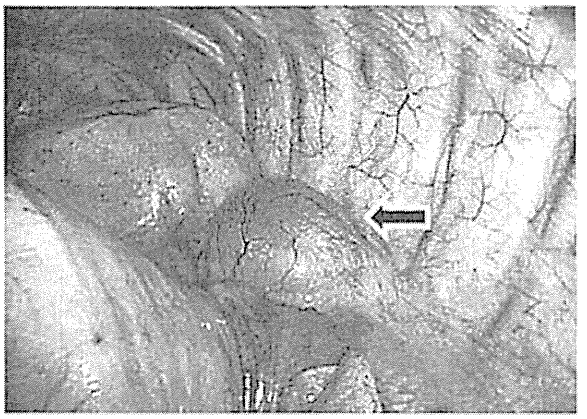

Fig. 4 Intraoperative findings: A posterior mediastinal tumor with a thin wall not showing adhesion to the surrounding tissue (arrow).

常なホルモン刺激で腔を形成する説，2．体腔上皮が漿液や円柱状の上皮に分化し周囲の組織内に陥入し嚢胞構造を形成する説，3．逆行性月経や骨盤内手術により異所性の子宮内膜組織が形成され同部より嚢胞が形成される説，を述べている[4]．後縦隔への発生機序は不明であるが，Batt らは Ludwig の説を支持している[3]．すなわち，Ludwig は詳細な発生学の研究のなかで，胎生16期に，第3から5胸椎体のレベルで，中腎ひだの頭側端に肥厚した体腔上皮が発達し，卵管の漏斗部原基を形成する，という説であり[4]，この説は嚢胞の卵管上皮構造の発生機序のみでなく，嚢胞が第4～6傍椎体に好発する説明になりうる．

後縦隔ミュラー管嚢胞は2005年に Hattori らが3例の報告をして以来[5,6]，これまでに計17例の報告がある[1,3-10]（Table 1）．いずれも女性で18歳から59歳，平均51歳と閉経前後の女性に多い．また肥満や婦人科疾患の合併を少なくとも9例に認めているが，婦人科疾患そのものとの関連は不明である．しかし，全例が女性であること，被覆上皮がエストロゲンレセプター，プロゲステロンレセプター陽性であることより，女性ホルモンとの関連が示唆される．自験例も，51歳で閉経直後，BMI25と軽度の肥満と，卵巣奇形腫合併例であった．報告例のなかに卵巣奇形腫はなかったが縦隔奇形腫合併を1例認めた．

術前診断は神経原性腫瘍10例，気管支原性腫瘍3例で，術前よりミュラー管嚢胞が疑われた例はない．術前診断は困難なため，診断を兼ねた摘出が望まれる．病理組織所見もミュラー分化した縦隔嚢胞は，線毛上皮のため気管支嚢胞と診断されやすい．鑑別に最も有用なのはエストロゲンレセプター，プロゲステロンレセプターによる免疫染色であり，確定診断となる．自験例も，術前診断は嚢胞変性した神経鞘腫，気管支嚢胞であったが，周囲神経との連続性がないこと，漿液性な内容物であったことより，他疾患の可能性を疑い文献的検索を行ったところ，ミュラー管嚢胞の可能性が高いと判断し，確定診断のため免疫染色を追加した．

発生部位は Th2～8の高さであるが，Th4/5レベルが最も多く，部位は椎体左側が9例，右側5例，前面が3例と左側に多い傾向がある．これまでの報告において，

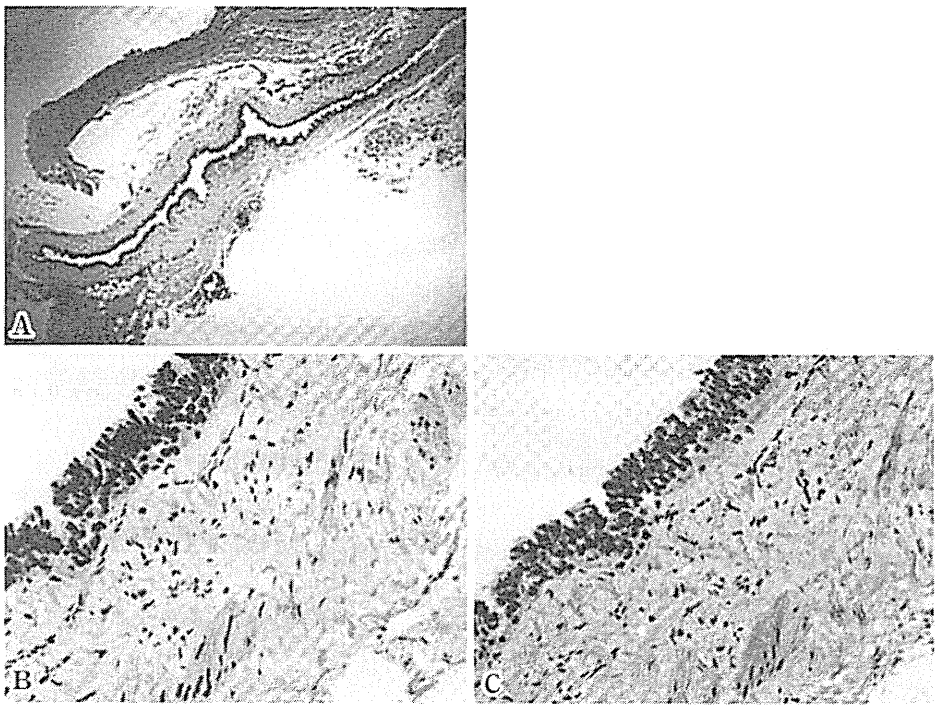

Fig. 5 Histological findings of the posterior mediastinal cyst with Mullerian differentiation. (A) The lesion was a thin-walled unilocular cyst with a circumferential smooth muscle layer (H&E staining, original magnification: ×40). (B) Immunohistochemical staining showing the positive reaction of the nucleus for ER and (C) PgR (original magnification: ×200).

Table 1 Characteristics of Mullerian cyst in the literature.

	Report	Age	Side	Level	Size (mm)	Preoperative diagnosis	Past history
1	Hattori[5, 6]	52	Right	T6	25	Neurogenic tumor	Hrt (−)
2		18	Right	T5	20	Neurogenic tumor	Hrt (−)
3		49	Left	T4	20	Neurogenic tumor	Hrt (−)
4	Vincent[7]	40	Left	T4	15	Neurinoma	2: overweight, 2: obese, 1: hysterectomy for cervical neoplasia, 1: hysterectomy for leiomyoma, 1: hysterectomy and hrt, 1: surgery for ovarian benign cyst, 1: abortion and endometrial polyp, 2: nulliparous
5		46	Left	T4	33	Neurinoma	
6		47	Right	T4/5	50	Neurinoma	
7		48	Left	T5	30	Bronchogenic cyst	
8		50	Right	T3/4	32	Neurinoma	
9		51	Left	T3/4	30	Cyst	
10		56	Left	T8	13	Neurinoma	
11		58	Prevertebral	T5	45	Bronchogenic cyst	
12		59	Right	T2-4	25	Neurinoma	
13	Adrian[8]	54	Left	T4-6	45	Neuroenteric cyst	Hrt (+)
14	Batt[3]	41	Left	T6	21		
15	Kobayashi[9]	53	Prevertebral	T5	20	?	Obese, mediastinal teratoma
16	Matthew[10]	52	Prevertebral	T5	41	Bronchogenic cyst	?
17		47	Left	?	50	?	?
18	Present case	51	Left	T5/6	40	Neurogenic tumor	Overweight, ovarian teratoma

*Hrt: hormone replacement therapy, Overweight: BMI25<, Obese: BMI30<

腫瘍サイズは15 mm～50 mm（平均30 mm）であり，摘出せず放置した場合巨大化するかは不明である．通常周囲との癒着は乏しいため胸腔鏡下手術のよい適応と考える．

結　　語

今回我々は稀な疾患である後縦隔ミュラー管嚢胞を経験した．後縦隔嚢胞の場合，性別，年齢，既往歴などからミュラー管嚢胞を疑い，摘出標本におけるホルモンレセプターの免疫染色で確定診断を行うことが重要である．

利益相反

本論文について申告する利益相反はない．

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Mullerian cyst in the posterior mediastinum

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The patient was a 51-year-old female. The chief complaint was atypical genital bleeding. Pelvic CT showed a tumor in the pelvis, and thoracic CT showed a posterior mediastinal tumor. She was recferred to gynecological and thoracic surgery departments for further examination. The preoperative diagnosis was a mature teratoma of the ovary and benign posterior mediastinal cyst. The patient underwent posterior mediastinal tumor resection following laparoscopic simple total hysterectomy and bilateral adnexotomy for the ovarian tumor. The mediastinal tumor had a thin wall and showed no adhesion to surrounding tissue. Pathological examination showed a mature teratoma of the ovary and a posterior mediastinal mullerian cyst, in which estrogen and progesterone receptors were positive on immunohistoligical examination. A Mullerian cyst in the posterior mediastinum is a new category of mediastinal cyst, initially reported by Hattori et al. in 2005. It occurs in perimenopusal females, especially in those with obesity or a gynecological history, but preoperative definitive diagnosis is difficult.

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ORIGINAL ARTICLE

Validity of using lobe-specific regional lymph node stations to assist navigation during lymph node dissection in early stage non-small cell lung cancer patients

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Abstract
Purpose The validity of our proposed lobe-specific regional lymph node stations (LSRLNS) was evaluated as a method for navigation during lymphadenectomy in patients with early stage non-small cell lung cancer (NSCLC).
Methods A total of 725 NSCLC patients with c-T2N1M0 or less extensive disease who had undergone a curative operation with complete mediastinal lymph node dissection (MLND) were studied. The LSRLNS were #2, #3, #4 and #10 for the right upper lobe, #11i, #11s, #7 and #8 for the right lower lobe, #4, #5 and #6 for the left superior division, #11, #5 and #7 for the left lingular division and #11, #7 and #8 for the left lower lobe.
Results If the LSRLNS were used for pathological examinations during surgery, 599 p-N0 and 39 p-N1 patients diagnosed with no metastasis would have been subjected to a selective MLND, while 20 p-N1 and 65 p-N2 patients who had a diagnosis of metastasis would have been navigated to a complete MLND. Two p-N2 patients with a diagnosis of no metastasis would have inappropriately undergone a selective MLND, resulting in the false negative rate at 0.3 %.
Conclusion Intra-operative pathological examination using our LSRLNS may accurately reveal the status of metastasis, and appropriately lead to a selective or complete MLND in patients with c-T2N1M0 or less extensive disease.

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Keywords Non-small cell lung cancer · Lymphadenectomy · Lobe-specific regional lymph node station

Introduction

The lymph node status is a major determinant of the stage and survival in patients with non-small cell lung cancer (NSCLC), and mediastinal lymph node dissection (MLND) is generally accepted for staging and is an important choice for standard treatment [1–3]. However, the extent of lymph node dissection remains controversial, especially in patients with early stage NSCLC [4–12].

We previously investigated which mediastinal lymph node stations should be examined during surgical intervention to diagnose N2 or less extensive disease in patients with NSCLC and proposed three regional lymph node stations for each lobe [5]. We then applied selective MLND in NSCLC patients, in whom no further MLND was performed when there was no metastasis shown in regional mediastinal lymph nodes examined pathologically during the surgery [6]. Although the results of that study were acceptable, there were several problems that needed to be solved, as follows: (1) The regional lymph node stations of the lower lobe included those located in the upper mediastinum; (2) The regional lymph node stations of the superior and lingular divisions in the left upper lobe were not separately determined; (3) The status of the visceral pleural invasion (VPI) of tumors was not considered when determining the regional lymph node stations. Therefore, we revised our originally proposed lobe-specific regional lymph node stations (LSRLNS) to increase their accuracy and usefulness.

The purpose of the present study was to validate the effectiveness of intra-operative pathological examinations

using our newly revised LSRLNS, which were determined using the data obtained at Wakayama Prefecture Medical College Hospital, as a navigation method for performing a selective or complete mediastinal lymphadenectomy in a large series of patients with c-T2N1M0 or less extensive disease, who had undergone surgery at Okayama University Hospital.

Methods

Patients

Between January 1995 and December 2009, 1061 patients underwent a curative operation for bronchogenic carcinoma at Okayama University Hospital. A curative operation was defined as the complete removal of the ipsilateral lobar, interlobar, hilar and mediastinal lymph nodes, together with a primary tumor, including negative bronchial margins. Since the number of N2 lung cancer patients with a tumor located in the middle lobe was so small in our previous study [5], the regional lymph node stations could not be reliably determined for the middle lobe. Therefore, 57 patients with a primary tumor in the middle lobe were excluded from the present study. Of the remaining 1004 patients, 725 with c-T2N1M0 or less extensive disease, including cases with VPI, were investigated in this study. The findings from these patients were not used to determine our original [5] or revised regional lymph node stations.

The patients underwent preoperative computed tomography (CT) of the chest and upper abdomen, magnetic resonance imaging (MRI) of the brain and bone scintigraphy examinations to determine the clinical staging. In recent cases, fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET) scanning was also employed. Mediastinoscopy was not performed for these early stage patients. The disease stage was based on the TNM classification of the Union Internationale Contre Cancer (UICC) [13]. The lymph node mapping proposed by Naruke [14] was employed, with the names and numbering shown in Table 1 [15]. This study was approved by the institutional review board of Okayama University Hospital.

Lobe-specific regional lymph node stations

The extent of lobe-specific complete MLND and our revised regional lymph node stations are shown in Fig. 1a–e. The complete MLND procedure included mediastinal lymph node stations #1–4 and #7 for a tumor located in the right upper lobe (Fig. 1a), #1–4 and #7–9 for one located in the right lower lobe (Fig. 1b), #4–7 for one located in the left upper lobe (Fig. 1c, d) and #4–9 for tumors located in the left lower lobe (Fig. 1e), as well as the lobar (#12), interlobar (#11) and hilar (#10) N1 lymph node stations [15]. The revised regional lymph node stations are shown by gray squares and circles in the figure. Hilar lymph node station #10 was added to the regional lymph node stations of the right upper lobe (Fig. 1a), because lymph from the right upper lobe flows into subcarinal lymph node station #7 through #10 [16]. Furthermore, we [5] and Hata [16] previously reported that cancer cells or lymph flow were transferred from the lower lobe to the superior mediastinal lymph node levels through #7 or the interlobar lymph node stations. Based on these findings, the interlobar lymph node stations #11s (superior) and #11i (inferior) were substituted for pretracheal lymph node station #3 in the right lower lobe (Fig. 1b), which allows the dissection of the upper mediastinum to be avoided.

The left upper lobe was divided into the superior and lingular divisions. Our previous study demonstrated that the left tracheobronchial (#4), subaortic (#5) and paraaortic (#6) lymph node stations often had metastasis when the tumor was located in the superior division [5]. Therefore, #4, #5 and #6 were determined to be the regional lymph node stations for the superior division (Fig. 1c). In the same manner as was used for the superior division, #5 and #7 were determined to be the regional lymph node stations for the lingular division (Fig. 1d) [5]. Left interlobar lymph node station #11, which is on the way to #4 [16], was substituted for #4 in the lingular division. In the left lower lobe, #11 was determined to be the regional lymph node

Table 1 Names and numbering of lymph node stations based on the Naruke mapping [15]

#1	Superior or highest mediastinal
#2	Paratracheal
#3	Pre-tracheal
#3P	Retrotracheal or posterior mediastinal
#3a	Anterior mediastinal
#4	Tracheobronchial
#5	Subaortic or Botallo's
#6	Paraaortic (ascending aorta)
#7	Subcarinal
#8	Paraesophageal (below carina)
#9	Pulmonary ligament
#10	Hilar (main bronchus)
#11	Interlobar
#11s	Interlobar, superior
#11i	Interlobar, inferior
#12	Lobar, upper lobe, middle lobe and lower lobe

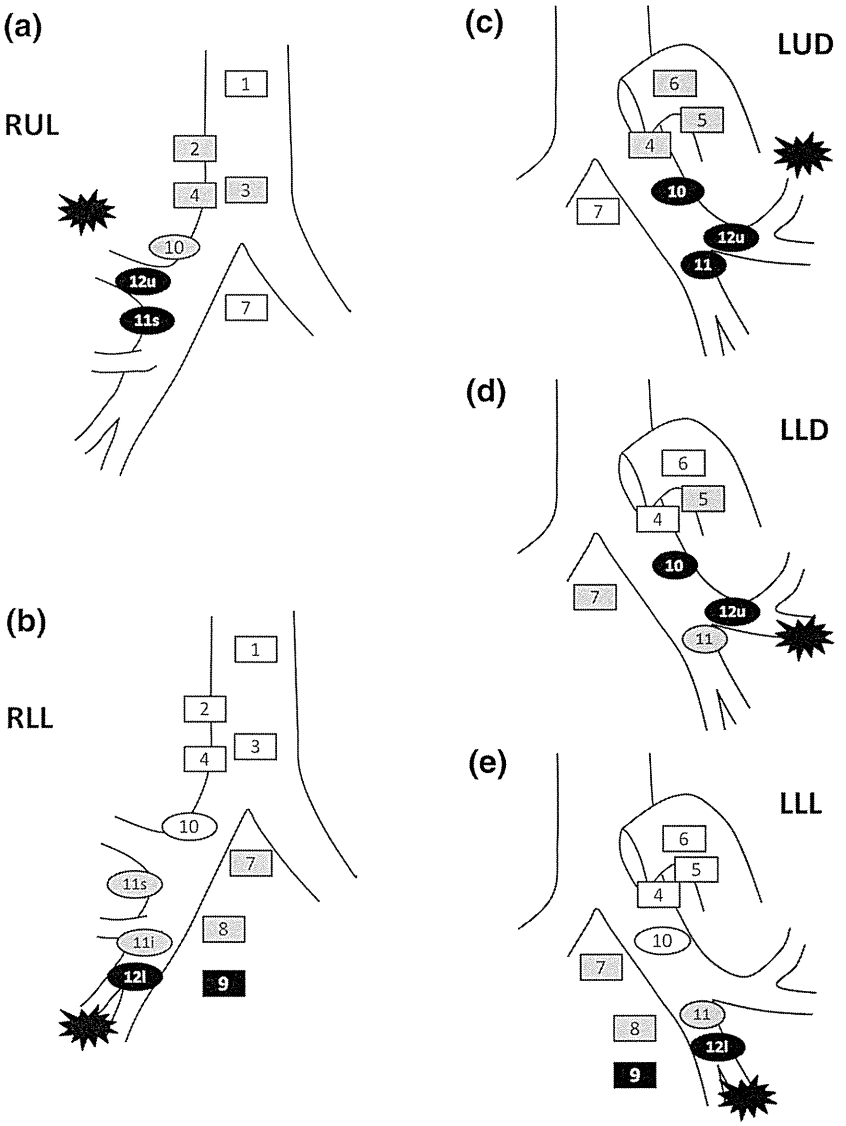

Fig. 1 The extent of lobe-specific complete mediastinal lymph node dissection [15] and our revised regional lymph node stations. The names and numbering of the lymph node stations shown in Table 1 [15] are used. *Squares* indicate mediastinal lymph node stations, while *circles* show hilar, interlobar and lobar lymph node stations. The present revised regional lymph node stations are shown with *gray squares* and *circles*

station to be used in place of #4 for the same reason (Fig. 1e).

Analyses

We analyzed the patterns of lymph node metastasis and VPI in p-N1 and p-N2 patients. According to the TNM staging system of the UICC [13], VPI is classified into the following stages: pl0, a lung tumor with no pleural involvement beyond its elastic layer; pl1, a lung tumor that extends beyond the elastic layer of the visceral pleura but is not exposed on the pleural surface; and pl2, a lung tumor that is exposed on the pleural surface but does not involve adjacent anatomic structures. We then investigated whether an intra-operative pathological examination using our revised LSRLNS would accurately diagnose the status of metastasis, and appropriately lead to the performance of a selective or complete mediastinal lymphadenectomy.

Results

Patient characteristics

The characteristics of our patients are shown in Table 2. Of the 725 patients studied, the primary tumor was located in the right upper lobe in 269, right lower lobe in 156, left superior division in 145, left lingular division in 36 and left lower lobe in 119. The tumor size of the 262 c-T2 patients was distributed mostly between 3 and 5 cm. P-T3 disease included invasion to the parietal pleura in nine patients and to the mediastinal pleura in two patients. Eight p-T4 patients had metastasis in the lobe with the primary tumor. Of the 682 c-N0 patients, 577 patients had p-N0, 47 had p-N1 and 58 had p-N2. Of the 43 c-N1 patients, 22 patients had p-N0, 12 had p-N1 and 9 had p-N2. Thus, 599 patients had no pathological metastasis to any lymph node station (N0), while 59 had metastasis to N1 and 67 to the

Table 2 The patient characteristics

Variables	No.
Median age (years), range	67, 24–86
Sex (male/female)	425/300
Histology	
Adenocarcinoma	558
Squamous cell ca.	144
Adenosquamous	12
Large cell ca.	10
Pleomorphic ca.	1
Location	
Right upper lobe	269
Right lower lobe	156
Left upper segment	145
Left lingular segment	36
Left lower lobe	119
TNM status	
c-T1/T2	463/262
c-T2: <3 cm[a]/3–5 cm/5–7 cm/<7 cm	21/221/20/0
c-N0/N1	682/43
p-T1/T2/T3/T4	446/260/11/8
p-N0/N1/N2	599/59/67

ca carcinoma

[a] Disease with a tumor size of 3 cm or <3 cm includes a radiological diagnosis of pleural invasion or atelectasis of the tumor-occupied lobe

mediastinal lymph node stations (N2). The patterns of lymph node metastasis and VPI in those p-N1 and p-N2 patients are depicted in Figs. 2a–e and 3a–e, respectively, according to the location of the primary tumor.

P-N1 patients (Fig. 2a–e)

Thirty-nine of the 59 p-N1 patients had metastasis to only the upstream N1 stations prior to the regional lymph node stations, while the remaining 20 had metastasis to the revised regional lymph node stations. It was noted that pl0, pl1 and pl2 were observed in 48, 7 and 4, respectively, of the p-N1 patients.

P-N2 patients

Right upper lobe (Fig. 3a)

All 21 p-N2 patients with a primary tumor in the right upper lobe had metastasis to a lymph node station in the superior mediastinum. The most common sites of metastasis were #2, #3 and #4, the combination of which is now defined as "4R" according to the 7th edition of the TNM classification of the UICC. Twelve patients had skip metastasis, which is defined as N2 metastasis without N1 metastasis. Only two patients had metastasis to #7, both of whom also had regional lymph node metastasis. There were no cases with metastasis to #10, which was newly added as a regional lymph node station. If the regional lymph node stations were examined in a pathological manner during the surgery, p-N2 would have been diagnosed in all patients.

Right lower lobe (Fig. 3b)

Fifteen of the 16 p-N2 patients with a primary tumor in the right lower lobe had metastasis to the subcarinal lymph node station #7, while all 4 patients with metastasis to the superior mediastinum were associated with metastasis to the regional lymph node stations. As in the cases with right upper lobe tumors, pathological examinations of the regional lymph node stations during the surgery would have resulted in a p-N2 diagnosis in all of these patients.

Left superior division (Fig. 3c)

Of the 18 p-N2 patients with a primary tumor in the left superior division, 16 had metastasis to #5, 5 to #6 and 4 to #4. Furthermore, seven patients had skip N2 metastasis without N1 metastasis. Since #4, #5 and #6 belong to regional lymph node stations, pathological examinations of those stations during the surgery would have resulted in a p-N2 diagnosis in all of these patients.

Left lingular division (Fig. 3d)

There was only one p-N2 patient with a primary tumor in the left lingular division. Since this patient had metastasis to regional lymph node station #5, a pathological examination of the regional lymph node stations during the surgery would have resulted in a diagnosis of p-N2.

Left lower lobe (Fig. 3e)

Of the 11 p-N2 patients with a primary tumor in the left lower lobe, 7 had metastasis to #7 and 6 had metastasis to #11. Furthermore, five of the 11 p-N2 patients had metastasis to the upper mediastinum, three of whom had metastasis to #7. Patient numbers 1 and 4 had metastasis to the superior mediastinum without metastasis to regional lymph node stations. Both patients had a primary tumor in segment 8 with a histological diagnosis of adenocarcinoma. Their preoperative TNM diagnoses were c-T1aN0M0 and c-T1bN0M0, which postoperatively changed to p-T1aN2M0 and p-T1bN2M0, respectively. The pathological examinations of the regional lymph node stations during the surgery would have resulted in a p-N2 diagnosis in nine of these patients, with false negative results obtained for the remaining two patients.

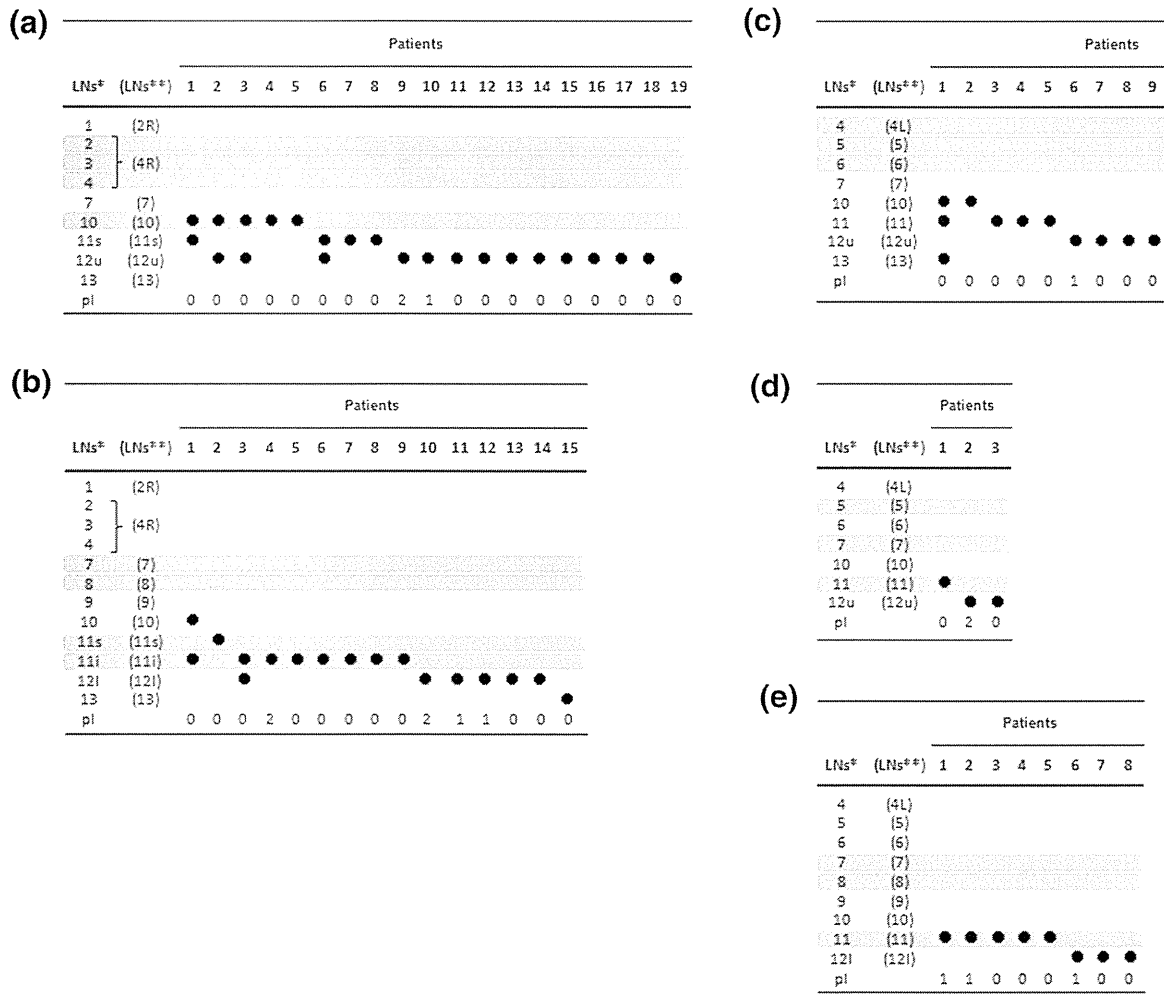

Fig. 2 The lymphatic metastatic patterns and visceral pleural invasion in NSCLC patients with p-N1 disease. The primary tumors were located in the right upper lobe in **a**, right lower lobe in **b**, left superior division in **c**, left lingular division in **d**, and left lower lobe in **e**. **LNs* lymph node stations dissected with a complete mediastinal lymphadenectomy (***LNs* expressed according to the 7th edition of UICC). *Gray bars* show lobe-specific regional lymph node stations

Visceral pleural invasion (VPI) in p-N2 patients (Fig. 3a–e)

We noted that pl0, pl1 and pl2 were observed in 46, 17 and 4, respectively, of the p-N2 patients. The p-N2 patients with pl1 or pl2 disease did not have any metastasis to downstream lymph node stations (distal side from tumors) beyond the regional lymph node stations with no metastasis.

Validity of the revised regional lymph node stations as a navigator to MLND

If the present revised regional lymph node stations were used for pathological examinations during the surgery with a frozen section technique, the 599 p-N0 and 39 p-N1 patients diagnosed with no metastasis would have been subjected to a selective MLND, while the 20 p-N1 and 65 p-N2 patients who had a diagnosis of metastasis would have been navigated to a complete MLND. Furthermore, two p-N2 patients with a diagnosis of no metastasis would have inappropriately undergone a selective MLND, thus leaving metastatic lymph nodes in the mediastinum. The false negative rates were extremely low at 0.3 % (2/682) for c-N0 disease and 0 % (0/43) for c-N1 disease, respectively.

Discussion

The lack of uniform terminology regarding mediastinal exploration during the resection of NSCLC has compounded the difficulties encountered when assessing the efficacy of the various techniques available for that

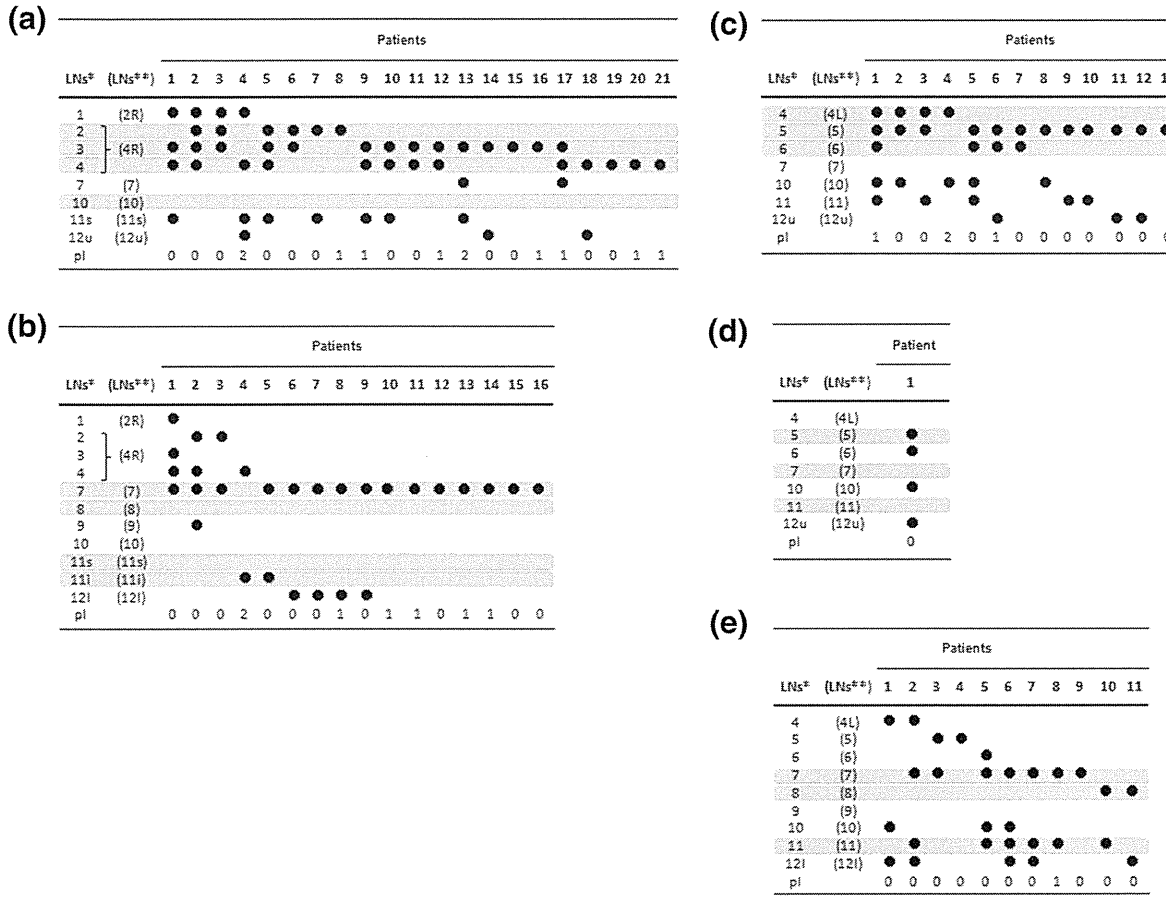

Fig. 3 The lymphatic metastatic patterns and visceral pleural invasion in NSCLC patients with p-N2 disease. The primary tumors were located in the right upper lobe in **a**, right lower lobe in **b**, left superior division in **c**, left lingular division in **d** and left lower lobe in **e**. **LNs* lymph node stations dissected with a complete mediastinal lymphadenectomy (***LNs* expressed according to the 7th edition of UICC). *Gray bars* show lobe-specific regional lymph node stations

purpose. Deslauriers [17] previously summarized those techniques, as shown in Table 3. Non-systematic sampling refers to a biopsy of only mediastinal nodes that appear obviously abnormal to visual or tactile inspection, while systematic mediastinal lymph node sampling (MLNS) is a routine biopsy of lymph nodes at pre-determined stations, regardless of the appearance of the node. MLND is the systematic removal of all ipsilateral lymph node-bearing tissues from multiple sites within the mediastinum. Recently, selective MLND has been applied to patients with early stage NSCLC, based on the lobar location of the primary tumor [5, 6, 9–12].

Resectable clinical N2 patients diagnosed using a non-invasive method, such as chest CT or FDG-PET generally, can undergo a pathological examination of the suspicious lymph nodes utilizing mediastinoscopy, video-assisted thoracic surgery, or more recently, endobronchial ultrasound-guided transbronchial needle aspiration biopsy techniques. If metastasis to those lymph nodes is confirmed, preoperative chemotherapy or chemo-radiotherapy therapy is often performed [18]. Resectable clinical T3 or 4 patients may also be good candidates for induction therapy [19]. MLND is always indicated for patients with resectable advanced NSCLC. On the other hand, the extent of mediastinal lymphadenectomy is controversial for other patients with c-T2N1M0 or less extensive disease who undergo initial surgery.

Table 3 Terminology for the nodal mediastinal exploration during surgery for non-small cell lung cancer [17]

(1) Non-systematic sampling: only nodes appearing abnormal in visual or tactile inspections are biopsied.
(2) Systematic sampling (MLNS): routine biopsy of nodes at pre-determined stations regardless of appearance.
(3) Lymph node dissection (MLND): removal of all ipsilateral lymph node-bearing tissues.
(4) Selective mediastinal node dissection (selective MLND): mediastinal node dissection based on the location of the primary tumor.
(5) Bilateral node dissection (BMD): includes the contralateral and supraclavicular nodes.

Table 4 Lymph node stations of MLND and MLNS used in the ACSOG Z0030 trial, and the selective MLND method used in the present study

MLND		MLNS		Present selective MLND				
Right	Left	Right	Left	Right		Left		
				UL	LL	UD	Lin	LL
2R	2L	*2R*						
3	3							
4R	4L	*4R*		**4R**		**4L**		
	5		*5*			**5**	**5**	
	6		*6*			**6**		
7	7	*7*	*7*		**7**		**7**	**7**
8	8				**8**			**8**
9	9				9			9
10R	10L	*10R*	*10L*	**10R**		10L	10L	
11s, 11i	11	11s, 11i	11	11s	**11s, 11i**	11	**11**	**11**
12	12	12	12	12u	12 l	12u	12u	12 l

Italicized entries show sampling lymph node stations. Bold entries show the present lobe-specific regional lymph node stations

MLND mediastinal lymph node dissection, *MLNS* mediastinal lymph node sampling, *UL* upper lobe, *LL* lower lobe, *UD* upper division, *Lin* lingular division

Of the 725 patients with c-T2N1M0 or less extensive disease in this study, 69 (9 %) had p-N2 disease. Oda et al. [20] reported that 13 % of their patients with clinical stage I had p-N2 disease and Asamura [9] demonstrated that 16.3 % of patients with NSCLC 3.0 cm or less in diameter had p-N2 disease. These high percentages of p-N2 disease in clinically diagnosed early stage NSCLC cases support the importance of MLND.

Recently, a large-scale multi-institutional randomized trial [American College of Surgery Oncology Group (ACOSOG) Z0030 Trial] was conducted to compare the prognosis of early stage (N0 or non-hilar N1, T1, T2) NSCLC patients between those who underwent MLND and MLNS. The lymph node stations of MLND and MLNS in the ACSOG Z0030 trial [8] are shown in Table 4, and are associated with our selective MLND based on the UICC 7th edition. That trial demonstrated that if a systematic and thorough pre-resection sampling of mediastinal and hilar lymph nodes is negative, MLND does not improve the survival in patients with early stage NSCLC. Although that study provided important findings, the extent of mediastinal exploration in MLNS and MLND was not determined based on each lobe with a primary tumor, only on the side on which the tumor was located. Therefore, with MLNS, #2R and #7 are sampled for a tumor in the right upper lobe, while those stations are not required for a pathological examination in our selective MLND technique. Exploration of #2R can cause recurrent nerve injury, while that of #7 can injure the bronchial artery or pulmonary rami of the vagal nerve. In contrast, the lower mediastinal lymph node stations, such as #8 and #9, are not sampled in MLNS, while those stations are included in our selective MLND for a lower lobe tumor, and metastasis to those lymph node stations was found present in our patients with that condition.

Okada et al. [11] also evaluated the possibility of lesser MLND for patients with clinico-surgical stage I NSCLC. They defined their selective MLND procedure as follows: dissection of the upper mediastinum for upper lobe tumors is performed, while it is not needed for lower-lobe tumors with intact hilar and lower mediastinal nodes. In addition, dissection of the lower mediastinum for an upper lobe tumor is not routinely required when the nodes in the hilum and upper mediastinum are negative. Basically, they diagnosed three stations (#10, #11, #12) of the N1 lymph nodes and one lobe-specific station of the N2 nodes using a frozen section technique to select the type of dissection. Ishiguro et al. [12] reported that selective MLND according to Okada's definition did not worsen the survival of patients with early stage NSCLC (clinical stage I: 95 %) in a large-scale retrospective cohort study.

Our selective MLND was able to be applied to c-T2N1M0 or less extensive disease, which covers nearly all cases indicated for initial surgery, and consists of a wider population compared with MLNS in the ACSOG Z0030 trial [8] and Okada's selective MLND [11]. Furthermore, the extent of our selective MLND is smaller than that of MLNS, because of its lobe-specific determination.

There is no doubt that a selective MLND was adequate as a curative operation for all p-N0 patients in the present study ($n = 599$). Since the 39 p-N1 patients had only metastasis to upstream hilar lymph node stations (proximal side to tumors) prior to the revised regional lymph node station classification, a selective MLND in association with a lobectomy can completely remove local cancer cells. Thus, 88 % (638/725) of the patients with c-T2N1M0 or less extensive disease would have benefitted from a selective MLND, while the 20 p-N1 and 65 p-N2 patients, who were diagnosed pathologically with metastasis to the revised regional lymph node stations, would have been navigated to undergo a MLND and also receive the benefits of a curative operation. The remaining two p-N2 patients, who had metastasis to downstream mediastinal lymph nodes (distal side from tumors) beyond the revised regional lymph node stations without metastasis, would have been navigated to a selective MLND, with a metastatic lymph node left in the mediastinum as a result. The false negative rate was 0.3 % (2/725), which is extremely low and acceptable in the clinical setting. One of the two false negative patients, one had a past history of lung tuberculosis in childhood and severe pleural adhesions were

recognized during the thoracotomy procedure. The findings in that case suggest that the normal lymph flow routes were changed because of the infection. The other false negative patient had a primary tumor in segment 8 and did not have any remarkable background factors.

We previously experienced two different errors in regard to the pathological diagnosis made using frozen sections during the surgery [6]. One was overlooked micro-metastasis in investigated regional lymph nodes, which is extremely difficult to diagnose using a frozen section technique. Thereafter, micro-metastasis was correctly diagnosed in that case by examining permanently fixed specimens. The other error was the wrong selection of a metastasized lymph node. In that case, a representative lymph node from each station considered to be most likely metastatic based on macroscopic findings was selected and studied for the pathological diagnosis, while the other lymph node in the same station had micro-metastasis, which was found by examining the permanently fixed specimen. Since these micro-metastasis areas were located within the area of the regional lymph node stations, we considered that the cancer cells could be completely removed. It is also important to note the risk of a lymph node located downstream of the regional lymph node stations that is macroscopically suspected to be metastatic during the surgery, and such nodes should be biopsied for a pathological diagnosis.

VPI is an indicator of the invasiveness and aggressiveness of NSCLC [21], and mediastinal lymph node involvement is often observed in affected cases [22, 23]. Two hypotheses have been proposed to explain the mechanism of mediastinal lymph node metastasis in VPI patients. One is the result of the desquamation of tumor cells within the pleural space, which are reabsorbed by lymphatic vessels of the parietal or diaphragmatic pleura. Such drainage involves the mediastinal lymph node stations, and theoretically induces skip N2 disease without N1 disease [24]. The other VPI tumor cell pathway is through the sub-pleural lymphatics, followed by the hilar lymph nodes and into the mediastinal lymph nodes. Such a pathway should result in less frequent skipping of N1 in VPI patients, because VPI tumor cells would travel through the hilar lymph nodes, in contrast to non-VPI patients [25]. In our series, contiguous (N1+) N2 disease was found in 56 % of the 46 pl0 patients, 29 % of the 17 pl1 patients and 100 % of the 4 pl2 patients, with no lymph node metastasis beyond the regional lymph node stations to the downstream mediastinal lymph node stations (distal side from tumors). Recently, Imai et al. [26] investigated the sub-pleural lymphatic flow to the mediastinum using indocyanine green with a near-infrared fluorescence imaging system in 17 NSCLC patients. They showed three cases with direct drainage to the mediastinum without passing through the hilar lymph nodes. All of their sentinel lymph nodes in the mediastinum belonged to our revised regional lymph node stations. Therefore, the lobe-specific revised regional lymph node stations can be applied to VPI cases as a method for patient navigation.

There are several limitations to this study. One is that it was a retrospective analysis. However, although the patient number was small, our previous study was performed prospectively [5, 6]. The second is that we used the UICC 6th edition for the TNM classification and Naruke lymph node mapping, where the definition of lymph node #10 was slightly different from that of the 7th edition. The lymph node station #10, newly defined as the regional lymph node station of the right upper lobe, is not located in the sub-carinal region, but on the cartilage of the right main bronchus, very close to the #10 lymph node station defined by UICC 7th edition. The third limitation is the small number of patients who underwent surgery for lung cancer in the left lingular division. We are now reviewing the cases of 160 patients with a tumor in the lingular division as part of a multi-institutional study. The preliminary results have shown that there were no false negative cases when the revised regional lymph node stations were applied for these patients.

Our preliminary study using the original regional lymph node stations demonstrated the efficacy of selective MLND for early stage NSCLC patients [6]. Thus, we consider that the present regional lymph node station revision has greater potential to accurately navigate patients to lymph node dissection during the surgery and may reduce lymph node dissection-related complications without lowering curability.

Conclusions

An intra-operative pathological examination using our newly revised LSRLNS may accurately diagnose the status of metastasis, and appropriately lead to a selective or complete MLND in patients with c-T2N1M0 or less extensive disease.

Conflicts of interest Shinichiro Miyoshi and the co-authors have no conflicts of interest to declare.

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Hereditary Lung Cancer Syndrome Targets Never Smokers with Germline *EGFR* Gene T790M Mutations

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Introduction: Hereditary lung cancer syndromes are rare, and T790M germline mutations of the epidermal growth factor receptor (*EGFR*) gene predispose to the development of lung cancer. The goal of this study was to determine the clinical features and smoking status of lung cancer cases and unaffected family members with this germline mutation and to estimate its incidence and penetrance.

Methods: We studied a family with germline T790M mutations over five generations (14 individuals) and combined our observations with data obtained from a literature search (15 individuals).

Results: T790M germline mutations occurred in approximately 1% of non–small-cell lung cancer cases and in less than one in 7500 subjects without lung cancer. Both sporadic and germline T790M mutations were predominantly adenocarcinomas, favored female gender, and were occasionally multifocal. Of lung cancer tumors arising in T790M germline mutation carriers, 73% contained a second activating *EGFR* gene mutation. Inheritance was dominant. The odds ratio that T790M germline carriers who are smokers will develop lung cancer compared with never smoker carriers was 0.31 ($p = 6.0E-05$). There was an overrepresentation of never smokers with lung cancer with this mutation compared with the general lung cancer population ($p = 7.4E-06$).

Conclusion: Germline T790M mutations result in a unique hereditary lung cancer syndrome that targets never smokers, with a preliminary estimate of 31% risk for lung cancer in never smoker carriers, and this risk may be lower for heavy smokers. The resultant cancers share several features and differences with lung cancers containing sporadic EGFR mutations.

Key Words: *EGFR* gene, Germline mutation, Hereditary lung cancer syndrome, Never smoker status, T790M mutation.

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Lung carcinomas that initially respond to tyrosine kinase inhibitors (TKIs) often harbor somatic gain-of-function mutations in the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) gene.[1,2] These mutations are more frequently associated with adenocarcinoma histology, female gender, East Asian ethnicity, and low or absent tobacco exposure.[3] Despite initial response to TKI therapy, the tumors usually recur or progress by development of acquired resistance.

The major mechanism of acquired resistance is the presence of a second *EGFR* point mutation T790M in exon 20.[4,5] T790M mutations usually occur with a common activating mutation, and they greatly increase the adenosince triphosphate (ATP)-binding affinity of the initial mutation.[4,6–9] T790M mutations may occur in treatment naïve tumors and cell lines,[10,11] and the mutant peaks in sequencing electropherograms are present in a minority of cells and may require sensitive detection methods. By contrast, the common activating mutations are often equal to or greater than the wild-type peak, a phenomenon we have termed as mutant allele-specific imbalance.[12] T790M germline mutations are present in 1% of lung cancers undergoing *EGFR* gene sequencing with the mutant allele occurring in about equal proportion to the wild-type allele.[13]

In this report, we describe the development of lung cancer in a young woman with a germline T790M mutation, and we combined an extensive study of her family pedigree with a review of the existing literature.

MATERIALS AND METHODS

Literature Search

We searched the PubMed database using combinations of the terms "Lung Neoplasms," "Receptor, Epidermal Growth Factor," "Germ-Line," and "T790M."

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Investigation of a Family with Germline T790M Mutation

Using an institutional review board–approved protocol and after informed consent, we were able to track a germline T790M mutation in the *EGFR* gene present in the proband and relevant history through five generations of her family. The family pedigree was constructed with the assistance of the proband and several family members. The smoking histories of deceased individuals were confirmed by at least two close relatives. Blood samples were obtained from 17 family members representing three generations. Genetic testing of the T790M mutation was performed by a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory utilizing *EGFR* exon 20-specific primers to sequence the region containing T790, using a standardized Sanger sequencing protocol (BigDye Terminator v3.1 Cycle Sequencing Kit Protocol, Applied Biosystems, Foster City, CA).

Detection of EGFR and Germline T790M Mutations in Japanese Patients

Paired tumor and nonmalignant lung samples from 629 Japanese patients with resected lung cancers were examined for *EGFR* mutations using previously published methods.[14–16] The study received local institutional review board permission and all patients gave informed consent for mutation testing. Testing was performed for therapy selection. Two patients were excluded because they had neuroendocrine tumors, leaving 627 patients with non–small-cell lung cancer (NSCLC).

RESULTS

Literature Search

A search of the PubMed database yielded five references for germline T790M mutations.[13,17–20]

The Proband

The proband was a 29-year-old woman with a total tobacco exposure of 0.1 pack-years. She presented with a 4.4-cm left upper lobe adenocarcinoma and biopsy-proven bilateral preneoplastic and preinvasive lesions (Supplemental Figure 2, Supplemental Digital Content, http://links.lww.com/JTO/A545). Analysis of tumor DNA for *EGFR* exons 18-21 revealed an L858R mutation in exon 21 (minor peak in comparison to the wild-type peak) and a T790M mutation (equivalent in height to the wild-type peak) (Figure 1). Mutation analysis of her blood mononuclear cells indicated a T790M mutation, with equivalent heights of the mutant and wild-type peaks, confirming the presence of a germline T790M mutation. No L858R mutation was detected in the mononuclear cells.

History and Mutation Status of Proband's Family

Information about the proband's family pedigree is presented in Figure 2 and Supplemental Table 1 (Supplemental Digital Content, http://links.lww.com/JTO/A545). Eight of 17 family members tested were positive for the mutation, including the proband's mother and brother. All family members tested received genetic counseling before testing and after the results were completed. Two of the family members were tested to establish lineage (IV:12, III:13) and were subsequently discarded from further analysis. Eight of 15 maternally related family members tested were mutation positive (53.3%), consistent for Mendelian inheritance of an autosomal gene, As documented in Figure 2, there are 14 family members who are tested, obligate, or assumed carriers of the T790M mutation. Four of these 14 (including the proband) developed lung cancer. CT scans, were available on five mutation carriers. All scans showed one or multiple small pulmonary lesions of uncertain diagnosis (Supplemental data, Supplemental Digital Content, http://links.lww.com/JTO/A545).

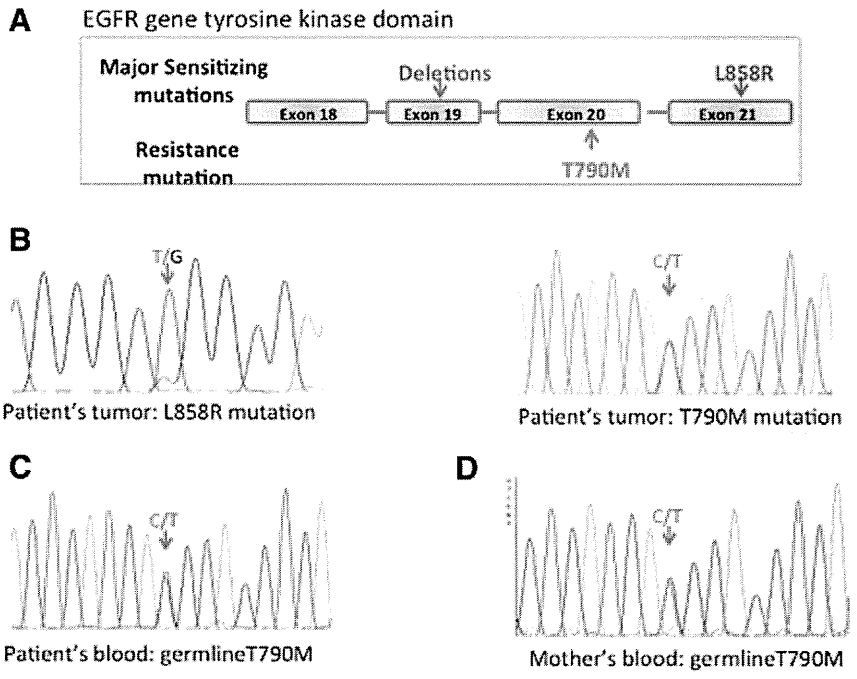

FIGURE 1. A cartoon of the location of the common mutations in the tyrosine kinase domain of the EGFR gene is shown in the upper part of the figure (*A*). Sequencing of the *EGFR* gene in the proband's adenocarcinoma revealed an activating mutation (L858R) in the *EGFR* gene (minor peak) (*B*) and a prominent T790M mutation equal in size to the wild-type peak (*B*). The T790M mutation substitutes methionine for threonine at position 790 (nucleotide c.2369). The finding in a lung cancer of a T790M mutant band equal in size to the wild-type band before tyrosine kinase inhibitor therapy suggests a germline mutation. Examination of the proband's blood cells confirmed a germline T790M mutation in the patient (*C*) and in her mother (*D*).

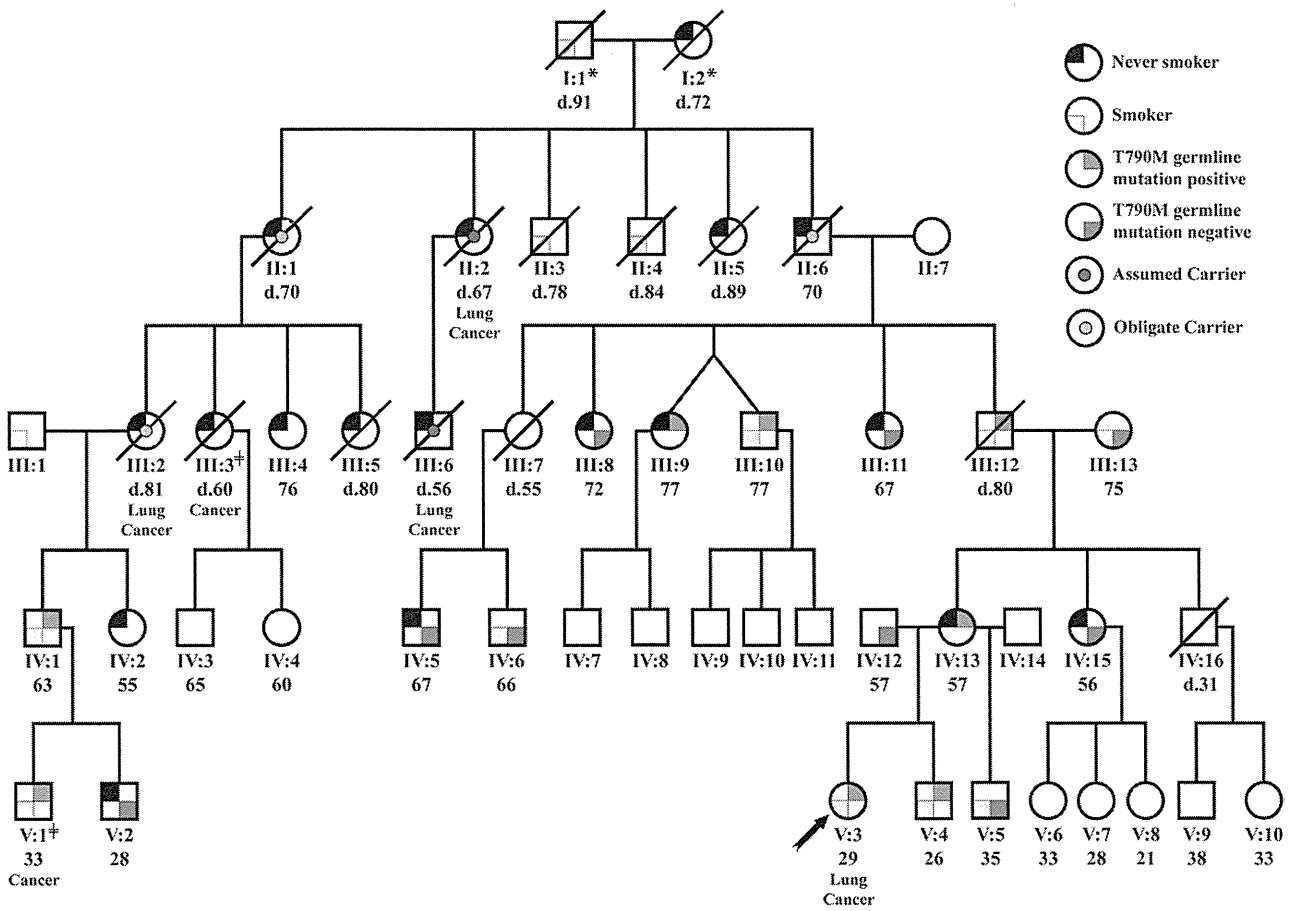

FIGURE 2. Pedigree of family with germline T790M mutation. Age, smoking history, mutation status, and other cancer history are recorded. *In addition to the three obligate carriers identified in the pedigree, either individual I:1 or I:2 is a obligate carrier based on pedigree analysis. Individual III:3 is reported to have died from bladder cancer. She also had lung cancer, but it is uncertain if this was a primary cancer or a metastasis. Individual V:1 had a lung carcinoid tumor. These two cases were not considered to be lung cancer cases. There are two never smokers who developed lung cancer in the family (II:2 and III:6), given the low likelihood that they represent two sporadic cases in this family we have assumed that they are also mutation carriers, thus for a total of 14 known, obligate, or assumed mutation carriers.

Germline T790M Mutations in Lung Cancer Cases and Controls

From our proband's family pedigree and the five reports in the literature, there are a total of 29 mutation carriers. Lung cancer developed in 19 of these carriers (referred to as lung cancer cases (Table 1). The remaining 10 T790M germline cases without lung cancer are referred to as controls. However, the gender and smoking status of some of the cases and controls from the literature are lacking or ambiguous. Thus, subgroup analysis contains varying number of cases and controls (Table 2). It should be noted that, as with other germline mutation analyses, some of the controls may ultimately develop cancer.

Characteristics of Lung Cancers Arising in T790M Germline Carriers

As demonstrated in Table 1, there are 19 cancer cases known to have arisen in germline carriers including our pedigree. All but one of the probands had a family history of lung cancer, and the germline mutation status of 11 was confirmed by sequencing, one was an obligate carrier, and seven family members are assumed to be carriers. In one case (case 8), both parents had lung cancer, and the parental inheritance could not be determined. Twelve of the 14 patients with known ethnicity were Caucasian and two were East Indian. Their ages ranged from 29 to 81, and there were 13 females, five males, and one of unknown gender. The median age of the cancer cases was about 63 years, with our proband at 29 years being the youngest case identified. Tumor histology was available for 14 patients, all of whom had NSCLC, and all of whom had one or more adenocarcinomas (case 9 also had a large cell neuroendocrine carcinoma). Three patients had multiple pathology-documented lung cancers, and three had documented invasive cancers and multiple bilateral nodules (biopsy proven to be microinvasive cancers in one of the cases).[21]

Of the 22 apparently individual tumors arising in 11 patients that were tested for EGFR gene mutations and

TABLE 1. Summary of Literature Regarding Lung Cancer Patients with Germline T790M Mutations and Family Member with Lung Cancer (Mutation Status Known and Assumed)

Reference	Case	Family	Other Family Members with Lung Cancer/ Relationship to Proband	Ethnicity	Age	Sex	Smoker	Tumor	T790M Germline Mutation	Second *EGFR* Gene Mutation	Comment
Bell et al.[21]	1	1	Proband	White	50	M	S	5 ADCs	Yes	5 tumors L858R in 2 of 5 delL747-T751 in 1 of 5	Multiple bilateral nodules
Bell et al.[21]	2	1	Yes, brother	White	55	M	?	ADC	Yes	G719A	Widespread metastases
Bell et al.[21]	3	1	Yes, mother	White	62	F	?	ADC	Mutation assumed	NA	
Bell et al.[21]	4	1	Yes, grandfather	White	72	M	?	ADC	Mutation assumed	NA	
Girard et al.[18]	5	2	Proband	East Indian	66	F	NS	ADC	Yes	L858R	Multiple bilateral nodules
Girard et al.[18]	6	2	Yes, father	East Indian	41	F	NS	NA	Mutation assumed	NA	
Girard et al.[18]	7	3	Proband	White	56	M	NS	ADC	Yes	L858R	Widespread metastases
Girard et al.[18]	8	3	Yes, either father or mother	White	72/80	M/F	S	N/A	Mutation assumed	NA	
Prudkin et al.[19]	9	4	Proband	?	72	F	NS	2 ADCs 1 LCNEC	Yes	None in 3 tumors	
Prudkin et al.[19]	10	4	Yes, sister	?	?	F	?	ADC	Mutation unknown	NA	
Oxnard et al.[14]	11–12	5, 6	One case had a sibling with cancer	?	44/73	F/M	NS/S	ADC	Yes	Both had exon 19 deletions	
Oxnard et al.[14]	13	7	No	?	44–73	F	NS	ADC	Yes	6 Nodules—4 had L858R, 2 exon 19 deletions	
Tibaldi et al.[20]	14	8	Proband	White	72	F	NS	ADC	Yes	Del; E746-A750 in exon 19	Bilateral pulmonary lesions
Tibaldi et al.[20]	15	8	Yes, sister	White	74	F	NS	NSCLC	Yes	None	
UTSW V-3	16	9	Proband	White	29	F	S	ADC	Yes	L858R	
UTSW II-2	17	9	Yes, great-great-aunt of proband	White	67	F	NS	NA	Mutation assumed	Unknown	
UTSW III-6	18	9	Yes, son of case 17	White	56	M	NS	NA	Mutation assumed	Unknown	
UTSW III-2	19	9	Yes, distant aunt of proband	White	81	F	NS	NA	Obligate carrier	Unknown	

Under "Family history of lung cancer," the number of family members with lung cancer is indicated in parentheses. The Oxnard reference only provides summary information about five germline mutation cases, two of which were previously reported by Girard et al., thus only recorded once. There is both a paternal and a maternal history of lung cancer in case 7 (Girard et al.) so the lineage is unknown; however, we would assume that one of the parents is a mutation carrier. Please see Supplemental data (Supplemental Digital Content, http://links.lww.com/JTO/A545) for discussion on potential problems with the report by Tibaldi et al.[20]

ADC, pulmonary adenocarcinoma; F, female; LCNEC, large cell neuroendocrine carcinoma; M, male; NA, not applicable; NS, never smoker; NSCLC, non–small-cell lung cancer; S, smoker; UTSW, University of Texas Southwestern Center.

16 (73%) had a second activating mutation in addition to the T790M germline mutation.

A logistic regression model was used to test the association between smoking status and lung cancer among the germline T790M carriers adjusting for sex, as there are more female cases whereas more males are represented in the mutation carriers that have not developed lung cancer (controls) (Table 2). Without adjusting for sex, the odds ratio (OR) for a 2-by-2 table can be calculated as ad/bc, which is $2\times4/(5\times11) = 0.15$. After taking gender into consideration, it became 0.31 (95% confidence interval: [0.04, 2.25]). Although this OR itself was not significant ($p = 0.34$), given the small sample size, it is significantly smaller ($p = 6.0E\text{-}05$ by a two-sample mean test) than the OR of 40.4 (95% confidence interval: [21.8, 79.6]) estimated from the general U.S. population.[22] There was an excess of never smokers (NSs) in lung cancer cases arising in germline T790M carriers. The proportions of smokers in all lung cancer cases were estimated to be 0.91 in males and 0.81 in females.[24] By contrast, only two out of 13 germline T790M derived cases (with both sex and smoking status known) were ever smokers