tissue and cell lines showed high cytoplasmic expression of caspase-3 and very low expression of cleaved caspase-3. We only found a positive correlation between AI and cleaved caspase-3 expression in mesothelioma tissue. Soini et al reported high expression of caspase-3 but no association was found between apoptotic index and caspase-3 immunoreactivity in mesothelioma[15]. They used an anti-caspase-3 antibody that detects both inactive 32 kD pro-enzyme and the active 17 kD fragment[15]. Our result using cleaved caspase-3 is more specific and, therefore, we found a positive correlation of AI to cleaved caspase-3 expression.

Mesothelioma tissue and cell lines showed high cytoplasmic expression of bax and low cytoplasmic expression of bcl-2. This result is similar to a previously published study of mesothelioma[16]. The previous report found no significant difference in bcl-2 mRNA expression between mesothelioma and normal pleural tissue[3], and suggested that apoptosis in mesothelioma has no direct relation to bax or bcl-2 expression. Survivin is the smallest protein among the IAPs[8], and it directly inhibits activation of caspase-3[8, 11]. In the present study, mesothelioma tissue and cell lines showed high cytoplasmic expression of survivin. The immunohistochemical expression of survivin is reinforced by mRNA expression by real time RT-PCR and protein expression by western blot. Falleni et al. have reported high expression of survivin mRNA in mesothelioma tissue compared to corresponding normal tissue[3].

In conclusion, apoptosis is an uncommon event in mesotheliomas. Although apoptosis-inducing proteins such as bax and caspase-3 were highly expressed in mesothelioma tissue and cell line, the expression of cleaved caspase-3 indicated that low activation of caspase-3 was responsible for the inhibition of apoptosis. Furthermore, high expression of survivin, a known inhibitor of caspases, may also play a role in the inhibition of apoptosis in mesothelioma.

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Association of *CYP19A1* polymorphisms with risks for atypical adenomatous hyperplasia and bronchioloalveolar carcinoma in the lungs

Takashi Kohno, Ryutaro Kakinuma[1], Motoki Iwasaki[2], Taiki Yamaji[2], Hideo Kunitoh[3,12], Kenji Suzuki[4,13], Yoko Shimada, Kouya Shiraishi, Yoshio Kasuga[5], Gerson Shigeaki Hamada[6], Koh Furuta[7], Koji Tsuta[8], Hiromi Sakamoto[9], Aya Kuchiba[9], Seiichiro Yamamoto[10], Yae Kanai[11], Shoichiro Tsugane[2] and Jun Yokota*

Biology Division, National Cancer Center Research Institute, Tokyo 104-0045, Japan, [1]Cancer Screening Technology and Development Division, National Cancer Center, Tokyo 104-0045, Japan, [2]Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045, Japan, [3]Thoracic Oncology Division and [4]Thoracic Surgery Division, National Cancer Center Hospital, Tokyo 104-0045, Japan, [5]Department of Surgery, Nagano Matsushiro General Hospital, Nagano 381-1231, Japan, [6]Nikkei Disease Prevention Center, São Paulo 04122-000, Brazil, [7]Clinical Support Laboratory and [8]Clinical Laboratory Division, National Cancer Center Hospital, Tokyo 104-0045, Japan, [9]Genetics Division, National Cancer Center Research Institute, Tokyo 104-0045, Japan, [10]Cancer Information Services and Surveillance Division, Center for Cancer Control and Information Services, National Cancer Center, Tokyo 104-0045, Japan and [11]Pathology Division, National Cancer Center Research Institute, Tokyo 104-0045, Japan

[12]Present address: Department of Respiratory Medicine, Mitsui Memorial Hospital, Kanda-Izumicho 1, Chiyoda-ku, Tokyo 101-8643, Japan

[13]Present address: Department of General Thoracic Surgery, Juntendo University School of Medicine, Hongo 3-1-3, Bunkyo-ku, Tokyo 113-8431, Japan

*To whom correspondence should be addressed. Tel: +81 3 3547 5272; Fax: +81 3 3542 0807;
Email: jyokota@ncc.go.jp

Estrogen has been indicated to play an etiological role in the development of lung adenocarcinoma (ADC), particularly bronchioloalveolar carcinoma (BAC), a type of ADC that develops from a benign adenomatous lesion, atypical adenomatous hyperplasia (AAH). Polymorphisms in the *CYP19A1* gene cause interindividual differences in estrogen levels. Here, 13 *CYP19A1* single-nucleotide polymorphisms (SNPs) were examined for associations with lung AAH risk. AAH is detected as ground-glass opacity (GGO) by computed tomography (CT) examination, and this study consisted of 100 individuals diagnosed with GGO in their lungs among 3088 CT-based cancer screening examinees and 424 without. Minor allele carriers for the rs3764221 SNP showed an elevated risk for GGO [odds ratio (OR) = 1.72, $P = 0.017$]. Associations of this SNP with risks for lung AAH and BAC in the lungs were next examined using 359 ADC cases whose resected lung lobes were subjected to a histological examination for AAH accompaniment and the presence of BAC components and 330 controls without cancer. The ORs were also increased for lung ADC accompanied by AAH (OR = 1.74, $P = 0.029$) as well as lung ADC with BAC components (OR = 1.41, $P = 0.091$). The minor allele was associated with an increased circulating estradiol level ($P = 0.079$) in a population of 363 postmenopausal women without cancer. These results indicate that *CYP19A1* polymorphisms are involved in the risk for lung AAH and BAC in the lungs by causing differences in estrogen levels.

Abbreviations: AAH, atypical adenomatous hyperplasia; ADC, adenocarcinoma; BAC, bronchioloalveolar carcinoma; CI, confidence interval; CT, computed tomography; ER, estrogen receptor; GGO, ground-glass opacity; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; OR, odds ratio; SNP, single-nucleotide polymorphism; SQC, squamous cell carcinoma.

Introduction

Adenocarcinoma (ADC) is the commonest histological type of lung cancer, comprising ~40% of lung cancer cases, among European, North American and Asian countries and is increasing in incidence (1). Development of ADC is more weakly associated with smoking than those of two other major histological types of lung cancer, squamous cell carcinoma (SQC) and small-cell lung carcinoma. Thus, effective ways of preventing ADC are being searched for. Recent studies indicate that estrogen plays a role in the growth of lung ADC cells (2,3). Estrogen receptor (ER) β is expressed in bronchiolar epithelial cells (4). ERβ expression was detected in >75% of lung ADC being more frequent than SQC and small-cell lung carcinoma, and the expression was preferentially observed in bronchioloalveolar carcinoma (BAC) (4), a differentiated type of lung ADC developed in the peripheral lung (5). ERβ expression was also detected in atypical adenomatous hyperplasia (AAH) (4), a possible precancerous lesion for BAC (6). Growth of lung ADC cells with ERβ expression was enhanced by estrogen, whereas it was suppressed by antagonizing estrogen (2,4). Therefore, estrogen is probably to play an essential role in the growth of lung ADC cells. In fact, an ER antagonist, fulvestrant, is being examined for its utility in the treatment of lung ADC (7).

Estrogen treatment significantly increased the development of adenoma and ADC in the lungs of ovariectomized female and male mice, therefore, estrogen is a risk factor for the development of lung ADC in mice (8). In a cohort study of 44 667 lifelong never-smoking women in Japan, women of either early age menarche or late age menopause showed significant increase in the risk for lung cancer, and involvement of the use of hormone replacement therapy in the risk for lung cancer of postmenopausal women was also suggested (9). Since ADC comprised >85% of lung cancer cases in this study, estrogen is a candidate risk factor for lung ADC also in the human. However, the involvement of endogenous and exogenous estrogen in the etiology of lung cancer of women has been inconsistent in other populations (10–18). In addition, the significance of estrogen on lung cancer risk of men has not been reported to our knowledge, although men have similar levels of circulating estrogen to postmenopausal women (19) and ERβ expression was detected in lung ADC both of men and women (4,20). Therefore, estrogen is a possible target for prevention of lung ADC, and the significance of estrogen on its etiology should be further investigated.

Polymorphisms in genes involved in estrogen metabolism have been suggested to be associated with circulating estrogen levels (19). Particularly, polymorphisms in the *CYP19A1* gene, encoding an aromatase responsible for the final step in the biosynthesis of estrogens, estradiol (E2) and estrone (E1) (21), have been most intensively investigated (22). A tandem repeat polymorphism, (TTTA)n, in intron 4 and a single-nucleotide polymorphism (SNP), rs10046, in the 3′-untranslated region of exon 10 were reported to be associated with circulating estrogen levels in postmenopausal women (23,24). The tandem repeat polymorphism was also associated with circulating estrogen levels in men (25). Recently, by a large-scale association study, in which >3000 postmenopausal women of European descent were analyzed for 103 SNPs dispersed in the *CYP19A1* gene, SNPs located in the 3′ region (i.e. exons 2–10) of the *CYP19A1* gene, such as rs10046 and four other SNPs (marked by blue lines in Figure 1), were defined as most strongly associated with serum E2 and E1 levels (26). Therefore, it was indicated that polymorphisms in the 3′ region of the *CYP19A1* gene are responsible for interindividual differences in circulating estrogen levels. On the other hand, in a recent association study involving 1068 men from Sweden and 2568 men from the USA, SNPs in the 3′ region of the *CYP19A1* gene, including rs10046, also showed associations with serum E2 and E1 levels in men (19).

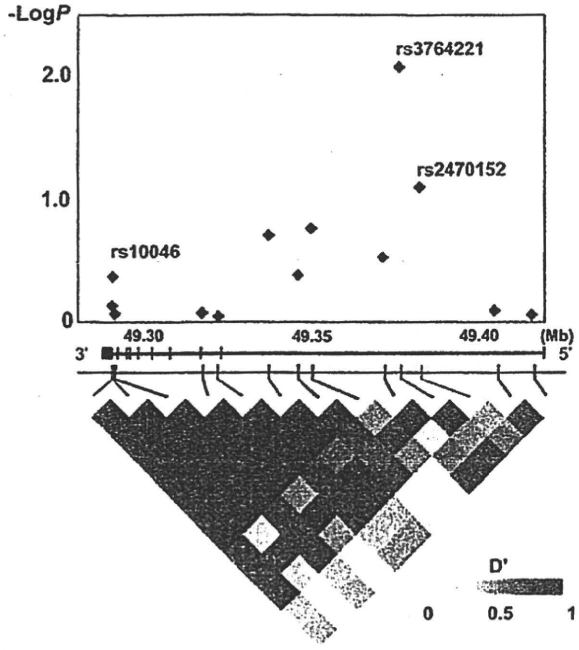

Fig. 1. Association of 13 SNPs in the *CYP19A1* gene with GGO risk. The top panel shows association results by trend test for SNPs and the location of SNPs. Black lozenges depict the results for risks for GGO. The bottom panel shows the LD structure in 424 control subjects. Boxes are shaded according to the pair-wise *D'* values.

However, the rs2470152 SNP located in intron 1 of the *CYP19A1* gene (marked by a green line in Figure 1) showed a stronger association than the SNPs in the 3′ region (19). Therefore, it was indicated that polymorphisms in intron 1 of the *CYP19A1* gene also affect the estrogen levels. Since the rs2470152 SNP was not examined in the association study above in postmenopausal women (26), polymorphisms responsible for estrogen levels remain unclear. However, these studies strongly indicate that *CYP19A1* polymorphisms are a critical determinant of interindividual differences in the serum estrogen levels both in men and women.

We investigated here the significance of *CYP19A1* SNPs on risks for AAH and ADC by conducting four independent association studies to further obtain information on estrogen in the etiology of lung ADC. (i) AAH in the lungs is detected as a ground-glass opacity (GGO) by helical computed tomography (CT) examination (6,27–30). Therefore, the first study was to examine association of *CYP19A1* SNPs with GGO risk in the lungs among examinees admitted to a single cancer screening center. This study consisted of 100 cancer screening examinees diagnosed with GGOs by a thin-section (high resolution) CT examination among 3088 examinees and 424 examinees without GGO who were matched to the GGO cases in sex and age categories. (ii) AAH is an incidental histologic finding detected in 16–35% of lungs bearing primary lung ADC (6,31). Therefore, the second study was to examine association of *CYP19A1* SNPs with the risk for ADC accompanied with AAH(s) in the lungs among patients admitted to hospital. This study consisted of 81 cases diagnosed with lung ADC accompanied with AAH(s) among 359 lung ADC cases who received lobectomy followed by a histological examination of resected lobes serially sliced at intervals of 5 mm and 330 controls without cancer. (iii) AAH has been considered as a precancerous lesion that particularly develops to BAC, a type of ADC. Therefore, the third study was to examine association with risk for BAC. This study consisted of 151 cases diagnosed with lung ADC containing BAC components among 172 cases diagnosed with small-sized ADC, which include BAC as the majority, and 330 controls without cancer.

Table I. Study subjects

Set	Subject	Category	All	Male (%)	Age (mean ± SD)	Smoker (%)
GGO	Case		100	45 (45)	57 ± 9	37 (37)
	Control		424	194 (46)	57 ± 9	197 (46)
Lung ADC	Case	All	359	193 (54)	59 ± 9	187 (52)
		AAH accompaniment				
		Present	81	39 (48)	60 ± 7	42 (52)
		Absent	278	154 (55)	58 ± 9	145 (52)
		BAC components				
		Present	151	70 (46)	59 ± 8	66 (44)
		Absent	21	11 (52)	61 ± 11	8 (38)
	Control		330	186 (56)	62 ± 11	154 (47)

(iv) Finally, *CYP19A1* SNPs that were associated with GGO, AAH and BAC risks were examined for association with circulating estrogen levels of 363 postmenopausal women without cancer.

Subjects and methods

Subjects for association study on GGO risk

Study subjects were Japanese and consisted of examinees who underwent helical CT examination of the lungs from 2005–07 as a cancer screening program provided by the Research Center for Cancer Prevention and Screening of the National Cancer Center, Japan. Details of the screening program have been described elsewhere (32). All examinees gave written informed consent to allow their data and materials collected through the screening program to be used for the purpose of medical research. The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan. Eligible examinees were individuals who underwent helical CT examination of the lungs. Details of the CT screening method were described previously (33). Examinees diagnosed with lung cancer or with a history of malignancies were considered ineligible. In a consecutive series of 3088 examinees aged from 40 to 79, 2822 fulfilled the necessary conditions above. One hundred and five examinees were defined as GGO cases because they had at least one GGO ≥5 mm in diameter by a screening CT examination followed by validation by a thin-section (high resolution) CT examination. Four hundred and forty examinees were chosen as control subjects from examinees without GGO by frequency matching to these GGO cases in sex and four age categories (ages 40–49, 50–59, 60–69 and 70–79 years). Genomic DNAs were available for 100 cases and 424 controls of these subjects for this study (the GGO set, Table I). One hundred and two examinees were chosen by a simple random sampling method from 512 examinees diagnosed as having at least one GGO <5 mm in diameter by screening and/or high-resolution CT examinations and were examined as a population containing GGO cases as a subset.

Before undergoing the screening program, examinees completed a self-administered questionnaire concerning medical history and lifestyle characteristics, including smoking habit. The composition of the questionnaire has been detailed elsewhere (32,34). The questionnaire inquired about smoking habits by first determining smoking status (current, former and never) and then expressing lifetime exposure to cigarette smoking among current- and former-smokers by pack-years, with one pack-year defined as the smoking of 20 cigarettes every day for 1 year. Both current- and former-smokers were expressed as smokers in this study.

Subjects for association study on lung ADC risk

All 359 cases and 330 controls were Japanese and were admissions to the National Cancer Center Hospital from 1999 to 2004. Cases were admissions who were diagnosed with lung ADC by histological examinations according to World Health Organization classification (5) and received lobectomies at National Cancer Center Hospital. Controls were admissions who were not diagnosed with lung cancers and had no history of cancers (the lung ADC set, Table I). They were individuals who had been suspected to carry lung or gastric cancer in other hospitals and were not diagnosed with these cancers in National Cancer Center Hospital by CT, endoscope examinations, etc. All cases and controls, from whom informed consent as well as blood samples were obtained, were consecutively included in this study without any exclusion criteria. The participation rate was nearly 80%. From each individual, a 20 ml whole-blood sample was obtained.

All 359 ADC cases were subjected to pathological search for AAH in the resected lobes as described (35). Briefly, resected lungs were inflated with 10%

formalin through bronchial cut ends, and after fixation for a few days were serially sliced at intervals of 5 mm, and each cut surface was macroscopically examined. Sliced lungs containing a lesion(s) suspected for AAH were further examined microscopically. Even in cases without macroscopic lesions, at least one tissue block was prepared from all sliced lungs and subjected to microscopic examination. The criteria for AAH were as follows and as described previously (36,37): (i) a localized lesion with well-defined boundaries; (ii) an alveolar wall slightly thickened with mild infiltration of inflammatory cells but without scar formation; (iii) proliferating atypical epithelial cells abutting each other but not as compact as in ADC; (iv) atypical epithelial cells that were cuboidal to low columnar or peg-shaped in appearance, resembling either type II pneumocytes or non-ciliated bronchiolar epithelial cells (Clara cells) and (v) the presence of some atypical cells with two or more nuclei, most of which had relatively smaller and smoother contours than those of ADC. These criteria are compatible with those described in the reference of World Health Organization classification of lung tumors as a proposal (6). In the lobes, AAH lesions were detected in 81 cases (23%), whereas no AAH lesion was detected in the remaining 278 cases (77%) (Table I). The 359 ADC cases included 172 cases of small-sized ADC (i.e. <2 cm in maximum diameter), and the information on the presence of BAC components in the tumor was available (Table I). One hundred and fifty-one cases (87%) contained BAC components in the tumor, whereas the remaining 21 cases (13%) did not.

The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan. Smoking histories of the case and control subjects were obtained via interview using a questionnaire. The definitions of never-smokers and smokers are described above.

Subjects for association with estrogen levels

Postmenopausal women who participated as controls in multicenter hospital-based case–control studies of breast cancer (38–40) were analyzed in the present study. This study was designed to determine lifestyle factors and genetic susceptibility to the risk for breast cancer and to compare potential risk factors among Japanese living in Nagano, Japan and Japanese Brazilians and non-Japanese Brazilians living in São Paulo, Brazil. Written informed consent was obtained from all these subjects. This study was approved by Comissão Nacional de Ética em Pesquisa (CONEP, National Committee of Ethics in Research), Brasília, Brazil and by the institutional review board of the National Cancer Center, Tokyo, Japan.

Estrogen (E2 and E1) levels in serum for Nagano and in plasma for São Paulo were determined by radioimmunoassay by Mitsubishi Kagaku Bio-Clinical Laboratories (Tokyo, Japan). Both the hormone levels and genomic DNA from peripheral blood cells of 185 Japanese, 44 Japanese Brazilians and 134 non-Japanese Brazilians were available for the present study.

SNP analysis

Genomic DNA was extracted from whole-blood cells using a Blood Maxi Kit (QIAGEN, Tokyo, Japan) according to the supplier's instructions. Thirteen SNPs located in the *CYP19A1* gene were selected. Five SNPs, rs4646, rs10046, rs2414096, rs727479 and rs1008805, were chosen since significant associations with serum estrogen levels of postmenopausal women were reported (26). rs2470152 was chosen since a significant association with serum estrogen levels of men was reported (19). The other seven SNPs were chosen based on the fact that their minor allele frequencies in the Japanese population were >0.1 in the GEMDBJ SNP database (https://gemdbj.nibio.go.jp/dgdb/). Genotyping of GGO set subjects for six SNPs, rs4646, rs10046, rs2414096, rs727479, rs1008805 and rs3764221 was performed by the Goldengate assay (Illumina, San Diego, CA) and that for the remaining seven SNPs was performed by the Taqman assay (Applied Biosystems, Foster City, CA) according to the supplier's instructions. Genotyping of lung ADC set subjects for the rs3764221 SNP and genotyping of the subjects for association of the rs3764221 and rs10046 SNPs with serum estrogen levels was performed by the Taqman assay.

Statistical analyses

A Hardy–Weinberg equilibrium (HWE) test was performed using the SNPAlyze version 3 software (DYNACOM, Chiba, Japan), and SNPs with a P value for deviation >0.05 were considered to be in HWE. Calculation of the D' and R^2 values between SNPs was performed by the expectation–maximization algorithm using the SNPAlyze version 3 software.

Associations of 13 SNPs in the *CYP19A1* gene with GGO risk were examined by a trend test adjusted for gender, age (≤49, 50–59, 60–69 and ≥70) and smoking (never-smoker versus smoker). Associations of the rs3764221 SNP with GGO and ADC risks were digitized as odds ratios (ORs) adjusted for gender, age (-49, 50–59, 60-69 and 70-) and smoking (never-smoker versus smoker) with 95% confidence intervals (CIs) by unconditional logistic regression analysis (41). ORs for ADC risk according to the accompaniment of AAH were assessed by the multinomial logistic regression model. These analyses were performed using the JMP version 6.0 software (SAS Institute, Cary, NC). Linear trends for estrogen levels according to increases in the number of minor alleles for the rs3764221 and rs10046 SNPs were tested in a multivariate regression model using SAS software version 9.1 (SAS Institute). Variables used for adjustment in each test are described in the footnotes to Tables II and III. A level of $P < 0.05$ in a test was judged as significant and that of $0.05 \leq P < 0.1$ was judged as marginal.

Results

Association of a CYP19 SNP with lung GGO risk

Thirteen SNPs dispersed in the *CYP19A1* gene region were examined for association with GGO risk in a case–control study that consisted of 100 examinees with GGO and 424 without (GGO set in Table I). All 13 SNPs were in HWE both in cases and controls. Significant association with GGO risk was observed for an SNP, rs3764221, located in intron 1 of the *CYP19A1* gene (P by trend test = 0.0085) (Figure 1; supplementary Table I is available at *Carcinogenesis* Online).

Five SNPs associated with estrogen levels in postmenopausal women of European descent (indicated by blue lines in Figure 1) were in strong linkage disequilibrium (LD) with each other (D' = 0.85–1.0) as reported (26). These five SNPs also showed LD with rs3764221 (D' = 0.75–0.92), however, none of them showed significant associations with GGO risk (supplementary Table I is available at *Carcinogenesis* Online). The rs2470152 SNP associated with estrogen levels in men from Sweden and the USA (indicated by a green line in Figure 1) were in a complete LD (D' = 1.0) with rs3764221, and this SNP showed a marginal association (P = 0.076) with GGO risk (supplementary Table I is available at *Carcinogenesis* Online).

Heterozygotes and homozygotes for the minor allele of the rs3764221 SNP showed increased ORs for the GGO risk (Table II), and the increase in the homozygotes was statistically significant. The OR in the dominant mode (C/T + T/T versus C/C) also

Table II. Association of CYP19A1 (rs3764221) genotypes with lung ADC risk

Category	Genotype	Control, *N* (%)	Case, *N* (%)	OR[a] (95% CI)	*P*
GGO	C/C	262 (62)	47 (47)	Reference	
	C/T	138 (33)	42 (42)	1.59 (0.99–2.56)	0.057
	T/T	24 (6)	11 (11)	2.47 (1.09–5.28)	0.030
	Dominant			1.72 (1.10–2.70)	0.017
	Recessive			2.03 (0.92–4.23)	0.077
ADC	C/C	187 (57)	184 (51)	Reference	
	C/T	123 (37)	145 (40)	1.21 (0.88–1.67)	0.24
	T/T	20 (6)	30 (8)	1.47 (0.80–2.77)	0.22
	Dominant			1.25 (0.92–1.70)	0.16
	Recessive			1.37 (0.76–2.53)	0.30
AAH accompaniment[b]					
Present	C/C		35 (43)	Reference	
	C/T		38 (47)	1.69 (1.01–2.85)	0.047
	T/T		8 (10)	2.05 (0.79–4.93)	0.12
	Dominant			1.74 (1.06–2.86)	0.029
	Recessive			1.66 (0.66–3.82)	0.26
Absent	C/C		149 (54)	Reference	
	C/T		107 (38)	1.10 (0.78–1.55)	0.60
	T/T		22 (8)	1.33 (0.68–2.60)	0.40
	Dominant			1.13 (0.81–1.57)	0.46
	Recessive			1.29 (0.68–2.47)	0.44
BAC components					
Present	C/C		72 (48)	Reference	
	C/T		64 (42)	1.34 (0.88–2.03)	0.17
	T/T		15 (10)	1.87 (0.88–3.90)	0.10
	Dominant			1.41 (0.95–2.09)	0.091
	Recessive			1.65 (0.80–3.35)	0.17

[a]Adjusted for age, sex and smoking.
[b]ORs according to the accompaniment of AAH were assessed by the multinomial logistic regression model.

showed a statistically significant increase [OR = 1.72 (1.10–2.70) $P = 0.017$] (Table II; supplementary Figure 1 is available at *Carcinogenesis* Online). The OR in the dominant mode was also calculated against 102 examinees with GGO < 5 mm in diameter by screening and/or high-resolution CT examinations. An increase in OR in the dominant mode was also observed [OR = 1.42 (0.90–2.23)]; however, the increase did not reach a statistical significance ($P = 0.13$).

Association of a CYP19 SNP with lung ADC risk

Association of the rs3764221 SNP with lung ADC risk was examined in a case–control study consisting of 359 lung ADC cases and 330 controls (Lung ADC set in Table I). This SNP was in HWE both in cases and controls. ORs of heterozygotes and homozygotes for the minor allele and those in both the dominant and recessive modes for the lung ADC risk were increased; however, the increases were not statistically significant (Table II; supplementary Figure 1 is available at *Carcinogenesis* Online).

All 359 lung ADC cases were informative for the presence of AAH in the lung lobe with primary ADC (Table I). Eighty-one (23%) cases had AAHs with primary ADC, consistent with previous reports that AAHs were detected in 16–35% of lungs with primary ADC (6,31). The ORs of heterozygotes and homozygotes for the minor allele and those in the dominant and recessive modes were higher for the risk for ADC with AAH than for ADC without AAH, although their 95% CIs overlapped (Table II; supplementary Figure 1 is available at *Carcinogenesis* Online). ORs of hetrozygotes and in the dominant mode for the risk for ADC with AAH were statistically significant.

Among the 359 cases, 172 cases had small-sized ADC (i.e. <2cm in maximum diameter) and were informative whether their tumors contained BAC components or not (Table I). Tumors of 151 cases were diagnosed as containing BAC components. The ORs of heterozygotes and homozygotes for the minor allele and those in the dominant and recessive modes were higher for ADC with BAC components than for overall ADC, although their 95% CIs overlapped (Table II; supplementary Figure 1 is available at *Carcinogenesis* Online). ORs in the dominant mode for the risk for ADC with BAC components were marginally significant. The number of ADC cases without BAC components was small; therefore, ORs for ADC without BAC components were not calculated.

Association of the rs3764221 SNP with estrogen level

Association of the rs3764221 SNP with GGO and ADC risks prompted us to examine whether this SNP is associated with estrogen levels or not. For this purpose, we examined the allele distribution of this SNP in 363 postmenopausal women, consisting of 185 Japanese, 44 Japanese Brazilians and 134 non-Japanese Brazilians, whose information on circulating E2 and E1 levels was available (38–40). We also examined the allele distribution of the rs10046 SNP because the E2 and E1 levels in heterozygotes and homozygotes for the minor allele had been shown previously to be significantly higher than those in major allele homozygotes (Caucasian in Table III) (26). Heterozygotes and homozygotes for the minor allele for the rs3764221 SNP in all subjects showed higher E2 and E1 levels as for rs10046 in the previous report (Table III) (26). The increase in the E2 level according to increases in the number of minor alleles in all subjects was marginally significant ($P = 0.078$), whereas that in the E1 level was not significant. Heterozygotes and homozygotes for Japanese subjects also showed higher E2 and E1 levels, although the differences were not statistically significant. On the other hand, heterozygotes and homozygotes for the minor allele for the rs10046 SNP showed only slightly increased levels of E1 and E2 in this study population.

Discussion

In this study, the rs3764221 SNP in the *CYP19A1* gene was shown to be associated with risk for GGO (Table II). AAHs are usually detected as GGOs by CT examinations and a subset of these AAHs progress to ADC, including BAC (28,30,42). Therefore, this SNP was suggested to be involved in the risk for the development of AAH and also of lung ADC, particularly of BAC in the lungs. This suggestion was supported by the following two findings. First, the rs3764221 SNP showed a significant association with the risk for ADC accompanied by AAH but not for ADC not accompanied by AAH (Table II). Second, this SNP showed a marginal association with the risk for ADC containing BAC components, and the association in this subset of ADC was more evident than that in overall lung ADC (Table II). This result is consistent with the concept that AAH is a precancerous lesion of ADC, preferentially of BAC (5,6,43). The frequency of having AAH in the lungs has been shown to be considerably higher in ADC patients than in individuals without cancer (6,31,36,37,44). Therefore, the susceptibility to the development of AAH is probably to be associated with that of ADC in the lungs. Thus, the rs3764221 SNP might confer lung ADC risk by affecting the susceptibility to the development of AAH that progress to ADC, preferentially BAC.

In the present study, the minor allele for the rs3764221 SNP was marginally associated with a higher estrogen level in postmenopausal women. Notably, rs3764221 was in complete LD ($D' = 1$) with rs2470152, whose association with serum estrogen levels in men had been reported (19). Accordingly, the rs2470152 SNP also showed a marginally significant association with risk for GGO (Figure 1).

Table III. Association of CYP19A1 SNPs with circulating estrogen levels SNP

	Population	Genotype	No. of subjects	Increase in estradiol (E2)	*P* for trend	Increase in estrone (E1)	*P* for trend
rs3764221	All	CC	220	Ref	0.078[a]	Ref	0.26[a]
		CT	120	+4.8%		+1.0%	
		TT	17	+16.0%		+13.4%	
	Japanese	CC	86	Ref	0.11[a]	Ref	0.30[a]
		CT	86	+6.6%		+1.2%	
		TT	12	+17.1%		+17.0%	
rs10046	All	GG	116	Ref	0.92[a]	Ref	0.36[a]
		GA	193	+0.04%		+1.1%	
		AA	54	+0.69%		+5.3%	
	Japanese	GG	61	Ref	0.83[a]	Ref	0.43[a]
		GA	93	+0.8%		+2.5%	
		AA	31	−2.3%		+6.1%	
	Caucasian[b]	GG	835	Ref	2.9×10^{-9}	Ref	1.1×10^{-8}
		GA	1691	+5.7%		+5.4%	
		AA	799	+12.8%		+11.7%	

[a]Adjusted for age, ethnic group, age at menarche, age at menopause, number of births, age at first birth, height, body mass index, smoking, alcohol drinking and physical activity in the past 5 years.
[b]Data from Haimann *et al.* (26).

Interestingly, intron 1 of the *CYP19A1* gene contains 10 tissue specific promoters, which have been indicated to play regulatory roles in *CYP19A1* gene expression differentially among diverse tissues (21,22). rs2470152 and rs3764221 SNPs are located, respectively, in and 3′ to the I.4 promoter, which enables *CYP19A1* expression in skin, testis and adipose tissues (21,45,46). Therefore, genetic variations in the region spanning these two SNPs might be responsible for differential *CYP19A1* expression among individuals, and this might cause interindividual differences in estrogen levels. In contrast to previous reports (26), the rs10046 SNP did not show association with estrogen levels in the present study. Such an inconsistency might have come from ethnic differences of subjects examined. Since the minor allele frequency for the3764221 SNP is considerably lower in Europeans (<0.05) than in Asians (>0.2) (http://www.ncbi.nlm.nih.gov/projects/SNP/), this SNP was not examined in previous association studies of Europeans (19,26). The rs3764221 SNP is in LD with SNPs located in the 3′ region of the *CYP19A1* gene, including rs10046, therefore, it is also possible that SNP(s) critical for estrogen levels is located in this 3′ region.

Interaction of *CYP19A1* genotypes with smoking and gender was also investigated. The ORs for the risks for GGO and lung ADC were consistently higher in never-smokers than in smokers, although their 95% CIs overlapped (supplementary Table II is available at *Carcinogenesis* Online). This result went along with the result of meta-analysis showing that hormone replacement therapy particularly increases lung ADC risk of never-smokers (10). This stronger association of *CYP19A1* genotypes with GGO and lung ADC risks in never-smokers than smokers might be due to the anti-estrogenic effect of smoking (47,48). Smoking has been indicated to be associated with low levels of estrogen and with decreased risks for estrogen-dependent cancers, such as endometrial cancers (49–51). On the other hand, risks for GGO and lung ADC were not consistently associated with gender (supplementary Table III is available at *Carcinogenesis* Online); therefore, the interaction of *CYP19A1* genotypes with gender remains unclear.

The present study proposes that *CYP19A1* polymorphisms are involved in the risk for AAH and BAC in the lungs by causing differences in estrogen levels. Association studies of a single population among *CYP19A1* genotypes, estrogen levels and the risk for AAH and BAC, by taking gender and smoking into account, will further authenticate the present results. The contribution of *CYP19A1* polymorphisms to cancer risks has been investigated in estrogen-dependent cancers, such as ADCs of breast and endometrium. The contribution has been indicated to be possible but remains inconclusive due to inconsistent results among studies (22,26,52). Studies of *CYP19A1* polymorphisms on risks for ADCs of a variety of organs, including the lungs, breast and endometrium, will further elucidate the significance of these polymorphisms and estrogen levels on cancer risks.

Supplementary material

Supplementary Figure 1 and Tables I–III can be found at http://carcin.oxfordjournals.org/

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The adenocarcinoma-specific stage shift in the Anti-lung Cancer Association project: Significance of repeated screening for lung cancer for more than 5 years with low-dose helical computed tomography in a high-risk cohort☆

Nobuhiko Seki[a,*], Kenji Eguchi[a], Masahiro Kaneko[b], Hironobu Ohmatsu[c], Ryutaro Kakinuma[d], Eisuke Matsui[e], Masahiko Kusumoto[f], Takaaki Tsuchida[b], Hiroyuki Nishiyama[g], Noriyuki Moriyama[d]

[a] Division of Medical Oncology, Department of Internal Medicine, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan
[b] Division of Endoscopy, National Cancer Center Hospital, Tokyo, Japan
[c] Division of Thoracic Oncology, National Cancer Center Hospital East, Chiba, Japan
[d] Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan
[e] Anti-lung Cancer Association, Tokyo, Japan
[f] Diagnostic Radiology, National Cancer Center Hospital, Tokyo, Japan
[g] Division of Thoracic Surgery, Social Health Insurance Medical Center, Tokyo, Japan

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ABSTRACT

Background: We investigated whether a stage shift occurs during long-term repeated screening for lung cancer with low-dose helical computed tomography (LDCT) in a high-risk cohort.
Methods: A total of 2120 subjects (mean age, 63 years; 87% male and 83% smokers) were continuously recruited and underwent repeated screening with LDCT from 1993 through 2004.
Results: Nineteen lung cancers were detected at baseline examinations (prevalence cancers), and 57 lung cancers were detected at subsequent examinations (incidence cancers). For both prevalence cancers and incidence cancers, adenocarcinoma (74% and 63%, respectively), especially invasive adenocarcinoma (42% and 23%, respectively), was the most common histological diagnosis, and stage IA was the most common pathological stage (58% and 79%, respectively). The detection rate of incidence cancers other than bronchioloalveolar carcinoma became significantly higher after 5 years of LDCT examinations ($r=0.50$, $P=0.020$). Moreover, both the percentage of cancers of stage II–IV and tumor size became significantly lower for invasive adenocarcinoma after 5 years of LDCT examinations ($r=-0.77$, $P=0.007$ and $r=-0.60$, $P=0.029$, respectively).
Conclusions: Repeated screening for more than 5 years might demonstrate the efficacy of LDCT screening for lung cancer through an adenocarcinoma-specific stage shift.

1. Introduction

Lung cancer is considered as an appropriate disease for screening because it is the leading cause of cancer death worldwide, symptomatic disease is generally lethal, localized disease can be managed curatively, and high-risk cohorts can be defined on the basis of tobacco consumption [1]. However, screening with chest X-ray films or sputum cytological examination has failed to reduce lung-cancer mortality rates in randomized, controlled trials [2–6].

Low-dose helical computed tomography (LDCT) is a promising screening method because a higher percentage of asymptomatic, X-ray–invisible, or stage IA lung cancers (mostly adenocarcinoma) are found with baseline or repeated computed tomography (CT) examinations than with conventional screening methods [7–11]. In fact, according to the results of the International Early Lung Cancer Action Program, the 10-year survival rate for all patients with lung cancer was 80% regardless of stage or treatment [12]. If the cancer was in clinical stage I and was promptly resected, the 10-year survival rate was 92%. However, because large, randomized, controlled trials of LDCT screening are still in progress [13,14], whether LDCT screening reduces lung-cancer mortality rates remains uncertain. Although mortality data are needed to determine whether LDCT screening is effective, indirect evidence for a possible mor-

Abbreviations: CT, computed tomography; LDCT, low-dose helical computed tomography; BAC, bronchioloalveolar cell carcinoma; ALCA, Anti-lung Cancer Association.

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* Corresponding author. Tel.: +81 3 3964 1211x1587; fax: +81 3 3964 7094.
E-mail address: nseki@med.teikyo-u.ac.jp (N. Seki).

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tality reduction can be obtained from a "stage shift," an increase in the detection rate of putatively curable early-stage lung cancers and a concomitant decrease in incurable late-stage cancers, leading to a decrease in the lung-cancer-specific mortality rate [15], which can be used as a surrogate endpoint even in a nonrandomized, uncontrolled trial.

Results of many single-armed, uncontrolled trials of annual screening with LDCT have been published [12,16–22]. However, none of these trials has documented a stage shift, perhaps because the number of lung cancers detected with repeated screening was too small (range, 4–35 cancers) or because the duration of repeated screening (range, 1–4 years) was too short. Thus, to determine whether a true stage shift occurs, a longer-term LDCT study with a larger number of detected lung cancers is required.

Furthermore, studies performed to date have not considered the effect of histological classification on the stage shift. Recent LDCT trials suggest that an increase in early-stage lung cancer might not be accompanied by a decrease in late-stage lung cancer (i.e., overdiagnosis) [15] and that the presence of localized bronchioloalveolar cell carcinoma (BAC) and mixed adenocarcinoma with BAC component might reflect overdiagnosis bias, although adenocarcinoma without BAC component behaves as aggressively as do other non-small cell carcinomas [23].

In the present study, on the basis of an update of the Anti-lung Cancer Association (ALCA) project [16], we investigated whether a stage shift occurs when lung cancers are stratified by histological subtype during long-term repeated LDCT screening for lung cancer in a high-risk cohort comprising mostly male smokers in their 60s.

2. Patients and methods

2.1. Study population

From September 1993 through August 2004, LDCT screening was performed semiannually by the ALCA in Tokyo. The ALCA is a for-profit organization established in 1975 to thoroughly screen for lung cancer in dues-paying participants. Because the participants are continuously recruited from members of the general population 40 years or older with a history of smoking (>20 pack-years) or a single episode of hemoptysis within the past 6 months, most participants are male smokers in their 60s. Written informed consent was obtained from each participant at baseline CT screening.

2.2. Screening procedures

Screening was performed as described previously [16]. Briefly, at baseline screening a simple questionnaire about smoking history and symptoms was completed, and LDCT, chest radiography (posterior–anterior position), and sputum cytological examination pooled for 3 days were performed. Participants were invited twice a year by mail after the baseline screening to repeat the same screening procedures. The CT scanner (TCT-900S Superhelix, Toshiba Medical, Tokyo, Japan) was used under the following conditions: 120 kVp, 50 mA, 10-mm collimation, 1 rotation of the X-ray tube per second, and a table speed of 20 mm/s (pitch, 2:1). Image construction was performed with 180° linear interpolation at 1-cm intervals. All CT images were examined by 2 of 7 readers (radiologists or thoracic physicians).

2.3. Evaluation of detected lung cancers

The staging and the histological classification of detected lung cancers were performed according to the International System for Staging Lung Cancer [24] and the World Health Organization lung tumor classification system [25], respectively. Cancers were classified as adenocarcinoma, squamous cell carcinoma, other non-small cell carcinoma, or small cell carcinoma. Moreover, adenocarcinoma was subclassified on the basis of the histological growth pattern as localized BAC, mixed adenocarcinoma with BAC component, and adenocarcinoma without BAC component (invasive adenocarcinoma).

Lung cancers detected at baseline screening were considered "prevalence cancers," whereas those newly detected at subsequent repeated LDCT screening examinations were considered "incidence cancers." Furthermore, lung cancers diagnosed outside our semiannual LDCT screening procedure within a screening interval were defined as "interval cancers," whereas those diagnosed outside our screening procedure after a period longer than the screening interval (due to refusal by ALCA participants) were not classified as "interval cancers." The presence or absence of interval cancers was confirmed through questionnaire when participants were invited twice a year by mail after the baseline screening to repeat the same screening procedures.

Excluded from analysis were 6 cases of hilar lung cancer detected on sputum cytological examinations or on evaluation of hemoptysis but not with LDCT.

2.4. Statistical analysis

Statistical *P* values for the differences in percentages and means were evaluated with the χ^2 test and the *t*-test, respectively. Survival curves were estimated with the Kaplan–Meier method, with survival time defined as starting from when microscopic evidence for malignancy was first obtained to the date of death or November 25, 2005, whichever came first. Differences in survival rates between groups were evaluated with the log-rank test. Multivariate Cox proportional hazards model analysis was performed to identify significantly independent prognostic factors for overall survival. Linear regression analysis with the least-squares method was performed for the relationships between groups. All calculations were performed with Stat View 5.0J software (SAS Institute Inc., Cary, NC). *P* values less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. Characteristics of participants

During the study period, 20,113 LDCT scans were performed for 2120 ALCA participants (mean age, 63 years; 87% male and 83% smokers), and 76 peripheral lung cancers were detected. Participants underwent LDCT screening a median number of 7 times (range, 1–22 times; Fig. 1A); a median number of 3 lung cancers were detected in each ordinal screening (range 0–9; Fig. 1B); a median of 3.5 years had passed since a participant's baseline screening (range, 0–10.5; Fig. 1C); and a median of 0.5 years had passed since a participant's previous screening (range, 0–10.0; Fig. 1D). Of the 2120 ALCA participants, 243 (11%) underwent only baseline LDCT screening, 753 (36%) underwent repeated LDCT screening for more than 5 years, and 322 (15%) underwent repeated LDCT screening for more than 10 years.

3.2. Comparison of results between baseline and subsequent LDCT screenings

The characteristics of all participants and of participants who underwent at least 1 subsequent LDCT screening examination are shown in Table 1. No significant difference was observed between these groups in terms of age, sex, or smoking status at baseline. However, the detection rate of lung cancer was significantly higher

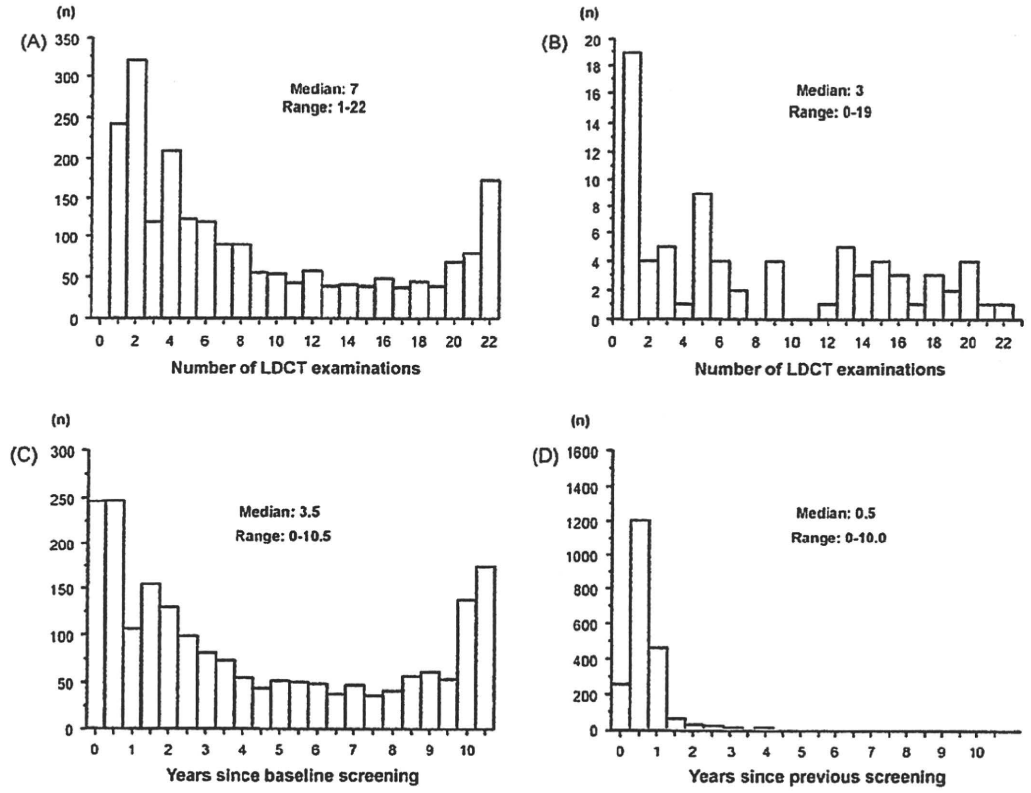

Fig. 1. Characteristics of repeated LDCT screening. (A) Distribution of the number of times participants underwent repeated LDCT screening (*X* axis indicates the number of LDCT examinations, and *Y* axis indicates the number of participants in each ordinal screening). (B) Distribution of the number of lung cancers detected in screening examinations grouped by ordinal number (*X* axis indicates the number of LDCT examinations, and *Y* axis indicates the number of lung cancers detected in each ordinal screening.). (C) Distribution of years since participants had undergone baseline screening (*X* axis indicates years since baseline screening, and *Y* axis indicates the number of participants in each ordinal screening period). (D) Distribution of years since participants had undergone previous screening (*X* axis indicates years since previous screening, and *Y* axis indicates the number of participants in each ordinal year since previous screening).

at baseline screening (0.90%: 19 prevalence cancers in 2120 participants) than at repeated screenings (0.32%: 57 incidence cancers in 1877 participants; $P<0.001$).

The characteristics of 76 patients with lung cancers detected at screening examinations are summarized in Table 2. The 19 patients with prevalence cancers and the 57 patients with incidence cancers did not differ in age, sex, or smoking status. However, both the percentage of positive chest X-ray films (53% vs. 16%, $P=0.004$) and tumor size (24 mm vs. 17 mm, $P=0.018$) were significantly less in patients with incidence cancers than in patients with prevalence cancers. Although neither histological diagnosis nor pathological stage differed significantly between patients with prevalence cancers and those with incidence cancers, in both groups of patients adenocarcinoma (74% and 63%, respectively), especially invasive adenocarcinoma (42% and 23%, respectively), was the most common histological diagnosis and stage IA was the most common pathological stage (58% and 79%, respectively).

Survival rates were compared between patients with prevalence cancers and those with incidence cancers. The 5- and 10-year survival rates were 84.5% and 84.5%, respectively, in patients with incidence cancers ($n=57$) and were 68.7% and 38.1%, respectively, in

Table 1
Characteristics of participants.

	Baseline LDCT	Repeated LDCT	*P*
No. of participants	2120	1877	
Age (years, mean ± SD)[a]	63 ± 11	64 ± 11	NS
Sex (% male)	87	88	NS
Smoking (% smokers)[a]	83	84	NS
No. of detected lung cancers	19	57	
No. of screenings	2120	17993	
Detection rate (%)	0.90	0.32	<0.001

[a] Fixed at baseline screening.

Table 2
Clinicopathological characteristics of patients with screening-detected lung cancer.

	Prevalence cancers	Incidence cancers	*P*
No. of patients	19	57	
Age (years, mean ± SD)[a]	66 ± 8	69 ± 9	NS
Sex (% male)	84	86	NS
Smoking (% smokers)[a]	89	93	NS
Positive X-ray (%)	53	16	0.004
Tumor size (mm, mean ± SD)	24 ± 15	17 ± 10	0.018
Histological type			NS
Adenocarcinoma	14 (74%)	36 (63%)	
BAC	2	11	
Adenocarcinoma with BAC	4	12	
Invasive adenocarcinoma	8	13	
Squamous cell carcinoma	4	12	
Other non-small cell carcinoma	1	5	
Small cell carcinoma	0	4	
Pathological stage			NS
IA	11 (58%)	45 (79%)	
IB	2	3	
II	0	3	
III	5	4	
IV	1	2	

BAC: bronchioloalveolar cell carcinoma.
[a] Fixed at baseline screening.

patients with prevalence cancers ($n=19$). No significant difference was observed between the groups (log-rank test, $P=0.208$). Multivariate analysis with the Cox proportional hazards model found that only pathological stage ($P=0.006$) was an independent prognostic factor for overall survival. The risk of death in patients with stage II–IV disease was increased 8.26-fold (95% confidence interval, 1.85–37.03). In contrast, age, sex, smoking status, tumor size, histological subtype (presence of BAC component), and screening type (baseline vs. repeated) were not independent prognostic factors.

No interval lung cancers were detected outside our semiannual LDCT screening procedure within a screening interval. However, 3 lung cancers were detected outside our screening procedure after a period longer than the screening interval. For these 3 lung cancers, the histological classification and stage, screening period from baseline to previous screening, and time since previous screening, respectively, were: invasive adenocarcinoma, stage IV, 5 years, and 4 years; squamous cell carcinoma, stage IA, 3.5 years, and 5 years; and other non-small cell carcinoma, stage II, 5 years, and 1.5 years.

3.3. The presence of an increased detection rate, a stage shift, and a size shift

The detection rate of all 57 incidence cancers was positively correlated with the duration of repeated screening ($r=0.50$, $P=0.020$) but remained uncorrelated if the duration of repeated screening was 5 years or less (Fig. 2A). In contrast, the detection rate of localized BAC showed a weak negative correlation with the duration of repeated screening ($r=-0.38$, $P=0.086$). Other histological subtypes, including invasive adenocarcinoma, showed no significant correlations.

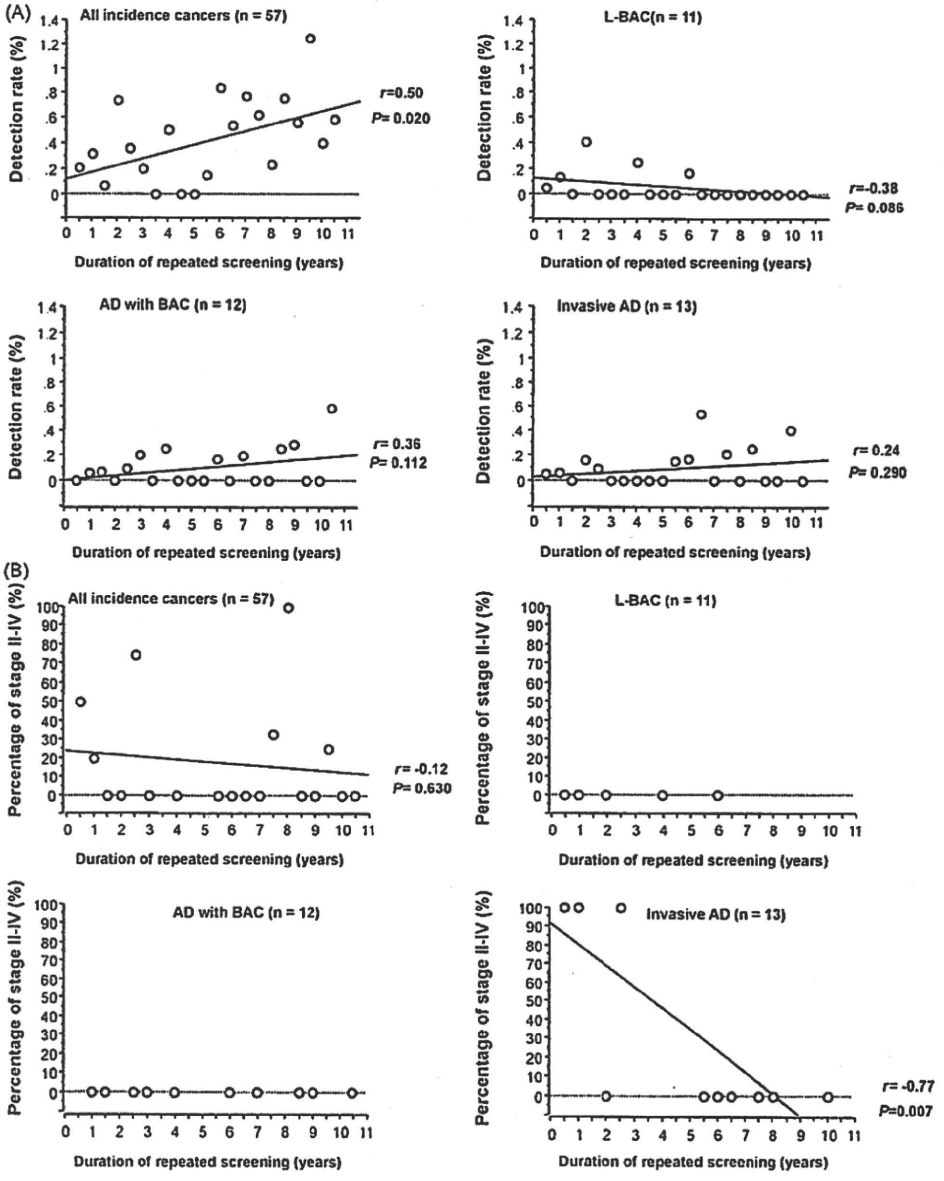

Fig. 2. Relationship between the duration of repeated screening and characteristics of incidence lung cancers. Correlations between the duration of repeated screening and the detection rate (A), the proportion of stage II–IV disease (B), and tumor size (C) were evaluated according to histological subtypes. L-BAC, localized bronchioloalveolar carcinoma; AD, adenocarcinoma.

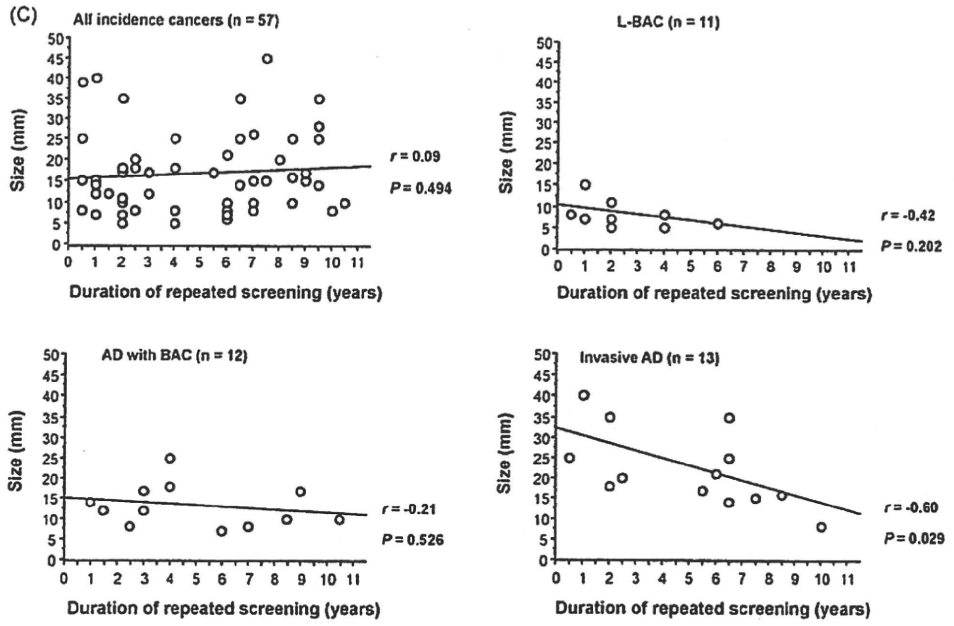

Fig. 2. (*Continued*).

Although the percentage of stage II–IV disease among all 57 incidence cancers was not correlated with the duration of repeated screening ($r=-0.12$, $P=0.630$), the percentage of stage II–IV disease among invasive adenocarcinoma was negatively correlated with the duration of repeated screening ($r=-0.77$, $P=0.007$) but remained uncorrelated if the duration of repeated screening was 5 years or less (Fig. 2B). In contrast, the percentage of stage II–IV disease among both localized BAC and mixed adenocarcinoma with BAC component remained 0% regardless of the duration of repeated screening. Neither squamous cell carcinoma ($r=-0.12$, $P=0.767$) nor small cell carcinoma ($r=-0.67$, $P=0.999$) showed a significant correlation between the percentage of stage II–IV disease and the duration of repeated screening.

Similarly, although tumor size among all 57 incidence cancers was not correlated with the duration of repeated screening ($r=-0.12$, $P=0.630$), the tumor size of invasive adenocarcinoma was negatively correlated with the duration of repeated screening ($r=-0.60$, $P=0.029$) but remained uncorrelated if the duration of repeated screening was 5 years or less (Fig. 2C). In contrast, other histological subtypes showed no significant correlations.

4. Discussion

In the present study involving 10 years of semiannual LDCT screening in a continuously recruited cohort comprising mostly male smokers in their 60s, increased detection rates were observed for lung cancers other than localized BAC. Moreover, both a stage shift and a size shift were observed for invasive adenocarcinoma of the lung. This report is, to our knowledge, the first to document the significance of long-term repeated screening for lung cancer with LDCT in a high-risk cohort.

Recently, Bach et al. have demonstrated that screening for lung cancer with LDCT may not meaningfully reduce the risk of advanced lung cancer or death from lung cancer [26]. Their conclusion was based on a model predicting deaths from lung cancer applied to 3 studies of LDCT screening in asymptomatic population at risk for lung cancer [20–22]. However, most importantly, the screening period of each of the 3 studies was less than 5 years. If each screening period had been 5 years or longer, Bach et al. might have instead confirmed a decrease in the lung-cancer-specific mortality rate. The screening period is important for other cancers for which the efficacy of screening has already been demonstrated; for example, the period of screening with fecal occult blood for colorectal cancer has been shown to be the important factor in a large randomized, controlled trial [27]. The initial protocol of the study specified 5 years of screening; however, the Policy and Data Monitoring Group recommended that screening be reinstituted because of the lack of statistical power regarding the mortality rate through 5 years of screening in the population. Screening then continued for 10 years, resulting in the finding of a lower mortality rate in screened subjects. Furthermore, meta-analysis of 8 randomized, controlled trials of screening mammography has demonstrated a statistically significant reduction in mortality rate among women aged 40–49 years at entry through screening for 10 years [28]. In particular, in 1 of these studies, the mortality rate from breast cancer was similar in screened group and the control group during the first 8 years but then became lower in screened group after 8 years [29]. Therefore, the efficacy of repeated screening for lung cancer might be demonstrated only with a long screening period.

To determine whether LDCT screening can reduce the mortality rate from lung cancer, a large, randomized, controlled trial has been started in the United States (National Lung Screening Trial) [13]. In this trial, 50,000 subjects at high risk for lung cancer were randomly assigned to undergo screening with chest radiography or LDCT at baseline and then annually for 2 additional years with annual telephone follow-up thereafter. Accrual was completed in February 2004, and final analyses are scheduled to be completed in 2009. In addition, a Dutch-Belgian randomized trial (NELSON trial) comparing CT screening with no screening at baseline and then 2 repeated screenings within 3 additional years in almost 20,000 subjects at high risk for lung cancer should be completed by 2010 [14]. However, if only long-term, repeated LDCT screening produces a stage shift, these 2 trials of short-term, repeated LDCT screening might fail to show any benefit. In fact, we should note that the detection rate of incidence lung cancers of all types remained unchanged if the duration of repeated screening was 5 years or less. Furthermore, neither a stage shift nor a size shift in invasive adenocarcinoma occurred if the duration of repeated screening was 5

years or less. Therefore, considering our present findings that the detection rate of incidence lung cancers in a cohort of mostly male smokers increased after 5 years of repeated LDCT screening and that the stage shift was observed for at least invasive adenocarcinoma after long-term, repeated LDCT screening for 5 years, we believe that proving the efficacy of LDCT screening would be difficult if the screening period is less than 5 years.

In the present study both a stage shift and a size shift were observed for invasive adenocarcinoma of the most common histological diagnosis. Considering direct evidence exists for a stage-size relationship in LDCT screen-diagnosed lung cancers [30], the fact that the stage shift was followed by a simultaneous size shift supports the occurrence of a stage shift in invasive adenocarcinoma. However, we wonder why this phenomenon was observed for only invasive adenocarcinoma. This question is difficult to answer, considering that invasive adenocarcinoma behaves as aggressively as do other non-small cell carcinomas. A possible explanation might simply be that the number of incidence lung cancers detected in our study lacks sufficient statistical power. However, some adenocarcinomas have higher volume-doubling times, grow more slowly, and are, therefore, diagnosed more easily at an early stage; another explanation could be length-time-biased sampling inherent to single-armed, uncontrolled trials. Thus, large, randomized, controlled trials on the basis of long-term repeated screening will be necessary to answer this question.

In the present study, we have performed semiannual LDCT screening to detect aggressive, fast-growing lung cancers at an early stage. However, no interval lung cancers were detected in our screening population. On the other hand, an interesting phenomenon is shown by the characteristics of 3 patients with lung cancers detected outside our screening procedure after a period longer than the screening interval. These lung cancers were detected after the patients had stopped undergoing semiannual LDCT screening because no abnormality was observed during the screening periods, which were 3.5 years in 1 patient and 5 years in 2 patients. Therefore, these facts suggest the efficacy of long-term repeated LDCT screening for more than 5 years.

We have several concerns about our study. The first concern is that, in addition to the stage shift caused by long-term repeated screening, we estimated the efficacy of long-term repeated screening could also be shown indirectly if the overall survival of patients with incidence cancers would be significantly longer than that of patients with prevalence cancers. So, we compared baseline screening with subsequent screening. However, multivariate Cox proportional hazard model analysis showed that the screening type (baseline vs. repeated screening) was not an independent prognostic factor for overall survival. A possible reason for this finding is the small number of participants and, therefore, the small number of deaths from lung cancer in both groups. Thus, larger studies involving larger numbers of participants are needed to investigate whether the overall survival of patients with incidence cancers is, in fact, significantly longer than that of patients with prevalence cancers because of the efficacy of long-term repeated screening. A second concern is that the partial-volume effect might affect the ability of screening CT images to demonstrate small nodules because only thick-section screening CT with image construction at 1-cm intervals was available during the screening period. Therefore, in a second ALCA study still in progress we have performed both chest radiography and LDCT to evaluate the detection power of LDCT in terms of the partial-volume effect. A third concern associated with long-term semiannually repeated LDCT screening is that a large number of healthy persons would be exposed to radiation and have an increased risk of radiation-induced lung cancer, although the risk of radiation-induced cancers other than lung cancer would be far lower [31,32]. According to one estimate, LDCT screening at a rate of 1.5 examinations per year would induce 4.5 lung cancers per year in 100,000 persons aged 60–70 years [33]. According to another estimate, annual LDCT screening would induce approximately 6.7 lung cancers per year in 100,000 persons if male current smokers aged 60 years undergo annual screening until age 75 years with a compliance rate of 50% [34]. In contrast, because our population with a median age of 64 years undergoes LDCT screening twice a year, the risk of radiation-induced malignancy would be slightly higher. However, assuming that our semiannual screening yielded 57 lung cancers in 1877 participants during a median follow-up period of 3.5 years, the yearly incidence of lung cancer in 100,000 participants would be 868. Furthermore, because the 13 incidence invasive adenocarcinomas detected with the benefits of a stage shift and a size shift in our study suggest an incidence of 198 cancers per year per 100,000 persons, which is far larger than that of radiation-induced lung cancers, we maintain that semiannually repeated LDCT screening is beneficial despite the potential harm of the radiation exposure.

In conclusion, we have demonstrated that both a stage shift and a size shift occur for invasive lung adenocarcinoma during long-term repeated LDCT screening in a high-risk cohort. Long-term repeated screening for more than 5 years might disclose the potential efficacy of LDCT screening for lung cancer as the truth has been disclosed for other types of cancers, including colorectal cancer and breast cancer.

Conflicts of interest

The authors indicated no potential conflicts of interest.

Acknowledgements

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Previously Reported Lung Cancer Growth Curves

Ryutaro Kakinuma, Noriyuki Moriyama, Kenji Eguchi, Hironobu Ohmatsu, Nobuhiko Seki and Masahiro Kaneko

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patient, the decisions to initiate and terminate SUP should be considered carefully and individually. We wholly agree with Dr Porath that both initiation of SUP and its continuation beyond the ICU and the hospital require meticulous attention in order to minimize individual and public burden from such costly nosocomial complications as CDI.

Marya D. Zilberberg, MD, MPH, FCCP
Amherst, MA
Andrew F. Shorr, MD, MPH, FCCP
Washington, DC

Affiliations: From EviMed Research Group, LLC (Dr Zilberberg); the University of Massachusetts (Dr Zilberberg); and the Washington Hospital Center (Dr Shorr).
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Correspondence to: Marya Zilberberg, MD, MPH, FCCP, University of Massachusetts, School of Public Health and Health Sciences, PO Box 303, Goshen, MA 01032; e-mail: Marya@evimedgroup.org

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Previously Reported Lung Cancer Growth Curves

To the Editor:

The article by Lindell et al[1] documenting the growth curves of 18 lung cancers that was recently published in *CHEST* (December 2009) is a well-designed and well-written study of the natural history of lung cancer. The results indicated that the growth of some of the lung cancers was not exponential and that their growth during certain intervals was not predictive of their future growth. Although the conclusion of the abstract states that this study was the first to document the growth curves of individual lung cancers, we would like to draw the authors' attention to the fact that the individual growth curves of 13 lung cancers had been documented in the January 2008 issue of *Clinical Radiology*.[2]

It would have been most beneficial, therefore, if the authors had included a discussion of the growth curve results reported in the earlier article. The authors of the *Clinical Radiology* article did not interpret the growth curves of the 13 lung cancers in detail, but the growth curve for each lung cancer was graphically depicted in their Figure 5. A case report published in the *Annals of Thoracic and Cardiovascular Surgery* in 2007 also described the growth curve of a minute small cell lung cancer that exhibited a latent phase in its early growth period.[3]

In regard to the nonexponential growth of lung cancers, at least two previous articles[4,5] have reported no growth or a decrease in volume during the progression of certain lung cancers. Although neither article specifically stated that such lung cancers were not limited to exponential growth, based on the changes observed, the authors inferred that the growth of some of the lung cancers was not exponential.

In the introduction of their article, Lindell et al[1] make a point of stating that "No studies have documented growth dynamics of screening-detected, untreated, subclinical lung cancers on computed tomography (CT)." With all due respect to the authors, we cannot agree with this statement, because at least two earlier articles[4,5] have reported progression of CT scan screening-detected, untreated, subclinical lung cancers.

Ryutaro Kakinuma, MD
Noriyuki Moriyama, MD
Kenji Eguchi, MD
Hironobu Ohmatsu, MD
Nobuhiko Seki, MD
Masahiro Kaneko, MD
Chuo-ku, Tokyo, Japan

Affiliations: From the National Cancer Center, Research Center for Cancer Prevention and Screening.
Financial/nonfinancial disclosures: The authors have reported to *CHEST* that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.
Correspondence to: Ryutaro Kakinuma, MD, National Cancer Center, Research Center for Cancer Prevention and Screening, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045 Japan; e-mail: rkaki@ncc.go.jp

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Lung Cancer Growth Curves Based on CT Imaging

To the Editor:

We read with interest the recent article in *CHEST* (December 2009) by Lindell et al[1] in which the authors state that this "study is the only one to have evaluated growth curves of lung cancers using multiple CT scans." We would like to point out our previously published article that examined growth rates in 54 lung lesions, including 33 primary non-small cell lung cancers, based on volumetric measurements from thin-section CT imaging.[2] Individual growth curves were plotted for the 20 lesions with ≥3 CT scans, including 13 non-small cell lung cancers. As in the study by Lindell et al, we found considerable variability in growth rates among the individual cancers that were analyzed. On the other hand, however, most of our lesions showed exponential growth, differing somewhat from the results of Lindell et al.

Lindell et al noted that their study was limited by the accuracy of two-dimensional measurements for volume calculation. Indeed, in our study, we found that calculated growth rates differed substantially, depending on the volume measurement technique used (ie, based on lesion diameter, lesion area, or direct volume measurement using automatic segmentation and direct volume measurement); presumably, direct volume measurements are superior to the other methods because primary lung cancers are not spherical and often grow asymmetrically. Despite the potential limitations of our studies, CT imaging-based growth rates are undoubtedly more accurate than the oft-quoted rates based on data derived from older chest radiography studies.[3-5]

Leslie E. Quint, MD
Ann Arbor, MI
Joan Cheng, MD
New York, NY
Andrew C. Chang, MD, FCCP
Greg P. Kalemkerian, MD
Ann Arbor, MI

Affiliations: From the Department of Radiology (Dr Quint), the Department of Thoracic Surgery (Dr Chang), and the Department of Internal Medicine (Dr Kalemkerian), University of Michigan Health System; and the Department of Radiology (Dr Cheng), Columbia University.
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Correspondence to: Leslie E. Quint, MD, Department of Radiology, University of Michigan Health System, 1500 E Medical Center Dr, Ann Arbor, MI 48109; e-mail: lequint@umich.edu

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Response

To the Editor:

I would like to thank Quint el al and Kakinuma et al for their letters and making us aware of the article by Quint et al titled "Lung Lesion Doubling Times: Values and Variability Based on Method of Volume Determination."[1] Their article's focus was on volume determination methods, whereas our article in a recent issue of *CHEST* (December 2009)[2] was on the lack of exponential growth of a subset of lung cancers as shown by growth curves and the implications that this could have on prior studies that advocated using two volume measurements to determine volume doubling time. While their study did include growth curves for 20 lesions, only 13 of those were lung cancers, and although it is difficult to be sure from their growth curves, it appears that most, if not all, of their lung cancers were followed with only three CT scan exams. An early criticism of our manuscript during peer review was that it would be difficult to prove or exclude exponential growth based on only three data points, and it was recommended that we include only cases with at least four CT scan exams. Therefore, although Quint and colleagues did generate growth curves, it is our position that based on peer review feedback they did not plot growth curves that were analogous to the curves we plotted. We did perhaps err in not referencing the paper and explaining our reasoning. We do agree with their conclusion that "given the very slow growth of some lung cancers, short term follow-up CT may not always be capable of detecting volume changes indicative of malignancy. Therefore, stability on short term follow-up exams should be interpreted with caution." However, based on our study we would expand on that to caution that even using a volume doubling equation based on two exams could potentially be misleading since the assumption of exponential growth has been called into question.

The differing results regarding exponential growth are interesting but perhaps somewhat explainable by our prospective screening method and different inclusion criteria. Compared with their study, our study's criteria resulted in a population of lung cancers with a smaller initial size (none>8 mm vs a mean of 11-17 mm) and slower growth (volume doubling time mean of 771 days vs a mean range of 58-128 days) that were followed on more CT scan exams for a longer period of time (mean 1,025 days, median 1,051 days, range 404-1,666 days, or 55.5 months if assuming 30 d/mo, vs a mean of 227 days, median 154 days, range 6 days-34.5 months). Since their study was retrospective and only included lesions with a histologic diagnosis, they selected for a different set of cancers. Cancers with a slower growth rate may not have changed sufficiently during the course of their study to have undergone resection and were therefore not included.