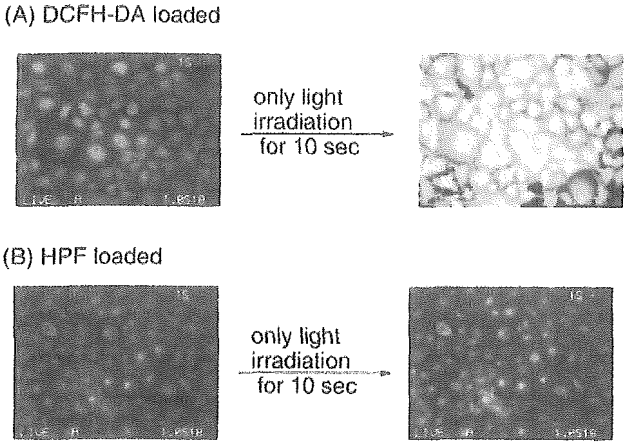

FIG. 4. **Light-induced autoxidation in DCFH-DA-loaded HLE cells (*A*) and HPF-loaded HLE cells (*B*).** HLE cells were loaded with DCFH-DA or HPF (10 μM; 0.1% DMF as a cosolvent) by incubation for 30 min at 37 °C in the dark. Fluorescence images were acquired. After that, the cells were laser-irradiated at 488 nm for 10 s, and fluorescence images were acquired again.

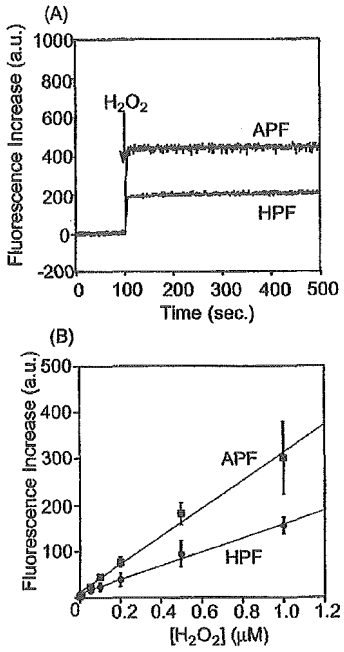

FIG. 5. **Detection of hROS in the HRP/H_2O_2 system using HPF and APF.** *A*, representative data are shown (n = 3). HPF (*lower line*) or APF (*upper line*) (final 10 μM; 0.1% DMF as a cosolvent) were added to sodium phosphate buffer (0.1 M, pH 7.4) containing HRP (0.2 μM). H_2O_2 (final 1 μM) was added at the time indicated by the *arrow*. The fluorescence intensity was determined at 515 nm with excitation at 490 nm. These reactions were performed at 37 °C. *B*, relation between the amount of added H_2O_2 and fluorescence increase in the HRP/H_2O_2 system using HPF (*circle*) and APF (*square*). Data are mean ± S.E. (n = 3). Dyes (final 10 μM; 0.1% DMF as a cosolvent) were added to sodium phosphate buffer (0.1 M, pH 7.4) containing HRP (0.2 μM). The fluorescence intensity was determined at 515 nm with excitation at 490 nm. These reactions were performed at 37 °C for 5 min.

fluorescence microscopy. We loaded HPF or DCFH-DA into HLE cells and irradiated the dye-loaded cells for 10 s. Fluorescein and dichlorofluorescein, the autoxidized products, form dianions in the buffer (pH 7.3) (36, 37), and therefore tend to remain in the intracellular medium. The results are shown in Fig. 4. HPF could permeate the cell membrane and enter into cells. DCFH was much more easily autoxidized by light irradiation than HPF in cells under conditions practically used for excitation in fluorescence microscopy.

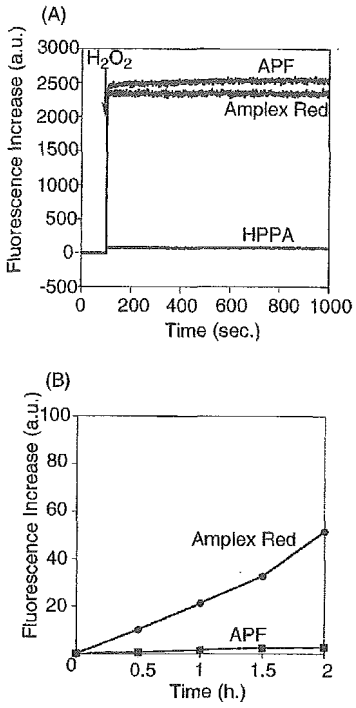

FIG. 6. **Comparison between APF, Amplex Red, and HPPA.** *A*, comparison of the sensitivity in the HRP/H_2O_2 system. Dyes (final 10 μM; 0.1% DMF as a cosolvent) were added to 0.15 M KCl, 25 mM Tris-HCl buffer (pH 7.4) containing HRP (0.2 μM). H_2O_2 (final 3 μM) was added at the time indicated by the *arrow*. The fluorescence intensity with APF, Amplex Red, and HPPA was determined at 515, 580, and 405 nm with excitation at 490, 570, and 320 nm, respectively. These reactions were performed at 37 °C. *B*, comparison of the lability of light-induced autoxidation. Dyes (final 10 μM; 0.1% DMF as a cosolvent) were added to 0.15 M KCl, 25 mM Tris-HCl buffer (pH 7.4). Dyes were placed under a fluorescent lamp for the indicated time.

Application of HPF and APF to an Enzymatic System—We investigated whether HPF and APF could detect hROS generated in an enzymatic system, *i.e.* the HRP/H_2O_2 system. In this system, two-electron oxidation of the native enzyme (HRP) to compound I is followed by two one-electron reductions to yield the resting state of HRP (38). H_2O_2 was added to buffer solutions of HPF and APF containing HRP. As shown in Fig. 5A, the fluorescence intensity increased immediately upon the addition of H_2O_2. Furthermore, it was found that HPF and APF could detect hROS generated in the HRP/H_2O_2 system in a dose-dependent manner (Fig. 5*B*). Thus, the data in Fig. 5 really show that HPF and APF can serve as substrates for horseradish peroxidase, and HPF and APF could detect hROS in a dose-dependent manner not only in a chemical system, but also in an enzymatic system.

Next, we compared the reactivity for hROS generated in the HRP/H_2O_2 system and the lability to light-induced autoxidation among our novel fluorescence probe APF and two widely used fluorescence probes for peroxidase (Amplex Red and HPPA) (39, 40). APF had slightly greater reactivity than Amplex Red for hROS generated in the HRP/H_2O_2 system and much greater reactivity than HPPA (Fig. 6A). Furthermore, APF had much greater resistance to autoxidation than Amplex Red (Fig. 6*B*). Therefore, APF is superior to the most widely used fluorescence probes for peroxidase.

Furthermore, we applied HPF and APF to the MPO/H_2O_2/Cl^- system. In the presence of Cl^-, ^-OCl is predominantly produced via the reactive intermediate, compound I. The re-

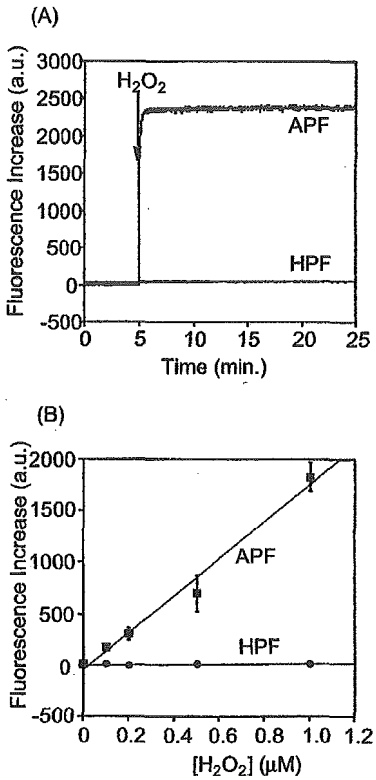

FIG. 7. **Application of HPF and APF to the MPO/H_2O_2/Cl^- system.** *A*, representative data are shown (n = 3). HPF (*lower line*) or APF (*upper line*) (final 10 μM; 0.1% DMF as a cosolvent) were added to sodium phosphate buffer (0.1 M, pH 7.4) containing MPO (11.2 nM) and NaCl (150 mM). H_2O_2 (final 1 μM) was added at the time indicated by the *arrow*. The fluorescence intensity was determined at 515 nm with excitation at 490 nm. These reactions were performed at 37 °C. *B*, relation between the amount of added H_2O_2 and fluorescence increase in the MPO/H_2O_2/Cl^- system using HPF (*circle*) and APF (*square*). Data are mean ± S.E. (n = 3). The buffer and wavelength for measurement were same as *A*. These reactions were performed at 37 °C for 25 min.

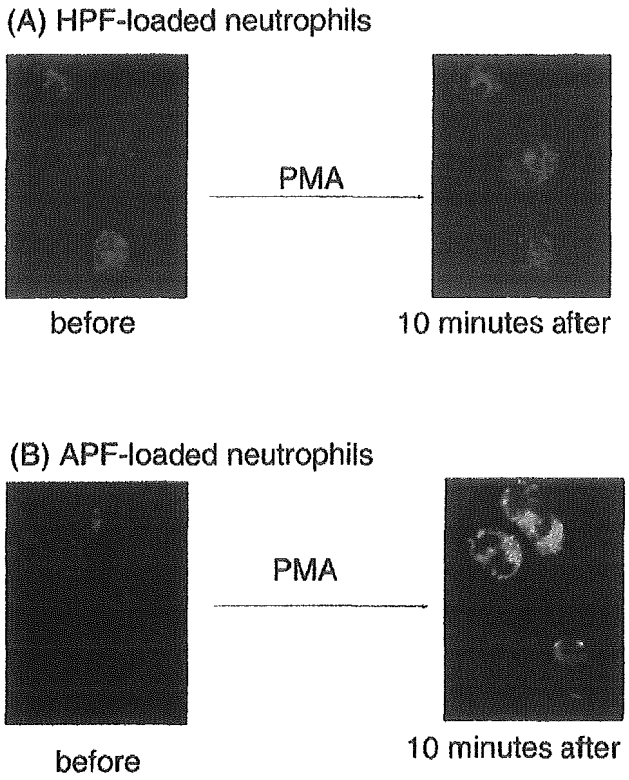

FIG. 8. **Fluorescence images of HPF- or APF-loaded neutrophils.** HPF or APF (final 10 μM; 0.1% DMF as a cosolvent) were loaded into neutrophils for 30 min at room temperature, and the dye-loaded neutrophils were stimulated with PMA (2 ng/ml). Fluorescence images were acquired before and 10 min after the stimulation with PMA.

sults are shown in Fig. 7. APF showed a dose-dependent fluorescence increase in this system, whereas HPF showed no fluorescence. These results correspond well with the reactivities of HPF and APF for $^-$OCl. Therefore, we succeeded in visualizing the production of $^-$OCl in the MPO/H_2O_2/Cl^- system.

Application of HPF and APF to Neutrophils—Neutrophils are a population of circulating blood cells, and their primary function is host defense against pathogenic microorganisms. Exposure of neutrophils to various stimuli such as PMA (41) and fatty acids (42) activates the "respiratory burst oxidase," NADPH oxidase, to generate $O_2^{\cdot-}$, which is then converted to H_2O_2 and O_2 (43, 44). As neither $O_2^{\cdot-}$ nor H_2O_2 is strongly microbicidal, these species are thought to be precursors of more potent oxidizing agents, such as $^{\cdot}$OH, $^-$OCl, and $^1O_2$. Neutrophils contain azurophilic granules, in which MPO exists abundantly, and MPO has been shown to catalyze the formation of $^-$OCl from H_2O_2 and Cl^- *in vitro* (45). Therefore, we tried to apply our novel fluorescence probes to neutrophils. We stimulated HPF- or APF-loaded neutrophils with PMA, and observed the dye-loaded neutrophils without washing out the extracellular medium. The results are shown in Fig. 8. It is noteworthy that the fluorescence intensity of HPF-loaded neutrophils did not change upon stimulation with PMA, whereas that of APF-loaded neutrophils greatly increased. Our results suggest that MPO produces $^-$OCl in the presence of both Cl^- and H_2O_2, which is generated by the stimulation with PMA, and we could identify this $^-$OCl production by using HPF and APF together. In other words, we could for the first time visualize $^-$OCl selectively, distinguishing it from other ROS, by using HPF and APF together, even in a biological system.

DISCUSSION

We have succeeded in developing novel autoxidation-resistant fluorescence probes, HPF and APF, that can reliably detect hROS and/or $^-$OCl selectively. Because it is likely that individual ROS have distinct roles in biological systems, the availability of selective fluorescence probes will be extremely useful. For example, by using HPF or APF, we can distinguish $^{\cdot}$OH from NO. This is very important, because DCFH reacts with both $^{\cdot}$OH and NO and so cannot be used reliably to study the biological role of $^{\cdot}$OH. In addition, the mere production of H_2O_2 is completely different in terms of cell damage from the situation in which H_2O_2 is converted into hROS in the presence of low-valent metal ions. We feel our probes are useful here, because they can distinguish these two situations. Furthermore, we can also distinguish $ONOO^-$ from NO or $O_2^{\cdot-}$. It has been reported that $ONOO^-$ can be generated from NO and $O_2^{\cdot-}$ *in vitro* and *in vivo* (46, 47), and therefore we will be able to visualize the production of $ONOO^-$ with a clear distinction from that of NO or $O_2^{\cdot-}$, and this will allow a reliable evaluation of the role of $ONOO^-$ in various processes. Furthermore, we could detect $^-$OCl selectively by using HPF and APF together, because HPF shows no fluorescence increase with $^-$OCl, whereas APF shows a dose-dependent increase. The ability to selectively detect individual species of ROS represents a major advance.

As shown in Table I and Fig. 4, the currently used fluorescence probe DCFH is easily autoxidized by light irradiation. This means that precautions must be taken to exclude light during incubation to load DCFH-DA into cells, and it is neces-

sary to change the visual field often during observations. However, HPF and APF are not autoxidized at all, as shown in Table I and Fig. 4. Therefore, we believe HPF and APF will contribute greatly to the elucidation of the roles of ROS in living cells by making it possible to see the generation of specific ROS with high resolution in time and space. Although the sensitivity of HPF and APF is inferior to that of DCFH (Table I), lability to autoxidation and selectivity among ROS, rather than sensitivity, are considered to be critical for fluorescence probes for ROS.

The question arises, why are HPF and APF selective for hROS, unlike DCFH? DCFH is nonfluorescent, and HPF and APF possess low fluorescence quantum efficiency, and all of them are converted to strongly fluorescent compounds, dichlorofluorescein or fluorescein, by oxidation. However, DCFH is converted to dichlorofluorescein, initially via abstraction of the hydrogen atom at the 9′-position, whereas HPF and APF are converted to fluorescein, initially via abstraction of the hydrogen atom of the phenolic hydroxy group or abstraction of one electron from the nitrogen atom. The hydrogen atom at the 9′-position of DCFH is readily abstracted because this hydrogen atom can be considered as being located at the central carbon of a triphenylmethane. It is therefore vulnerable even to a weakly oxidizing species, and this is the reason why DCFH lacks the selectivity among ROS. However, a strongly oxidizing species is required for the *ipso*-substitution reaction of HPF and APF. Therefore, we conclude that the difference of oxidizing power required for oxidation reaction used for detection causes the difference of selectivity among ROS. Furthermore, the fact that HPF shows no fluorescence increase with $^{-}$OCl, whereas APF does (Fig. 3 and Table I), reflects the difference in lability to oxidation between an aryloxyphenol and an aryloxyaniline.

HPF and APF could detect hROS generated in the HRP/H_2O_2 system (Fig. 5). HRP is often used as an enzyme label in immunohistochemical studies, 3,3-diaminobenzidine is commonly used as a substrate for measurement of the peroxidase activity. However, 3,3-diaminobenzidine can be detected only by absorbance measurement and is easily autoxidized by light irradiation. Because HPF and APF permit fluorescence detection, which has higher sensitivity than absorbance detection, and they are not autoxidized by light irradiation at all, they are likely to be more effective reagents for immunohistochemistry using peroxidase than 3,3-diaminobenzidine and related compounds.

We also used HPF and APF to visualize the production of $^{-}$OCl from neutrophils (Fig. 8). Dye-loaded neutrophils weakly fluoresced before the stimulation with PMA, because the dyes were taken up by pinocytosis and MPO was slightly released into pinocytic vacuoles. Nevertheless, the fluorescence intensity of APF-loaded neutrophils markedly increased, in contrast to little fluorescence increase of HPF-loaded cells upon stimulation with PMA. $^{-}$OCl is believed to play important roles not only in bacterial killing bacteria by neutrophils but also in injury to the venular endothelial surface in platelet-activating factor-induced microvascular damage (48). However, it has been difficult to draw firm conclusions concerning direct participation of $^{-}$OCl because a completely selective detection method for $^{-}$OCl has never been developed. Therefore, our finding that we could detect $^{-}$OCl selectively by using HPF and APF together will make it possible for the first time to elucidate reliably the roles of $^{-}$OCl in biological systems such as neutrophils.

In summary, we have developed novel fluorescence probes, HPF and APF, that can selectively and dose dependently detect certain species among ROS and that are highly resistant to autoxidation. They can be used in enzymatic and cellular systems. They are greatly superior to the existing fluorescence probes for ROS, and are expected to have many chemical and biological applications.

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Mechanism of Persistent Ischemic Mitral Regurgitation After Annuloplasty: Importance of Augmented Posterior Mitral Leaflet Tethering

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Mechanism of Persistent Ischemic Mitral Regurgitation After Annuloplasty

Importance of Augmented Posterior Mitral Leaflet Tethering

Fang Zhu, MD*; Yutaka Otsuji, MD*; Goichi Yotsumoto, MD; Toshinori Yuasa, MD; Takayuki Ueno, MD; Bo Yu, MD; Chihaya Koriyama, MD; Shuichi Hamasaki, MD; Sadatoshi Biro, MD; Akira Kisanuki, MD; Shinichi Minagoe, MD; Robert A. Levine, MD; Ryuzo Sakata, MD; Chuwa Tei, MD

Background—We hypothesized that surgical annuloplasty for ischemic mitral regurgitation (MR) that displaces the posterior annulus anteriorly can potentially augment posterior leaflet (PML) tethering, leading to persistent MR. Relationships between leaflet configurations and persistent ischemic MR after the annuloplasty were investigated.

Methods and Results—In 31 patients with surgical annuloplasty for ischemic MR and 20 controls, posterior and apical displacement of the leaflet coaptation, the anterior leaflet (AML) and PML tethering angles relative to the line connecting annuli, coaptation length (CL), and the MR grade were quantified before and early after surgery in echocardiographic left ventricular long-axis views. Six of the 31 patients showed persistent MR despite annuloplasty. Compared with patients without persistent MR, those with MR showed no improvement in the left ventricular ejection fraction and systolic volume, similar reduction in the annular area, significant increase in posterior displacement of the coaptation ($P<0.01$), no improvement in AML tethering, greater worsening in PML tethering ($P<0.01$), and no increase in the CL. All tethering variables were significantly correlated with both preoperative and postoperative MR in univariate analysis, and reduced CL was the primary independent determinant of both preoperative and postoperative MR. Although increased AML tethering was the primary determinant of the preoperative CL ($r^2=0.46$, $P<0.0001$), increased PML tethering was the primary determinant afterward ($r^2=0.60$, $P<0.0001$).

Conclusion—Although tethering of both leaflets is the major determinant of ischemic MR before surgical annuloplasty, both leaflets tethering but with predominant and augmented PML tethering is related to persistent ischemic MR after the annnuloplasty. **(*Circulation*. 2005;112[suppl I]:I-396–I-401.)**

Key Words: mitral valve ■ echocardiography ■ valuloplasty

Current surgical approaches for the treatment of ischemic mitral regurgitation (MR) mainly focus on annular size reduction, which is usually effective.[1–3] A considerable number of patients, however, show persistent or recurrent MR despite annuloplasty,[3–6] which adversely affects patients' outcomes,[7] and its mechanism has not been fully investigated.

The basic mechanism of ischemic MR is leaflet tethering by the outward displacement of papillary muscles (PM) due to left ventricular (LV) remodeling.[8–13] Surgical mitral annuloplasty, which is expected to hoist the posterior annulus anteriorly but may not cause significant positional changes to the anterior annulus fixed at the aortic root, can potentially augment tethering of the posterior mitral leaflet (PML) and restrict its anterior excursion toward coaptation while keeping the tethering of anterior leaflet (AML) unchanged (Figure 1).[6,14] We hypothesized that ischemic MR without annuloplasty is related to tethering of both leaflets and that MR after surgical annuloplasty is also related to tethering of both leaflets, but especially with tethering of the PML. Therefore, the purpose of this study was to investigate AML and PML configurations in patients with ischemic MR with and without surgical annuloplasty, and to clarify the characteristics of leaflet configurations responsible for persistent ischemic MR afterward.

Methods

Subjects

Subjects were 31 consecutive patients who had undergone surgical annuloplasty for ischemic MR at our hospital and 20 normal controls. Posterior annuloplasty with a flexible linear reducer (stain-

From the Department of Cardiovascular, Respiratory, and Metabolic Medicine (F.Z., Y.O., T.Y., B.Y., S.H., S.B., A.K., S.M., C.T.), Department of Public Health (C.K.), Department of Cardiovascular Surgery (G.Y., T.U., R.S.), Graduate School of Medicine, Kagoshima University, Kagoshima, Japan; and Massachusetts General Hospital (R.A.L.), Boston, Mass.

*The first 2 authors contributed equally to this work.

Correspondence to Yutaka Otsuji, MD, Department of Cardiovascular, Respiratory and Metabolic Medicine, Graduate School of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima City, 890-8520, Japan. E-mail yutaka@m.kufm.kagoshima-u.ac.jp

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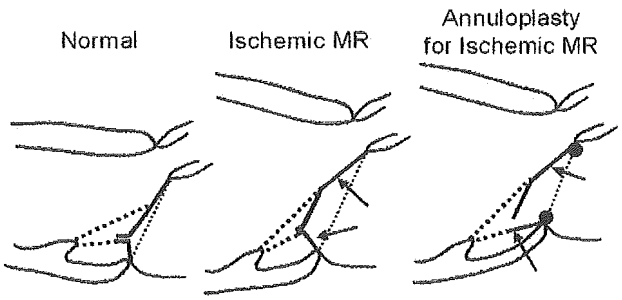

Figure 1. Potentially augmented tethering of the posterior mitral leaflet (PML) induced by surgical ring annuloplasty. The outward displacement of papillary muscles (PM) similarly tethers both anterior leaflet (AML) and PML with increased angles between the leaflets and the line connecting the mitral annuli (middle). Anteriorly hoisted posterior mitral annulus by ring annuloplasty may increase the distance between the PM tip and posterior annulus, thereby increasing tethering of the PML, with unchanged tethering of the AML (right).

less steel wire seemed with a polyester sheath), in addition to a Carpentier Edwards semi-rigid ring and a Duran flexible ring, was performed.[15] Patients profiles are summarized in Table 1. Ischemic MR was diagnosed by echocardiography using the following criteria: (1) The presence of LV dilatation and/or dysfunction, (2) the presence of apical displacement of mitral leaflets,[8] and (3) the absence of organic leaflet lesions. The control subjects had normal echocardiogram without known cardiovascular disease. After cardiovascular surgery with ring annuloplasty, no patient had a subsequent myocardial infarction or required additional revascularization. Concomitant coronary artery bypass grafting was performed in all patients and LV plasty with Dor's, overlapping of anterior wall,[16] or plication of posterior aneurysm was performed in 11 patients. Patients were managed after surgery with standard medications. Written informed consent was obtained from all patients.

Measurements by Echocardiography

Two-dimensional and Doppler echocardiographic examinations were performed in all patients using 2- to 3-MHz transducers and commercially available phased array sector scanners (ATL HDI 3000; Toshiba SSH 380A; Philips Medical Systems Sonos 5500; Aloka SSD-5500; Siemens Sequoia 512) 1 week before and 2 weeks to 2 months after the surgery.

LV end-diastolic volume, end-systolic volume (LVESV), and ejection fractions (EF) were determined by the modified biplane Simpson's method. The LV sphericity was assessed by its short-to-long axis dimension ratio in the end-systolic apical 4-chamber view. Mid-systolic mitral annular dimension was measured in the apical 4- and 2-chamber views, to calculate its area with an elliptical assumption.[17]

TABLE 1. Patient Profile

	All Subjects (n=31)	After Operation MR(+) (n=6)	After Operation MR(−) (n=25)	*P* value
Age	41 to 80 (63±12)	55 to 78 (69±8)	41 to 80 (62±12)	N.S.
Male/Female	25/6	5/1	20/5	N.S.
NYHA class	2 to 4 (3.0±1.0)	2 to 4 (3.0±1.0)	2 to 4 (3.0±1.0)	N.S.
Angina Pectoris	15	1	14	N.S.
Associated prior infero-posterior MI	11	2	9	N.S.
Prior anterior MI	13	1	12	N.S.
Prior anterior + infero-posterior MI	7	3	4	N.S.
Time lapse from first AMI, years	0.6 to 16 (7±6)	0.6 to 7 (5±3)	0.6 to 16 (7±7)	N.S.
Time lapse between surgery and post-operative echo, months	0.5 to 2.0 (1.0±0.5)	0.5 to 2.0 (1.0±0.5)	0.5 to 2.0 (1.0±0.5)	N.S.
Coronary risk factors				
Hypercholesterolemia	5	0	5	N.S.
DM	7	0	7	N.S.
Hypertension	9	1	8	N.S.
Smoking	10	2	8	N.S.
LV plasty	11	2	9	N.S.
Dor's	6	1	5	N.S.
Overlapping	4	0	4	N.S.
Plication of posterior aneurysm	1	1	0	N.S.
Number of CABG	1 to 5 (3.0±1.4)	1 to 3 (2.0±1.4)	1 to 5 (3.0±1.3)	N.S.
MV plasty				
Semi-rigid ring	12	2	10	N.S.
Carpentier Edwards ring size (mm)	26 to 32 (29±2)	26 to 32 (29±3)	26 to 32 (28±2)	N.S.
Flexible ring	19	4	15	N.S.
Duran ring (29 mm)	2	2	0	N.S.
Posterior annuloplasty with linear reducer	17	2	15	N.S.

NYHA indicates New York Heart Association; MI, myocardial infarction; AMI, acute myocardial infarction; DM, diabetes mellitus; LV, left ventricle; CABG, coronary artery bypass grafting; MV, mitral valve.

P values were obtained by χ^2 test.

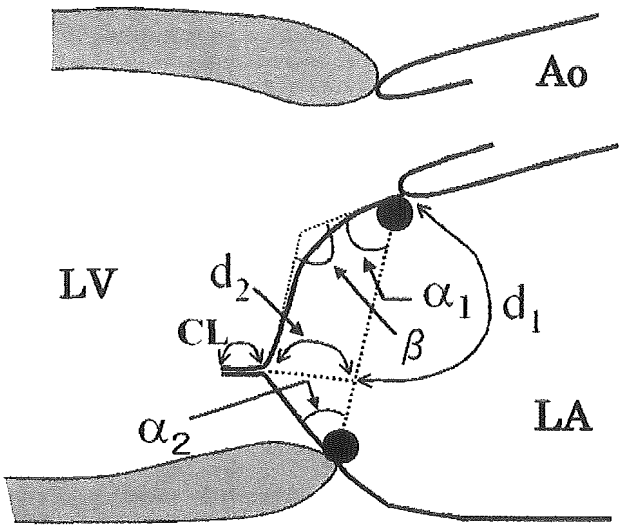

Figure 2. Methods to quantify the mitral leaflet configurations in the parasternal long axis 2-dimensional echocardiogram. Ao indicates aorta; LV, left ventricle; and LA, left atrium.

Mitral Leaflet Configuration and Mobility

Mitral leaflet configuration in mid-systole was quantified in the parasternal long-axis view (Figure 2). The angles α_1 and α_2 represent the grade of AML and PML tethering, respectively. The bending angle β between the tangent lines of proximal and distal AML represent the grade of AML tethering from secondary chordae.[18] The distances d_1 and d_2 represent posterior and apical displacement of the coaptation, respectively. Leaflet excursion or changes in α_1 and α_2 from diastolic maximal opening to systolic closure was evaluated. Coaptation length (CL) was also measured.

Quantification of MR and Its Jet Direction

MR was quantified by the vena contracta width or the narrowest jet origin in a parasternal or apical long-axis view perpendicular to the coaptation line.[6] Vena contracta width ≥3 mm was considered significant. MR jet direction was visually evaluated as anterior, central, or posterior in the color Doppler long-axis view. Echocardiographic measurements were averaged over 3 cardiac cycles for each measurement.

Statistical Analysis

Results were expressed as mean±SD. Comparisons of continuous variables among 3 or more groups were performed by Kruskal-Wallis test. When the Kruskal-Wallis test gave significant results, Scheffé's test was conducted for multiple comparisons. For comparison of each variable between before and after operation, we used Wilcoxson test. Determinants of the degree of preoperative and postoperative MR were explored by multiple stepwise regression analysis, entering all measured echocardiographic variables. A $P<0.05$ was considered statistically significant.

Results

Changes in LV Volume, Mitral Leaflet Configuration, and MR

The severity of MR significantly decreased in patients without postoperative MR ($P<0.01$) but did not decrease in 6 patients with postoperative MR by definition (Table 2). The LVESV and EF significantly improved in patients without postoperative MR ($P<0.01$) but did not improve in those with it. The mitral annulus area similarly and significantly decreased in both groups ($P<0.05$).

D_1 significantly decreased in patients without postoperative MR ($P<0.01$) but significantly increased in those with postoperative MR ($P<0.05$). D_2 similarly and significantly decreased in both groups ($P<0.05$). The α_1 significantly decreased in patients without postoperative MR ($P<0.01$) but did not decrease in those with postoperative MR. The AML excursion increased significantly in patients without postoperative MR ($P<0.01$) but did not increase in those with. The

TABLE 2. Echocardiographic Findings Before and After Surgical Annuloplasty

		All Subjects (n=31)		After Operation MR(−) (n=25)		After Operation MR(+) (n=6)	
	Control	Before Operation	After Operation	Before Operation	After Operation	Before Operation	After Operation
LVEDV/BSA, ml/m²	52±9	96±23*	90±21*†	94±21*	85±18*†	106±30*	111±24*‡
LVESV/BSA, ml/m²	18±4	65±16*	57±18*†	64±15*	52±14*†	71±19*	79±15*‡
LV EF, %	66±6	32±8*	37±9*†	32±8*	39±8*†	33±11*	32±8*‡
LV D/L	0.43±0.06	0.65±0.07*	0.62±0.09*†	0.65±0.07*	0.60±0.08*†	0.66±0.09*	0.68±0.11*‡
MAA/BSA, cm²/m²	5.6±0.8	7.2±1.2*	2.8±0.3*†	7.2±1.2*	2.8±0.3*†	7.3±0.7*	2.8±0.3*†
MR jet width/BSA, mm/m²	0	2.3±1.0*	0.5±1.1†	2.3±1.0*	0†	2.4±0.5*	2.7±0.7*†‡
Mitral Leaflet Configuration							
d_1/BSA, mm/m²	13.0±1.1	13.6±1.7	12.9±1.5	13.4±1.8	12.4±0.9†	14.3±1.2	15.0±1.6*†‡
d_2/BSA, mm/m²	3.9±0.7	6.8±0.9*	3.9±0.6†	6.8±0.9*	3.8±0.6†	7.0±0.8*	4.5±0.5†‡
α_1	12±3	33±4*	29±5*†	33±4*	28±5*†	33±4*	33±3*‡
AML-exursion	78±11	27±8*	30±13*	28±8*	33±12*†	26±4*	18±10*‡
α_2	30±5	57±9*	88±14*†	56±9*	83±7*†	60±12*	111±13*†‡
PML-excursion	58±8	29±11*	19±8*†	30±12*	22±6*†	26±7*	7±2*†‡
β	186±4	151±8*	155±16*	152±9*	160±12*†	149±5*	133±9*†‡
CL/BSA, mm/m²	5.2±0.6	2.0±0.6*	4.0±1.2*†	2.1±0.7*	4.5±0.5*†	1.9±0.5*	1.9±0.7*‡

*$P<0.05$ relative to normal controls; †$P<0.05$ relative to before operation value; ‡$P<0.05$ relative to after operation MR (−).

MR indicates mitral regurgitation; LVEDV, left ventricular end-diastolic volume; BSA, body surface area; ESV, end-systolic volume; EF, ejection fraction; D/L, short to long axis dimension ratio; MAA, mitral annular area; AML, anterior leaflet; PML, posterior leaflet; CL, coaptation length.

α_2 significantly increased in both groups ($P<0.05$), with a greater increase in patients with postoperative MR ($P<0.01$). The PML excursion significantly decreased in both groups ($P<0.05$), with a greater decrease in patients with postoperative MR ($P<0.01$). The β significantly increased in patients without postoperative MR, whereas it significantly decreased in those with it ($P<0.05$). The CL significantly increased in patients without postoperative MR ($P<0.01$), whereas it failed to increase in those with.

The preoperative α_1 and α_2 in patients with ischemic MR were both significantly increased compared with the normal values, with significant but only modest predominance of PML tethering (plus 21±4 versus 27±9 degree, $P<0.01$), and the preoperative AML and PML excursion were similarly reduced. Therefore, preoperative tethering was approximately similar between AML and PML. Postoperative α_1 and α_2 in patients with persistent MR, however, were significantly increased with advanced predominant PML tethering (plus 21±3 versus 81±13 degree, $P<0.01$). In addition, postoperative PML excursion was significantly smaller compared with that in AML in both groups. Therefore, postoperative tethering was significantly predominant for PML.

Determinants of Preoperative and Postoperative MR

Multiple regression analysis identified primary independent contribution from decreased CL along with increased d_2 for preoperative MR (Table 3). Multiple regression identified primary contribution from increased α_1 along with LV end-diastolic volume for the preoperative CL. These suggest that tethering of both leaflets was the main determinant of preoperative MR.

Multiple regression analysis identified decreased CL as the primary factor determining postoperative MR, in addition to increased d_1, increased α_2, and increased β. Multiple regression identified increased α_2 as the primary determinant of postoperative CL, in addition to decreased PML excursion and EF. These facts suggest that tethering of both leaflets, but especially augmented PML tethering, was the main determinant of the postoperative MR (Figure 3).

Figure 4 demonstrates representative patients. The patient in the upper panels without persistent ischemic MR was associated with relatively mild PML tethering, whereas the patient in the middle panels with persistent MR was associated with highly advanced PML tethering.

Change in LV Volume and Change in Mitral Leaflet Tethering After Surgery

Reduction in LVESV after surgery was significantly correlated with less leaflet tethering (ΔMR jet width: $r^2=0.38$, $P=0.0003$; $\Delta\alpha_1$: $r^2=0.18$, $P=0.02$; $\Delta\alpha_2$: $r^2=0.25$, $P=0.005$; Δd_1/BSA: $r^2=0.14$, $P=0.04$; Δd_2: $r^2=0.19$, $P=0.02$; $\Delta\beta$: $r^2=0.20$, $P=0.01$; ΔCL: $r^2=0.38$, $P=0.0003$).

MR Jet Direction

Preoperatively, MR jet direction was central in 24 patients and posterior in 7 patients with the absence of anterior jet. Postoperative jet direction, however, was anterior in 2, central in 4, and posterior in no patients with significant difference in the incidence of posterior jet ($P<0.01$).

TABLE 3. Determinants of the Severity of MR

	Univariate		Multivariate
	r^2 Value	*P* Value	*P* Value
Preoperative MR			
LVEDV/BSA	0.40	0.0002	N.S.
LVESV/BSA	0.29	0.0021	N.S.
LV EF	0.005	0.70	N/A
D/L	0.14	0.04	N.S.
Mitral annular area/BSA	0.28	0.003	N.S.
d_1/BSA	0.58	<0.0001	N.S.
d_2/BSA	0.53	<0.0001	<0.0001
α_1	0.50	<0.0001	N.S.
AML-excursion	0.15	0.04	N.S.
α_2	0.23	0.008	N.S.
PML-excursion	0.20	0.01	N.S.
β	0.22	0.01	N.S.
CL/BSA	0.73	<0.0001	<0.0001
Persistent MR after annuloplasty			
LVEDV/BSA	0.25	0.003	N.S.
LVESV/BSA	0.41	0.0001	N.S.
LV EF	0.20	0.01	N.S.
D/L	0.20	0.01	N.S.
Mitral annular area/BSA	0.009	0.62	N/A
d_1/BSA	0.59	<0.0001	<0.0001
d_2/BSA	0.21	0.01	N.S.
α_1	0.20	0.01	N.S.
AML-excursion	0.26	0.003	N.S.
α_2	0.74	<0.0001	<0.0001
PML-excursion	0.57	<0.0001	N.S.
β	0.50	<0.0001	<0.0001
CL/BSA	0.80	<0.0001	<0.0001

MR indicates mitral regurgitation; LVEDV, left ventricular end-diastolic volume; BSA, body surface area; ESV, end-systolic volume; EF, ejection fraction; D/L, short-to-long axis dimension ratio; AML, anterior leaflet; PML, posterior leaflet; CL, coaptation length; N/A, not applicable.

Discussion

Different Mitral Leaflet Configurations in Preoperative and Postoperative Ischemic MR

This study has demonstrated that ischemic MR without surgical ring annuloplasty is associated with similarly augmented AML and PML tethering. After the surgery, PML tethering significantly increased, but there was no major change in AML in patients with persistent MR. Therefore, AML and PML tethering is highly asymmetric, with PML predominance in patients with persistent ischemic MR. Augmented posterior displacement of the coaptation after annuloplasty in patients with persistent MR can be explained as a result of restricted PML excursion toward coaptation. This augmented PML tethering contributed to the reduced CL with persistent MR after ring annuloplasty.

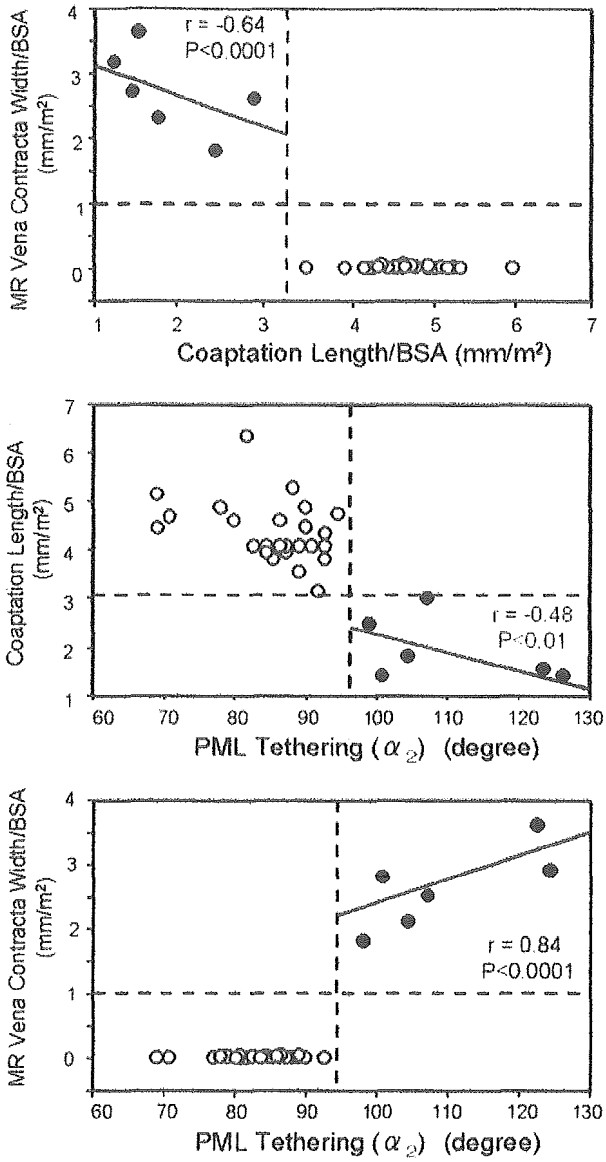

Figure 3. Scattergraphs showing the postoperative relationships between coaptation length (CL), PML tethering, and MR. CL and PML tethering were clearly different between patients without and with postoperative MR. CL, PML tethering, and MR were significantly correlated with each other in patients with postoperative MR.

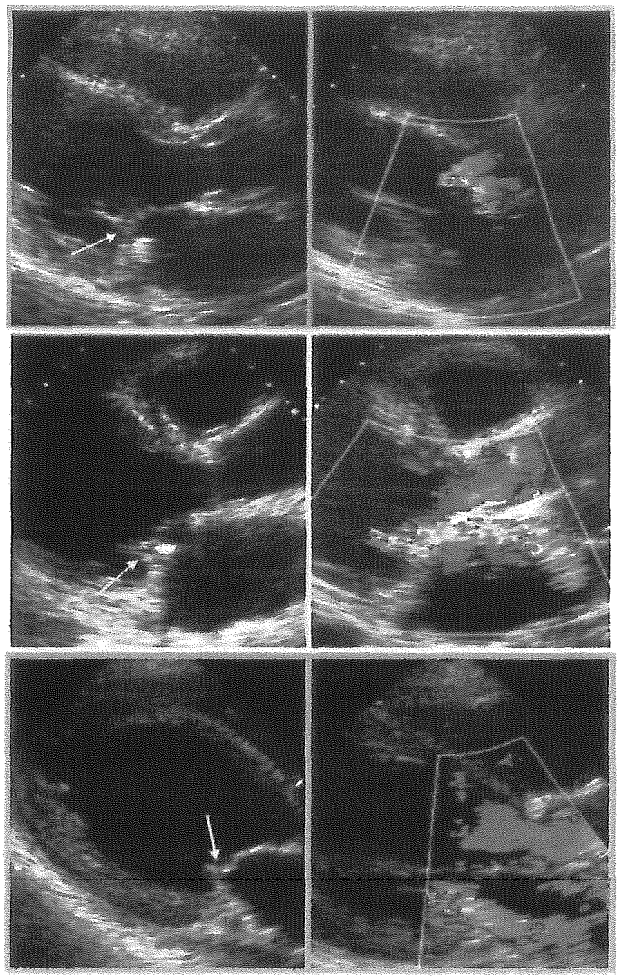

Figure 4. The upper panels show a patient with only modest PML tethering (arrow) without significant MR. The middle panels show a patient with advanced PML tethering (arrow) and significant persistent MR despite ring annuloplasty. The lower panels show a patient with posterior directed ischemic MR without annuloplasty. In both middle and lower panels, AML coapts with middle portion of PML and MR jet direction is parallel to PML.

Relation to Previous Studies

Hung et al[6] has found that ischemic MR can occasionally develop even with surgical ring annuloplasty and is related to leaflet tethering, as is the case for ischemic MR in patients without annuloplasty. Restricted PML motion has been observed by Green et al[14] in normal animal hearts after surgical ring annuloplasty, suggesting an important role of PML tethering in postoperative ischemic MR. The results of the current study are consistent with these reports and further revealed the importance of augmented PML tethering that contributes to the persistent ischemic MR after ring annuloplasty.

Usual AML prolapse develops posterior jet direction. Even in ischemic MR with tethering of both leaflets, less tethering of AML with its relative prolapse cause posterior jet (Figure 4, lower panels).[13] In this case, AML tip coapts with the body of PML, creating an MR orifice that resembles a funnel pointed posteriorly. Leaflet configuration of persistent ischemic MR in this study is a different form of malcoaptation, which can be described as "asymmetric PML tethering." In this case, AML tip also coapts with the body of the PML, creating an MR orifice that also resemble a funnel. This funnel is pointed anteriorly, however, because of the advanced PML tethering and causes central to anterior jet. Change in MR jet direction from central to posterior in the preoperative phase to central to anterior afterward suggests a mechanistic change of MR. The precise mechanism of MR jet direction and relations between the direction and the severity of MR or undersized ring annuloplasty remain uninvestigated.

Clinical Implications

Mitral annuloplasty per se does not relieve ventricular tethering; however, it is effective in the repair of ischemic MR, because it can reduce the anteroposterior diameter of the

annulus and restore reduced CL by ventricular tethering.[5] Disappearance of MR in most patients in this study confirmed the effects of ring annuloplasty for ischemic MR. The tethering of PML, however, was significantly increased afterward. Therefore, mitral annuloplasty reduces the anteroposterior diameter of the annulus and MR at the expense of augmented PML tethering. When the former effect is predominant, MR can be eliminated. When the former is not predominant, persistent MR may develop. The results of this study suggest the need for aggressive undersized annuloplasty, because restricted PML forces AML to cover whole annulus alone. AML longer than the anteroposterior diameter of the annulus is required, and undersized annuloplasty will have a beneficial effect. At the same time, a more posterior location of the coaptation ($\alpha_2 > 90°$) and significant bending of AML because of its tethering from basal chordae in patients with persistent ischemic MR in the present study suggest that AML considerably longer than the anteroposterior diameter of the annulus is required to prevent leakage when the tethering is advanced. In addition, reduction in LVESV after the surgery was associated with less tethering. Therefore, the results of the present study also encourage interventions for addressing ventricular tethering. Such approaches may include LV plasty with volume reduction, chordal elongation or cutting, PM displacement, and leaflet elongation procedures.[19–23] Because of the importance of augmented PML tethering in persistent ischemic MR, evaluation of both AML and PML tethering and interventions to specifically attenuate tethering of PML are also encouraged.[23]

Limitations

The current study addressed the mechanism of persistent ischemic MR early after surgery but did not address late-onset MR afterward. Multiple factors, such as the loss or deformity of physiological 3-dimensional saddle shape of the annulus, were not evaluated. The number of patients is small and they had heterogeneous etiology of ischemic MR. The procedures were not randomly performed and were heterogeneous, with multiple types of LV plasty without highly undersized annuloplasty. Therefore, the incidence or mechanisms of persistent ischemic MR after isolated annuloplasty in a different location of myocardial infarction, effects of rigid or semi-rigid and flexible ring annuloplasty with or without aggressive undersizing, and effects of different types of LV plasty were not accurately evaluated. Nevertheless, the purpose of this study was achieved by demonstrating augmented PML tethering by surgical ring annuloplasty and its significant contribution for the persistent ischemic MR afterward.

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Lung cancer death rates by smoking status: Comparison of the Three-Prefecture Cohort study in Japan to the Cancer Prevention Study II in the USA

Tomomi Marugame,[1,10] Tomotaka Sobue,[1] Hiroshi Satoh,[2] Shoko Komatsu,[4] Yoshikazu Nishino,[3] Haruo Nakatsuka,[5] Tomio Nakayama,[6] Takaichiro Suzuki,[6] Toshiro Takezaki,[7] Kazuo Tajima[8] and Suketami Tominaga[9]

[1]Statistics and Cancer Control Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104–0045, [2]Environmental Health Sciences, [3]Division of Epidemiology, Department of Public Health and Forensic Medicine, Tohoku University Graduate School of Medicine, 2–1 Seiryo-machi, Aoba-ku, Sendai 980–8575, [4]Faculty of Physical Education, Sendai College, 2-2-18 Funaokaminami, Shibata-machi, Shibata, Miyagi, 989–1693, [5]Miyagi University, 1 Gakuen, Taiwa-cho, Kurokawa, Miyagi, 981–3298, [6]Department of Cancer Control and Statistics, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3 Nakamichi, Higashinari-ku, Osaka 537–8511, [7]Department of International Island and Community Medicine, Kagoshima University Graduate School of Medical and Dental Science, 8-35-1 Sakuragaoka, Kagoshima 890–8520, [8]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1–1 Kanokoden, Chikusa-ku, Nagoya 464–8681, [9]Aichi Comprehensive Health Science Center of the Aichi Health Promotion Foundation, 1–1 Gengoyama, Morioka, Higashiura, Aichi 470–2101, Japan

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Cigarette smoking is an established risk factor for lung cancer. However, the magnitude of the relative risk (RR) on lung cancer mortality in relation to cigarette smoking is reported to be lower in Japan than in Western countries. We investigated whether this discrepancy could be explained by differences in the exposure to cigarettes smoked, by differences in sensitivity to smoking, or by differences in lung cancer mortality among non-smokers. We examined the 10-year follow-up data on 88 153 participants in a Japanese population-based prospective study conducted in three prefectures. Data used as a Western counterpart was retrieved from a published report of the US Cancer Prevention Study (CPS)-II. Although there was a significant increased risk of lung cancer death among current smokers compared with non-smokers, the observed RR in the Three-Prefecture Study were much lower than RR reported in the CPS-II. Lung cancer mortality of our Japanese sample was lower among current smokers and higher among non-smokers regardless of age and sex. Current smokers in our sample had initiated smoking at an older age and smoked fewer cigarettes per day for shorter durations than those in the CPS-II sample. The Poisson regression model (controlling for age, number of cigarettes smoked per day and duration of smoking) showed that male current smokers in our sample had a lower risk of lung cancer compared with those in the CPS-II sample (rate ratio 0.34 [95%CI 0.27–0.43]). These findings might explain why Japanese risks of lung cancer are lower than those observed in Western countries. (*Cancer Sci* 2005; 96: 120–126)

Numerous epidemiological studies have consistently reported smoking as a risk factor for lung cancer. Three prospective studies[1–3] and several case-control studies[4–6] in Japan have shown that the magnitude of the relative risk (RR) associated with cigarette smoking is lower than those in Western countries.[2] For example, in the Six-Prefecture Study[3] and the Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC),[1] the RR of lung cancer death among smokers compared to non-smokers was estimated at 4.5 for men, whereas the RR for men ranged from 11.6 to 23.2 in prospective studies conducted in the USA[7–9] and the UK.[10] For women, the RR were 2.3 in the Six-Prefecture Study[3] and 3.6 in the JACC study,[1] while corresponding RR ranged from 2.7 to 12.8 in the USA.[7,9] The first aim of this study was to verify these figures by evaluating lung cancer death and smoking habits with a new large-scale, population-based prospective survey (The Three-Prefecture Cohort Study), conducted in three prefectures in Japan.

The RR expresses a single summary estimate of the effects of smoking on lung cancer. However, the RR is computed by simply dividing the death rate among smokers by that among non-smokers. For a better understanding of the reasons for the lower RR of lung cancer among the Japanese, it would be more accurate to compare the death rates by smoking status. Furthermore, exposure levels to smoking might account for differences in the risk of lung cancer between Japanese and Western current smokers. It is well known that lung cancer risk depends on the amount, duration, and initiation age of smoking. Thus, to determine the reason for the lower RR associated with smoking in Japanese subjects, it is also important to compare the exposure levels to smoking as well as the lung cancer death rates between Japanese and Western subjects.

The second aim of this study was to compare death rates by smoking status and smoking exposure levels with published data from a large American prospective sample, the Cancer Prevention Study II (CPS-II),[9] which began at nearly the same time as the Three-Prefecture Cohort Study (1982). Finally, we examined whether any discrepancy in the RR of lung cancer between the studies could be explained by the difference in death rates due to smoking status (i.e. non-smokers vs smokers) and smoking exposure level between the Japanese and the US samples.

Materials and Methods

Study population. The Three-Prefecture Cohort Study collected data from February 1, 1983 to November 1, 1985, in selected areas of three prefectures in Japan: Miyagi, Aichi, and Osaka. The study areas of each prefecture included six areas of a city and two towns in Miyagi Prefecture, five elementary school districts in one area of a city and two areas of a city in Aichi Prefecture, and three towns in Osaka Prefecture. An additional study cohort was sampled in December 1, 1990, in one city in the Osaka Prefecture. The study population included all persons aged 40 years or older, who resided in the study areas according to each town's residential registry. A self-administered questionnaire was distributed to 130 839 persons, and 108 774 (50 544 men and 58 230 women) of them responded (83.1%). We then excluded individuals under 40 years (one man and one woman) and over 80 years of age (1 427 men and 2 465 women), any who

[10]To whom correspondence should be addressed. E-mail: tmarugam@gan2.res.ncc.go.jp

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moved out before the start of the follow up (five men and three women), and any whose information on smoking status at enrollment was incomplete (4 660 men and 12 059 women). After exclusion of these individuals, 44 451 men and 43 702 women remained in the analysis. This study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Follow up. Information on whether each subject was still alive and living in the same location was obtained from residential registries. If the subject had died, we then searched the population-based cancer registry in each prefecture and ascertained whether they had died from lung cancer. Sites of any cancers were coded using the International Classification of Disease and Injuries–ninth revision (ICD-9), except for one city in Osaka where the ICD 10th revision was used. Study subjects were followed for 10 years in each area. Therefore, the end of the study period varied from January 31, 1993 to October 31, 1995 (and February 28, 2000 for the one city in the Osaka Prefecture) according to the dates of enrollment. During the follow up, 8 836 (15.6%) individuals moved out of the study areas.

Smoking Information. At enrollment, study participants completed a self-administered questionnaire, including demographic information such as sex, date of birth, and smoking habits. The smoking habits questions were the same in each study area, except for one town in the Osaka Prefecture. All participants were asked: 'Do you smoke?' Response categories included: (1) yes; (2) smoked but quit; and (3) never smoked. We defined participants who chose response (1) as current smokers; those who chose response (2) as former smokers; and those who chose response (3) as non-smokers. For one city in the Osaka Prefecture, the response categories were: (1) yes (smoking every day); (2) yes, but occasionally; (3) smoked, but quit; and (4) never smoked. We defined participants who chose response (1) and (2) as current smokers, those who chose response (3) as former smokers, and those who chose response (4) as non-smokers.

The ages at initiation of smoking and the average number of cigarettes smoked per day for current and former smokers were obtained. The number of years of smoking that current smokers had smoked prior to enrollment was calculated by subtracting the age at initiation of smoking from the age at enrollment. Pack-years were defined as the number of years of smoking multiplied by the number of packs of cigarettes per day.

Cancer Prevention Study II. The CPS-II[9] is a prospective cohort study, conducted by the American Cancer Society (ACS). It was selected as the Western counterpart to our Japanese prospective cohort study because it contained detailed data on lung cancer mortality by sex, age group and smoking status, as well as data on smoking patterns of current smokers by sex and age group. The CPS-II data for the comparison were retrieved from the Smoking and Tobacco Control Monograph no. 8. Study participants were friends, neighbors, and acquaintances of ACS volunteers. Approximately 1.2 million men and women were enrolled in 1982. Enrollment included all household members 30 years of age or older if at least one family member was 45 years of age or older. Study participants completed an initial questionnaire including smoking habits and other lifestyle factors. The vital status of study participants was determined through personal inquiry by the volunteers. The underlying cause of death was obtained through death certificates. During the 6-year follow up of 711 363 current cigarette smokers and lifelong non-smokers, 3 229 died of lung cancer.

Statistical Analysis. Person years during the follow-up were counted from the date of enrollment into the study until the date of death, migration from the study areas, or the end of the study period, whichever came first. The RR was estimated with a Cox proportional hazards model with adjustments for age (continuous variable) and prefecture. Non-smokers were used as a reference category. A dose–response relationship among current smokers was examined in terms of the number of pack-years.

Using data from the CPS-II, we compared the baseline data on smoking patterns among current smokers and the follow-up data on lung cancer deaths among non-smokers and current smokers. Follow-up data were restricted to the first 6 years, the duration of the CPS-II. The mean number of cigarettes smoked per day and the mean number of years of smoking were calculated within the 5-year age groups fixed at the baseline. The age-adjusted number of cigarettes smoked per day and the age-adjusted number of years of smoking was obtained by directly standardizing to the combined distribution of age groups of the Japanese and US cohorts. Because the mean age at initiation of smoking among the CPS-II subjects was provided as 10-year birth cohorts, we calculated mean age of initiation in the Japanese study in the same way.

Sex- and age-specific death rates of lung cancer (per 100 000) were computed for non-smokers and current smokers. Calculation of the number of person years at risk was based on attained age. To compare the death rates of the Japanese and US cohorts, cumulative death rates between 40 and 84 years were presented. Rate ratios of the Japanese cohort to US cohort were calculated by using a Poisson regression model.

Lung cancer death rates were computed for male current smokers, stratified by the duration of smoking and the number of cigarettes smoked per day. Because of limited CPS-II data, only subjects who smoked 20 or 40 cigarettes per day were analyzed. To compare the lung cancer risks among male current smokers in Japan to those in the USA, adjusted rate ratios were obtained by Poisson regression analysis. The model included the natural logarithm of the number of lung cancer deaths as a response variable and the natural logarithm of person-years as an offset. Indicator variables for age group, number of cigarettes per day, and duration of smoking were used as covariates. Statistical computations were carried out using the SAS statistical package (version 8.02; SAS Institute, Cary, NC, USA).

Results

Current and former smokers in the Three-Prefecture Cohort Study showed a significantly increased risk of lung cancer death for both men and women compared with non-smokers (Table 1). A statistically significant dose–response trend of RR was observed for men and women current smokers (Table 2).

In the first 6 years of follow up, the Three-Prefecture Cohort Study had 341 deaths due to lung cancer (260 men and 81 women). Adjusted RR for current smokers versus non-smokers were 3.16 (95%CI 1.29–3.64) for men and 2.68 (95%CI 1.58–4.53) for women. Corresponding reported RR in the CPS-II study were 23.2 (95%CI 19.3–27.9) for men and 12.8 (95%CI 11.3–14.7) for women.

Death rates among current smokers and non-smokers were calculated, based on attained age (Fig. 1). Compared with the CPS-II, death rates among Japanese current smokers were lower in all age groups, with the exception of the youngest and oldest female age groups. In contrast, death rates among Japanese non-smokers were higher than those in the USA, for both men and women regardless of age. Cumulative death rates between 40 and 84 years and rate ratios are presented in Table 3. Compared with US non-smokers, Japanese non-smokers had a higher cumulative mortality of lung cancer with an approximately threefold increased risk for men and a twofold increased risk for women. However, Japanese current smokers were at a significantly 60% lower risk of lung cancer compared to those in the USA.

The mean number of cigarettes smoked per day (Fig. 2a) decreased with age for men and women in both Japan and the USA. However, current smokers in Japan had a lower daily

Table 1. Relative risk of lung cancer death associated with cigarette smoking, Three-Prefecture Cohort Study, Japan

Smoking status	No. subjects	Person-years	No. lung cancer deaths	Crude mortality rates	Relative risk† (95%CI)
Men					
Non-smokers	7 590	64 645	23	35.6	1.00
Former smokers	11 164	91 792	102	110.9	2.60 (1.65–4.10)
Current smokers	25 697	215 139	341	158.5	5.10 (3.34–7.79)
Women					
Non-smokers	36 884	321 170	79	24.6	1.00
Former smokers	1 630	13 258	13	98.1	2.94 (1.63–5.31)
Current smokers	5 188	42 931	40	93.2	3.66 (2.50–5.35)

†Adjusted for age and prefecture.

Table 2. Relative risk of lung cancer death by pack-years among current smokers, Three-Prefecture Cohort Study, Japan

Pack-years of smoking	No. subjects	Person-years	No. lung cancer deaths	Crude death rate	Relative risk† (95%CI)
Men‡					
<20	3 982	33 592	19	56.6	1.16 (0.72–1.88)
20–39	12 066	101 910	113	110.9	2.10 (1.62–2.71)
40–59	6 574	54 374	129	237.2	2.86 (2.23–3.65)
60 +	2 765	22 770	78	342.6	4.44 (3.34–5.89)
P for trend					<0.0001
Women§					
<20	3 136	26 212	12	45.8	1.75 (0.96–3.19)
20–39	1 545	12 642	15	118.7	3.92 (2.27–6.76)
40 +	397	3 157	10	316.8	7.22 (3.75–13.9)
P for trend					<0.0001

†Adjusted for age and prefecture. Reference category was non-smokers. ‡310 men were excluded because of missing data. §110 women were excluded because of missing data.

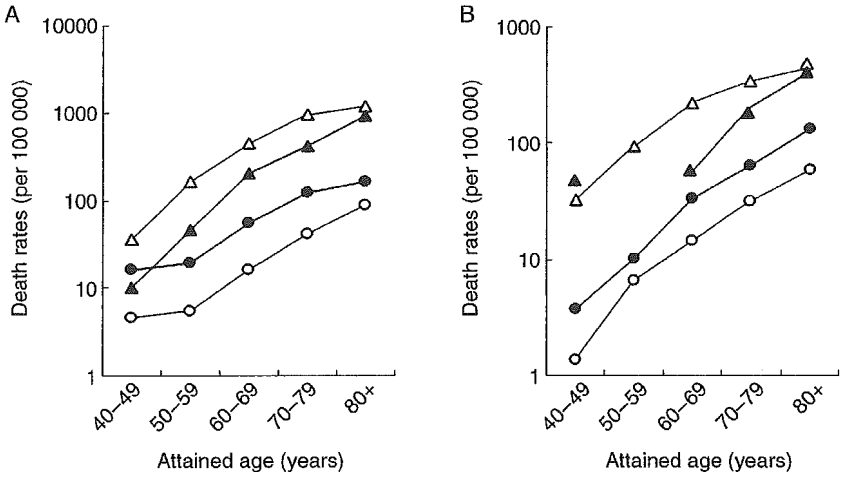

Fig. 1. Age-specific death rates due to lung cancer by attained age among current smokers and non-smokers in the Three-Prefecture cohort in Japan and Cancer Prevention Study II (CPS-II) in the USA. (a), Death rates of men; (b), death rates of women. (▲), Three-Prefecture cohort current smokers; (●), Three-Prefecture cohort nonsmokers; (△), CPS-II current smokers; (○), CPS-II non-smokers.

cigarette consumption for all age groups and for both men and women than current smokers in the USA. The differences ranged from 0.8 (aged 40–44 years) to 4.4 (aged 55–59 years) for men. Daily consumption of cigarettes in the youngest male age group showed the least difference. Japanese women constantly used approximately five fewer cigarettes per day in all age groups. The age-adjusted number of cigarettes per day for the Japanese and US cohorts were 21.5 and 24.8 for men, respectively, and 14.1 and 19.4 for women, respectively.

The mean number of years of smoking was slightly lower among Japanese men in all age groups than those in the USA (range 0.8–2.1) (Fig. 2b). Except for the youngest and oldest age groups, Japanese women had smoked for a much shorter time than comparable women in the USA. The range of differences was from 1.7 (aged 75–79 years) to 8.9 (aged 55–59 years). The age-adjusted years of smoking for the Japanese and US smokers were 37.1 and 38.6 years for men, respectively, and 26.8 and 34.2 years for women, respectively.

Japanese smokers in all age groups started smoking later than their counterparts in the USA, and this was especially true for women (Fig. 2c). While the age at initiation of smoking for Japanese women gradually became younger in recent birth

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Table 3. Cumulative mortality and rate ratios for lung cancer among non-smokers and current smokers, Three-Prefecture Cohort Study in Japan compared to Cancer Prevention Study II in the USA

	Non-smokers		Current smokers	
	Three-Prefecture	CPS-II	Three-Prefecture	CPS-II
Men				
Cumulative mortality rate (%)†	3.0	1.1	11.6	27.5
Rate ratio‡ (95%CI)	2.95 (1.79–4.87)	1.00	0.38 (0.32–0.41)	1.00
Women				
Cumulative mortality rate (%)†	1.9	0.8	5.3	11.6
Rate ratio‡ (95%CI)	2.10 (1.56–2.82)	1.00	0.42 (0.27–0.67)	1.00

Analysis restricted to first 6 years of follow-up to enhance comparability to Cancer Prevention Study II (CPS-II) data. †Cumulative mortality rates between 40 and 84 years. ‡Estimated based on Poisson regression model.

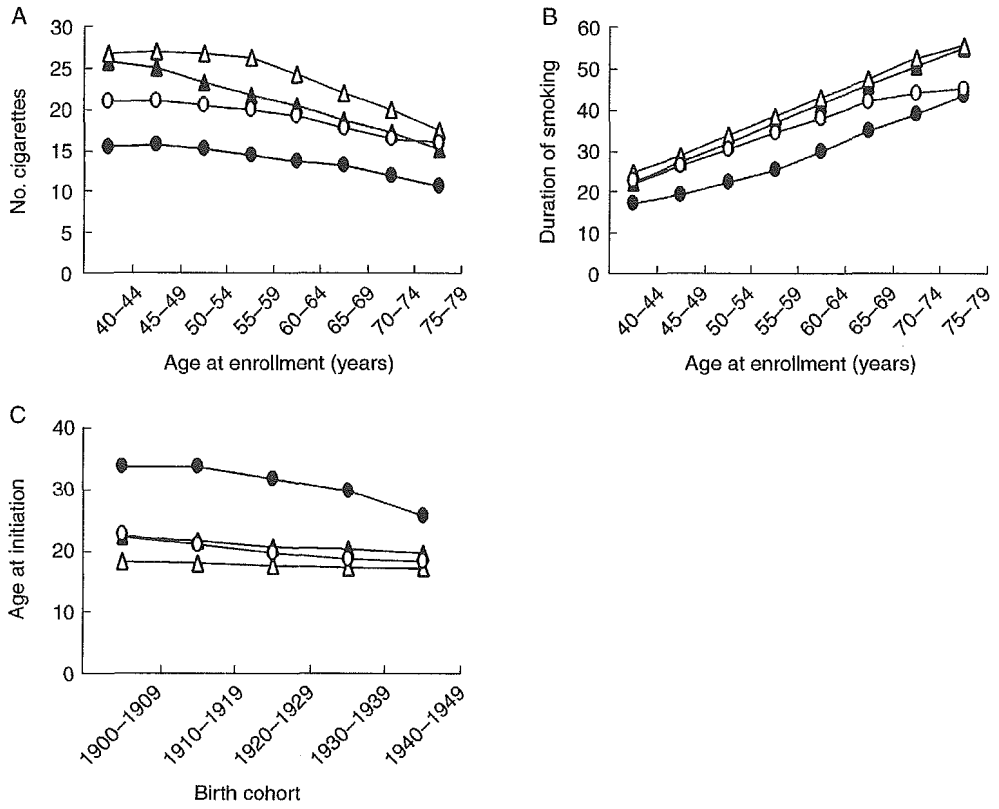

Fig. 2. Comparison of smoking patterns of current smokers at baseline between Three-Prefecture study in Japan and Cancer Prevention Study II (CPS-II) in the US. (a), Mean number of cigarettes smoked per day by age at enrolment; (b), mean duration of smoking by age at enrolment; (C), mean age of initiation of smoking by birth cohort. (▲), Three-Prefecture cohort men; (●), Three-Prefecture cohort women; (△), CPS-II men; (○), CPS-II women.

cohorts, they still began smoking much later than US women. The mean age at initiation of smoking among Japanese men in all birth cohorts was slightly older than those in the USA.

Finally, we calculated lung cancer death rates by years of smoking among current male smokers who had consumed 20 cigarettes per day (Table 4). Similar calculations for men who had smoked 40 cigarettes per day are not presented because there were too few of these men. We were unable to calculate lung cancer death rates in strata where no deaths occurred. For strata where calculations could be made, death rates of current Japanese smokers were lower than those in the USA. Rate ratios in all strata were less than 0.6. After controlling for age, duration of smoking and number of cigarettes smoked per day by the Poisson regression analysis, rate ratios of male Japanese current smokers relative to those in the USA was 0.34 (95%CI 0.27–0.43).

Discussion

The present large-scale, population-based prospective study confirmed an increased lung cancer risk among smokers, as compared with non-smokers, in Japan. The RR observed for Japanese smokers was lower than that observed in the USA. This finding is consistent with other studies conducted in Japan.[1–6] Comparison of death rates and exposure levels of current smokers in the two samples revealed one reason for the lower RR in Japan, namely, higher death rates among non-smokers

Table 4. Death rates by duration of smoking among current male smokers of 20 cigarettes per day, Three-Prefecture Study in Japan compared to the Cancer Prevention Study II in the USA

Attained age (years)	Three-Prefecture Duration*			CPS-II Duration†			Rate ratio Duration†		
	30–39	40–49	50 +	30–39	40–49	50 +	30–39	40–49	50 +
50–59	42.0	—	—	143.1	267.3	483.1	0.29	—	—
60–69	119.0	170.1	—	215.7	452.3	848.5	0.55	0.38	—
70–79	180.5	142.1	590.6	455.9	702.1	1149.0	0.40	0.20	0.51

†Duration of smoking was fixed at enrollment. —, no lung cancer deaths observed (Three-Prefecture cohort study), or no data available because of five or fewer deaths observed (Cancer Prevention Study II).

combined with lower death rates among smokers. A lower exposure level to smoking was responsible for the lower death rates among current smokers. However, even after adjustment for age, duration of smoking and daily cigarette consumption, male Japanese current smokers had a lower risk of lung cancer compared to those in the USA.

Death rates for non-smokers in all Japanese age groups were higher than those for non-smokers in the USA. The CPS-II used more detailed questions regarding smoking habits. For example, the CPS-II questionnaire clearly asked whether or not participants had smoked at least one cigarette per day for 1 year.[9] However, the questionnaire in our study did not specify the number of cigarettes or the duration of smoking. Therefore, the definition of non-smokers in the CPS-II was more strictly limited in terms of lifelong non-smokers, while non-smokers in our study might have included former smokers who had quit and not smoked for a long time. Such a difference in classification of non-smokers might have led to overestimation of death rates among Japanese non-smokers. Second-hand smoking might also have contributed to the difference. The prevalence of current smokers among Japanese subjects was higher than in the CPS-II. Among Japanese men, the prevalence was 58% for current smokers and 83% for ever smokers (ever smokers = current + former smokers); somewhat higher than the prevalence reported in the CPS-II (24% for white, male current smokers, 75% for white, male ever smokers, 36% for black, male current smokers, and 73% for black, male ever smokers).[9] Therefore, Japanese non-smokers might have had more opportunity to be exposed to environmental tobacco smoke (ETS). Furthermore, it was only in 2003 that Japanese law promoted the separation of smoking and non-smoking areas at the workplace and in public places. As well, since Japanese residences are small, Japanese non-smokers who had lived with parents or a spouse who smoked would have been exposed to concentrated tobacco carcinogens. Some, but not all, Japanese studies showed higher RR associated with spousal ETS,[11] and a pooled RR calculated from Japanese studies (1.41) was higher than the pooled RR calculated from US studies (1.19).[11] Therefore, until recently, Japanese non-smokers would have had a much higher cumulative exposure to ETS at home and in the workplace than their US counterparts.

Other risk factors, such as air pollution, radon and asbestos, do not offer a clear explanation for the observed differences. Several observational studies have shown an association between air pollution levels and lung cancer.[12,13] Even if a difference in air pollution levels exists between the two countries, it is unlikely that this small difference could account for the large difference in the risk of lung cancer among non-smokers given the only moderate association between air pollution and lung cancer.[14] The level of indoor radon in Japan, a known risk factor for lung cancer in Western countries[15] is much lower than in the USA.[16] Although asbestos consumption per capita was higher in Japan than in the USA during the mid-1970s,[17] it remains unknown whether low environmental exposure to asbestos (in contrast to heavy occupational exposure) causes lung cancer.[18]

In contrast to non-smokers, death rates among current smokers in our sample were lower than those observed in the CPS-II sample, regardless of age and sex. Because lung cancer risk and exposure level to smoking are clearly dose-related, the discrepancy in exposure levels among current smokers is probably a major factor explaining the difference in death rates among current smokers. However, considering lower exposure as a reason for the lower death rates among current smokers assumes that individuals with similar exposure levels have the same risk of lung cancer. However, the risk of lung cancer among male Japanese current smokers was lower than those in the USA, even after adjustment for age, duration of smoking and number of cigarettes smoked per day.

Although the difference in smoking patterns between the Japanese and US samples was greater among women than among men, the rate ratio for the current smokers was not very different between men and women. We have no clear explanation for this. However, the unit change in the lung cancer risk between Japanese female smokers and US female smokers with low levels of smoking exposure might not have the same magnitude as the unit change seen between Japanese male smokers and US male smokers with high levels of smoking exposure. Furthermore, Japanese women might under-report their smoking history. A single inquiry about smoking at baseline might not reflect the whole smoking history of individuals in either the Japanese or US samples.

Caution is advised when exposure levels to smoking are assessed, based on self-reported smoking history collected from a single questionnaire at the point of enrollment. Cigarette consumption per capita was much lower in Japan than in the USA from 1920 to 1970,[19] when the participants in these two cohorts were in adolescence to young adulthood. Furthermore, Japanese smokers experienced an extreme tobacco shortage during and immediately after World War II. It was not until the late 1970s that Japanese cigarette consumption per capita caught up with US consumption levels. Japanese participants classified in the same strata by smoking exposure undoubtedly experienced periods of cigarette shortage, and this bias toward overestimation of exposure may have produced spurious lower lung cancer death rates in our sample. Similarly, possible bias in the CPS-II sample may have included smokers who underreported usage of cigarettes due to strong social prohibitions to smoking in the USA.

Changes in tar content and the prevalence of filter-tipped cigarettes were also influential. The sales-weighted average yields of tar in the 1980s, and the reduction in tar levels during the 1960s and 1970s were similar in Japan and the USA.[20,21] Filter-tipped cigarettes were first marketed in the 1950s and their market share grew to more than 80% in the 1970s, reaching over 90% in both countries. However, as Stellmen *et al.* have noted, American manufactured cigarettes contain higher tobacco-specific nitrosamines than Japanese cigarettes.[22] Furthermore,

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charcoal filters, which remove certain compounds that inhibit lung clearance, are more widely used in Japanese cigarettes than American cigarettes.

Causes of death, other than lung cancer, might be influential in the estimation of lung cancer death rates among current smokers. Coronary heart disease (CHD) was the second leading cause of death among CPS-II smokers.[9] Premature death from CHD among CPS-II smokers might have led to somewhat lower lung cancer death rates in the USA. An increase in the discrepancy of lung cancer death rates among current smokers might have occurred, because death rates from CHD in Japan are not as high as in the USA.[23]

Other confounding factors, such as lifestyle or genetic factors, might also lower lung cancer death rates among Japanese smokers. The traditional Japanese diet, which is low in fat and high in several phytochemicals, might help decrease the risk of death due to lung cancer.[24–27] Deletion-type polymorphism CYP2A6, the principal enzyme in the metabolic activation of tobacco-specific nitrosamines, was found to be inversely associated with lung cancer among Japanese male smokers.[28] It has been demonstrated that the frequency of occurrence of this variant is higher amongst Japanese than among Caucasians.[29] However, caution is required, because diet and the odds of having CYP2A6 can be assumed to be constants (i.e. would be equally likely to affect non-smokers) and non-smokers presented the opposite pattern to current smokers.

Another potential explanation is different histological distribution of lung cancer between American and Japanese populations.[30] Adenocarcinoma, which is less strongly related to smoking than squamous cell carcinoma,[2] contributes to a larger proportion of Japanese lung cancer than US lung cancers. The relatively lower incidence of squamous cell carcinoma among Japanese smokers would reduce the overall Japanese lung cancer incidence for the same level of exposure to smoking as in the US cohort.

Generally, in Western countries non-smokers have a higher socioeconomic status than smokers. People with a high socioeconomic status tend to have more health conscious lifestyles, such as a higher intake of fruits and vegetables, as well as lower occupational exposures to other factors, such as asbestos. In the USA, the socioeconomic gap between smokers and non-smokers is much larger due to a strong societal antismoking campaign. This larger disparity of background risk factors resulted in a larger difference of lung cancer mortality between US non-smokers and smokers, as compared with Japanese non-smokers and smokers.

Finally, the comparability of the Japanese and US samples should be considered. A potential advantage was that both studies were conducted using a prospective design during approximately the same time period. Dates of birth of participants covered approximately the same years. Because cigarette types, such as non-filtered versus filtered cigarettes changed similarly in both the USA and Japan from the 1950s to the 1970s,[31] different study periods or birth cohorts might have weakened the comparability, especially in terms of exposure. In addition, lung cancer deaths were basically diagnosed by the same ICD-9 codes. Lung cancer deaths were determined based on death certificates for the US sample, and the Japanese sample lung cancer deaths were determined using the cancer registry, which was based on death certificate data. Death certificates were usually considered complete both in the US and Japan. As well, the cause of death was also considered to have been identified with reasonable accuracy. In 1988, the percentage of deaths with no classifiable diagnosis, including unknown cause of morbidity and mortality (ICD-9: 780–799) was 3.9% for Japan and 1.4% for USA. Therefore, both studies appeared to be equal in their precision of determining lung cancer deaths. Finally, follow-up periods were restricted to 6 years in both studies. However, over this relatively short time interval, there were too few deaths among the Japanese cohort to produce stable and informative estimates of death rates, especially at high exposure levels. To solve this problem, further investigation with samples as large as the CPS-II sample, or the pooling of several studies, are needed.

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Charcoal cigarette filters and lung cancer risk in Aichi Prefecture, Japan

Joshua E. Muscat,[1,5] Toshiro Takezaki,[3] Kazuo Tajima[4] and Steven D. Stellman[2]

[1]Department of Health Evaluation Sciences, Penn State Cancer Institute, Division of Population Sciences, Penn State College of Medicine, Room C3739, MC H069, 500 University Drive, Hershey, PA 17033, [2]Department of Epidemiology, Mailman School of Public Health, Columbia University, 722 West 168th Street, New York, NY 10032, USA; [3]Department of International Island and Community Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890–8544, and [4]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1–1 Kanokoden Chikusa-ku, Nagoya 464–8681, Japan

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The lung cancer mortality rate has been lower in Japan than in the United States for several decades. We hypothesized that this difference is due to the Japanese preference for cigarettes with charcoal-containing filters, which efficiently absorb selected gas phase components of mainstream smoke including the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. We analyzed a subset of smokers (396 cases and 545 controls) from a case-control study of lung cancer conducted in Aichi Prefecture, Japan. The risk associated with charcoal filters (73% of all subjects) was evaluated after adjusting for age, sex, education and smoking dose. The odds ratio (OR) associated with charcoal compared with 'plain' cigarette filters was 1.2 (95% confidence intervals [CI] 0.9, 1.6). The histologic-specific risks were similar (e.g. OR = 1.3, 95% CI 0.9, 2.1 for adenocarcinoma). The OR was 1.7 (95% CI 1.1, 2.9) in smokers who switched from 'plain' to charcoal brands. The mean daily number of cigarettes smoked in subjects who switched from 'plain' to charcoal brands was 22.5 and 23.0, respectively. The findings from this study did not indicate that charcoal filters were associated with an attenuated risk of lung cancer. As the detection of a modest benefit or risk (e.g. 10–20%) that can have significant public health impact requires large samples, the findings should be confirmed or refuted in larger studies. (*Cancer Sci* 2005; 96: 283–287)

Most cigarette brands that are manufactured and sold in Japan contain activated carbon (charcoal) granules embedded in the filter. The charcoal filter efficiently absorbs gas phase toxins in mainstream smoke including hydrogen cyanide, formaldehyde, ammonia and crotonaldehyde. Under standard US Federal Trade Commission (FTC) machine-smoking conditions, selected Japanese cigarettes with charcoal filters delivered similar yields of carbon monoxide and nicotine but substantially lower yields of the pulmonary carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone than selected American filter cigarettes.[1] The charcoal filter possibly limits the adverse health effects from smoking, but direct claims of risk reduction have not been made as empiric evidence is lacking. The charcoal also absorbs volatiles that flavor the cigarette and consequently the bland flavor and taste is considered unacceptable to American consumers. Japanese smokers perceive the taste as smoother than American brands.[2] Charcoal cigarettes make up <1% of all cigarette sales in the United States and about 70% in Japan. (Before 1981 there was no information on sales data in Japan. Since 1981, the annual market share of charcoal cigarettes has risen slightly from about 68% to over 70%.)

The higher smoking prevalence in Japanese men compared with American men, but lower rates and risk of lung cancer, has long been considered a 'paradox'.[3–6] It has been suggested that Japanese cigarettes are less toxic than Western brands. Because both charcoal filter cigarettes and 'plain' filter cigarettes are smoked in Japan, we determined the risk of lung cancer associated with filter type in a Japanese population. The overall methods for this study were described previously.[5]

Materials and Methods

Subject recruitment. We conducted a case-control study of cigarette smoking and lung cancer in Aichi Prefecture, the third largest metropolitan area in Japan. Aichi Prefecture has over five million residents, including two million in its largest city, Nagoya, and 300 000 in Okazaki City.[7] The Aichi Cancer Center, National Nagoya Hospital, First Red Cross Hospital, Aichi Prefecture Hospital and several smaller hospitals recruited newly diagnosed incident patients with histologically confirmed lung cancer. The hospital staff, physicians, nurses and study interviewers identified eligible patients between 1993 and 1998 from surgical schedules and admission rosters. The case eligibility included an age of 20–81 years, no previous diagnosis of lung, oral, kidney, bladder or pancreas cancer, and ability to participate and provide informed consent. The staff abstracted information on the diagnosis and histology from the pathology reports and medical records. The response rate was approximately 90%.

The study included both hospital controls and community-based controls. The eligibility criteria of the two control groups were the same as for cases except that the hospital controls were admitted for non-malignant diseases or conditions unrelated to cigarette smoking. A patient with a non-tobacco-related cancer was selected only if there were no other available control. The controls were identified from admission rosters and matched to cases by age (within 5 years), sex, hospital and date of interview (±4 months). The patient's physician was contacted to obtain consent for the interview. The controls were grouped by ICD-9 code categories. These included genitourinary system disorders such as kidney calculus and renal failure (37% of controls), digestive system disorders such as hernia, cholelithiasis and cirrhosis (16%), symptoms, signs and ill-defined conditions (13%), injuries and poisoning (11%), musculoskeletal and connective tissue diseases (11%), diabetes and other endocrine disorders (6%), nervous system disorders (4%) and cancer (2%). The response rate was approximately 90% but because physician consent was not obtained for all controls, the sample size of hospital controls was smaller than that for cases.[5]

We selected community controls using a stratified sampling scheme that was based on the age (within 5 years), sex and residential district of the hospital where the cases were admitted. Within each stratum, two controls were selected randomly from the Aichi Prefecture electoral records that are kept in Nagoya

[5]To whom correspondence should be addressed. E-mail: jmuscat@hmc.psu.edu

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Table 1. Characteristics of smokers in cases, hospital controls and community controls, Aichi Prefecture, Japan

Subject characteristic	Cases $n = 396$ (%)	Hospital controls $n = 224$ (%)	Community controls $n = 321$
Sex			
Men	348 (87.9)	201 (89.7)	280 (87.2)
Women	48 (12.1)	23 (10.3)	41 (12.8)
Mean age in years	61.5 ± 9.9	57.0 ± 10.1	61.6 ± 10.0
Mean years of education	11.1 ± 2.9	11.8 ± 2.9	12.0 ± 2.9
Smoking status			
Current	297 (75.0)	135 (60.3)	177 (55.1)
Former	99 (25.0)	89 (39.7)	144 (44.9)
Histology			
Adenocarcinoma	168 (42.5)		
Squamous cell carcinoma	109 (27.6)		
Small cell carcinoma	91 (23.0)		
Other/mixed	28 (6.9%)		

and Okazaki City. Each electoral record includes name, mailing address and birth date. The interviewers placed telephone calls to enlist participation. The telephone numbers were obtained from the information service of the telephone company. Forty percent of the community controls were interviewed. The same study interviewer assigned to a case patient made an appointment to visit the control subject at home.

All subjects signed an informed consent form that was approved by their respective hospital's Institutional Review Board. After consent was obtained, subjects were interviewed in person using a structured questionnaire that contained detailed items on smoking history including cigarette brand, years of smoking, cigarettes per day (cpd) and year of smoking cessation.

Statistical analysis. This analysis included subjects who reported smoking cigarettes regularly, defined as at least one cigarette per day for one or more years. Never smokers were excluded. The sample included 396 cases, 224 hospital controls and 321 community controls. The cigarette box label identifies whether the brand is manufactured with a charcoal filter. Of 1133 ever smokers, 941 (82.7%) of the current and former smokers reported that their most recent brand was a filter cigarette (82.7%). One hundred and ninety-two (17.3%) reported that their most recent brand was a non-filter cigarette or could not identify the filter type. These subjects were not included in the analysis.

Univariate analysis of the data included means and standard deviations. Odds ratios (OR) and 95% confidence intervals (CI) were derived from unconditional logistic regression analysis. The main effect variable was coded as '1' for charcoal filters and '0' for 'plain' cigarettes. The OR were adjusted for sex, age, education, smoking status and pack-years of smoking. We also modeled the risk adjusted for sex, age, education and years since quitting smoking. In the latter analysis, index variables were created for years since quitting smoking (<5, 6–10, 11–20 and >20), with current smokers serving as the referent group. Histologic-specific risks were calculated using smoking and other information from the entire control group. All statistical tests were two-sided.

For subjects who smoked more than one brand in their lifetime, we carried out an analysis based on the two most recent types of cigarettes smoked. The main effect variables were classified as 'charcoal only' or 'mixed' (e.g. charcoal and 'plain'). The referent group was 'plain only.' Those subjects who reported that their most recent brand was a filter cigarette but that their second most recent brand was a non-filter cigarette were further excluded.

Results

The distribution of sex, age, education and smoking history are shown in Table 1. Almost 90% of both cases and controls were men, reflecting the historically low prevalence of smoking among Japanese women. The average age was approximately 61 years in cases and 60 years in controls, Controls had a higher mean level of education. Seventy-five percent of cases, 60% of hospital controls and 55% of community controls were current smokers. The most common histopathologic types of lung cancer were adenocarcinoma (43%), squamous cell carcinoma (28%) and small cell carcinoma (23%).

The characteristics of the subjects were compared by cigarette filter type and are shown in Table 2. For 680 subjects (73%), the most recent brand of cigarette was a charcoal brand whereas for 257 subjects (27%) it was a 'plain' brand. Data on cigarette amount was missing for four subjects. Current smokers were more likely to smoke charcoal brands than former smokers. There were few differences in filter preference by sex, age and education (Table 2).

The overall OR associated with the most recent brand of cigarette was 1.2 (95% CI 0.9, 1.6, Table 3) for charcoal versus 'plain'. The OR was 1.1 (95% CI 0.7, 1.7) when the analysis was limited to cases and hospital controls only, and 1.3 (95% CI 0.9, 1.9) when the analysis was limited to cases and community controls only. In a model that substituted years since quitting for pack-years and smoking status, the overall OR associated with charcoal filter versus 'plain' filter was 1.1 (95% CI 0.8, 1.6; Table 3). In an analysis limited to men only, the OR for charcoal filter versus 'plain' filter was 1.1 (95% CI 0.8, 1.6). In histologic-specific analyses, the risk associated with charcoal versus 'plain' filter of the most recent brand of cigarette was 1.3 (95% 0.9–2.1) for adenocarcinoma, 1.2 (95% CI 0.7, 2.1) for squamous cell carcinoma, and 0.6 (95% CI 0.4, 1.1) for small cell carcinoma (Table 3).

In an examination of the two most recent cigarette brands smoked, 198 of the 941 smokers of filter cigarettes smoked a non-filter brand previously. Of the remaining 743, 361 were classified as smoking charcoal brands only (this number includes 45 subjects whose smoking history was only one brand of charcoal cigarette), 259 smoked both 'plain' and charcoal brands, and 123 smoked 'plain' brands (this number includes 44 subjects whose smoking history was only one brand of 'plain' cigarettes). Subjects who smoked both charcoal and plain brands were classified as 'switchers.' In the majority of cases, the switchers were smokers who changed from smoking a 'plain'

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Table 2. Characteristics of smokers by case-control status and filter type (e.g. charcoal vs 'plain'), Aichi Prefecture, Japan

Subject characteristic	Cases		Controls	
	Charcoal n = 304 (%)	'Plain' n = 92 (%)	Charcoal n = 379 (%)	'Plain' n = 166 (%)
Sex				
Men	261 (85.9)	87 (94.6)	331 (87.3)	150 (90.4)
Women	43 (14.1)	5 (5.4)	48 (12.7)	16 (9.6)
Mean age in years	61.2 ± 10.0	62.6 ± 9.5	58.8 ± 10.3	61.6 ± 9.4
Mean years of education	11.0 ± 2.6	11.2 ± 3.5	11.9 ± 2.9	12.0 ± 2.8
Smoking status				
Current	235 (77.3)	62 (67.4)	257 (67.8)	55 (33.1)
Former	69 (22.7)	30 (32.6)	122 (32.2)	111 (66.9)
Cigarettes per day	28.3 ± 15.1	31.6 ± 16.4	24.9 ± 14.7	25.5 ± 17.4

Based on the most recent brand of cigarette.

Table 3. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for filter type and lung cancer histology

Cigarette filter	All		AC		SCC		SmCC	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Most recent brand (n = 941)								
'Plain'	1.0		1.0		1.0		1.0	
Charcoal	1.2	0.9, 1.6	1.3	0.9, 2.1	1.2	0.7, 2.1	0.6	0.4, 1.1
Last two brands (n = 743)								
'Plain' only	1.0		1.0		1.0		1.0	
Mixed	1.7	1.1, 2.9	2.1	1.1, 4.0	2.5	1.0, 6.3	0.6	0.3, 1.5
Charcoal only	1.4	0.8, 2.2	1.6	0.8, 3.1	1.6	0.6, 4.1	0.6	0.3, 1.3

Odds ratios were adjusted for sex, age, education, smoking status (current vs former) and pack-years. The odds ratio was 1.1 (95% CI 0.8–1.6) after adjustment for sex, age, education, cigarette amount and years since quitting. Odds ratios associated with the last two brands of cigarettes were based on 743 subjects (includes 654 subjects who smoked two brands, 45 subjects who smoked only one brand of charcoal and were classified as charcoal only, and 44 subjects who smoked only one brand of 'plain' cigarettes and were classified as 'plain' only). AC, adenocarcinoma; SCC, squamous cell carcinoma; SmCC, small cell carcinoma.

Table 4. Average number of cigarettes smoked per day (cpd) for the two most recent brands

Cigarette history	Filter type	n	cpd	Filter type	n	cpd
Most recent brand	Charcoal	543	27.2 ± 15.0	'Plain'	111	24.3 ± 13.9
Previous brand	'Plain'	220	25.1 + 13.0	Charcoal	32	25.0 ± 12.2
	Charcoal	316	26.1 ± 13.8	'Plain'	78	24.0 ± 14.6

A few subjects had missing data for cpd on the previous brand smoked.

brand to a charcoal brand. Only 32 subjects switched from a charcoal to a 'plain' brand. Many switchers had a history of smoking three brands. In nearly all cases, the third most recent brand was also a 'plain' cigarette. Only nine subjects reported switching from a charcoal to a 'plain' back to a charcoal brand. Excluding the subjects whose second previous brand was a non-filter cigarette, the overall OR for the last two brands was 1.4 (95% CI 0.8, 2.2) for charcoal only versus 'plain' only, and 1.7 (95% CI 1.1, 2.9) for mixed versus 'plain' only (Table 3). Similar findings were observed in separate analyses using hospital controls only and community controls only (data not shown). A significant increased risk for adenocarcinoma of the lung was observed in subjects who switched from 'plain' to charcoal versus subjects whose last two brands were 'plain' cigarettes (Table 3).

The mean number of cigarettes per day in smokers who smoked two or more brands is shown separately for subjects who smoked charcoal brands only, mixed smokers, and 'plain' brands only (Table 4). The mean number of cigarettes smoked per day was 27.2 for charcoal brands and 24.3 for 'plain' brands. For smokers of charcoal brands that had switched from a previous brand, the mean number of cigarettes smoked per day increased by approximately one to two, regardless of the filter type of the previous brand.

Discussion

The introduction of filter cigarettes into the US market approximately 50 years ago was anticipated to reduce the future incidence rate of lung cancer. There are conflicting findings on whether this occurred in smokers who switched from high-tar cigarettes to low-tar cigarettes.[8] In Japan, the risk of lung cancer for those who smoked filter cigarettes all their life compared with subjects who smoked both non-filter and filter cigarettes was 0.70 (95% CI 0.4–1.2).[9] These findings indicate that while filtration substantially reduces exposure to tobacco carcinogens, the possible benefits might be lower than anticipated because of compensatory smoking behaviors.

Another technological approach to reduce the hazards from smoking is the development of a more efficient filtration system than that provided by a typical acetate filter.[10] The charcoal filter reduces exposure to several gas phase volatile compounds under FTC machine-smoking conditions. Selected Japanese charcoal brands deliver 30% lower yields of both tar and CO,