AhR-mediated signaling. Because of the small sample size, this is not a conclusive study, and these results need to be investigated further in large studies that also consider ethnic variation.

In summary, *AhRR* codon 185 polymorphism is associated with susceptibility to and severity of endometriosis in Japanese women. *AhR* codon 554 and *ARNT* codon 189 polymorphisms appeared not to be associated with endometriosis. The pathogenesis of endometriosis is still not clearly understood. The *AhRR* codon 185 polymorphism could be a useful genetic marker to predict the susceptibility to and severity of endometriosis.

ACKNOWLEDGEMENTS

We thank Tomoyuki Hanaoka, MD, PhD, Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, for helpful discussions and Hiroyuki Nakao, PhD, Department of Public Health, Miyazaki Medical College, University of Miyazaki, for statistical advice and analysis.

This work was supported in part by a Grant-in-Aid for Risk Analysis Research on Food and Pharmaceuticals and Cancer Research from the Ministry of Health, Labour and Welfare of Japan.

References

1. Wheeler JM. Epidemiology of endometriosis-associated infertility. J Reprod Med 1989;34:41-46.

2. Rawson JM. Prevalence of endometriosis in asymptomatic women. J Reprod Med 1991;36:513-515.

3. Kennedy S. The genetics of endometriosis. Eur J Obstet Gynecol Reprod Biol 1999;82:129-33.

4. Zondervan K, Cardon L, Kennedy S. Development of a Web site for the genetic epidemiology of endometriosis. Fertil Steril 2002;78:777-81.

5. Arvanitis DA, Koumantakis GE, Goumenou AG, Matalliotakis IM, Koumantakis EE, Spandidos DA. CYP1A1, CYP19, and GSTM1 polymorphisms increase the risk of endometriosis. Fertil Steril 2003;79:702-9.

6. Chang CC, Hsieh YY, Tsai FJ, Tsai CH, Tsai HD, Lin CC. The proline form of p53 codon 72 polymorphism is associated with endometriosis. Fertil Steril 2002;77:43-5.

7. Georgiou I, Syrrou M, Bouba I, Dalkalitsis N, Paschopoulos M, Navrozoglou I, et al. Association of estrogen receptor gene polymorphisms with endometriosis. Fertil Steril 1999;72:164-6.

8. Elizondo G, Fernandez-Salguero P, Sheikh MS, Kim GY, Fornace AJ, Lee KS, et al. Altered cell cycle control at the G(2)/M phases in aryl hydrocarbon receptor-null embryo fibroblast. Mol Pharmacol. 2000;57:1056-63.

9. Telakowski-Hopkins, C.A., R.G. King, and C.B. Pickett. Glutathione S-transferase Ya subunit gene: Identification of regulatory elements required for basal level and inducible expression. Proc. Natl. Acad. Sci 1988;85:1000-1004.

10. Whitlock JP Jr. Induction of cytochrome P4501A1. Annu Rev Pharmacol Toxicol. 1999;39:103-25.

11. Alexander DL, Eltom SE, Jefcoate CR. Ah receptor regulation of CYP1B1 expression in primary mouse embryo-derived cells. Cancer Res. 1997;57:4498-506.

12. Lucier GW, Portier CJ, Gallo MA. Receptor mechanisms and dose-response models for the effects of dioxins. Environ Health Perspect 1993;101:36-44.

13. Ohtake F, Takeyama K, Matsumoto T, Kitagawa H, Yamamoto Y, Nohara K et al. Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature 2003;423:545-50.

14. Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to

2,3,7,8-tetrachlorodibenzo-p-dioxin.Fundam Appl Toxicol. 1993;21:433-41.

15. Pauwels A, Schepens PJ, D'Hooghe T, Delbeke L, Dhont M, Brouwer A et al. The risk of endometriosis and exposure to dioxins and polychlorinated biphenyls: a case-control study of infertile women. Hum Reprod. 2001;16:2050-5.

16. Lebel G, Dodin S, Ayotte P, Marcoux S, Ferron LA, Dewailly E. Organochlorine exposure and the risk of endometriosis. Fertil Steril. 1998 ;69:221-8.

17. Wilson CL, Safe S. Mechanisms of ligand-induced aryl hydrocarbon receptor-mediated biochemical and toxic responses. Toxicol Pathol 1998;26:657-71.

18. Mimura J, Ema M, Sogawa K, Fujii-Kuriyama Y. Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. Genes Dev 1999;13:20-5.

19. Khorram O, Garthwaite M, Golos T. Uterine and ovarian aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator (ARNT) mRNA expression in benign and malignant gynaecological conditions. Mol Hum Reprod. 2002;8:75-80.

20. Kawajiri K, Watanabe J, Eguchi H, Nakachi K, Kiyohara C, Hayashi S. Polymorphisms of human Ah receptor gene are not involved in lung cancer.

Pharmacogenetics 1995;5:151-8.

21. Scheel J, Hussong R, Schrenk D, Schmitz HJ. Variability of the human aryl hydrocarbon receptor nuclear translocator (ARNT) gene. J Hum Genet 2002;47:217-24.

22. Cauchi S, Stucker I, Cenee S, Kremers P, Beaune P, Massaad-Massade L. Structure and polymorphisms of human aryl hydrocarbon receptor repressor (AhRR) gene in a French population: relationship with CYP1A1 inducibility and lung cancer. Pharmacogenetics 2003;13:339-47.

23. Watanabe T, Imoto I, Kosugi Y, Fukuda Y, Mimura J, Fujii Y. Human arylhydrocarbon receptor repressor (AHRR) gene: genomic structure and analysis of polymorphism in endometriosis. J Hum Genet 2001;46:342-6.

24. The American Fertility Society. Revised American Fertility Society classification of endometriosis: Fertil Steril. 1985;43:351-2.

25. de Kok JB, Wiegerinck ET, Giesendorf BA, Swinkels DW. Rapid genotyping of single nucleotide polymorphisms using novel minor groove binding DNA oligonucleotides (MGB probes). Hum Mutat. 2002;19:554-9.

26. Vessey MP, Villard-Mackintosh L, Painter R. Epidemiology of endometriosis in women attending family planning clinics. BMJ. 1993;306:182-4.

27. Eskenazi B, Warner ML. Epidemiology of endometriosis. Obstet Gynecol Clin North Am. 1997;24:235-58.

28. Goodman LA, Kruskal WH. Measures of association for cross classifications. J Am Stat Assoc. 1954;49:732-764.

29. Zondervan KT, Cardon LR, Kennedy SH. What makes a good case-control study? Design issues for complex traits such as endometriosis. Hum Reprod. 2002;17:1415-23.

30. Schenken RS, Williams RF, Hodgen GD. Effect of pregnancy on surgically induced endometriosis in cynomolgus monkeys. Am J Obstet Gynecol 1987;157:1392-6.

31. Barragan JC, Brotons J, Ruiz JA, Acien P. Experimentally induced endometriosis in rats: effect on fertility and the effects of pregnancy and lactation on the ectopic endometrial tissue. Fertil Steril. 1992;58:1215-9.

32. Hornstein MD, Gleason RE, Orav J, Haas ST, Friedman AJ, Rein MS et al. The reproducibility of the revised American Fertility Society classification of endometriosis. Fertil Steril. 1993;59:1015-21.

Table 1 Primers and probes used for real-time PCR analysis

Primers	Sequence
AhR codon 554	AGA to AAA, Arg to Lys
Forward primer	5' -AAA AAC AGT GAC TTG TAC AGC ATA ATG A-3'
Reverse primer	5' -CTG AAG TCA ACC TCA CCA GAA AAA T-3'
Probe: G allele	5' -FAM-TGA AGA CAT CAG ACA CAT-MGB-3'
Probe: A allele	5' -VIC-AGA CAT CAA ACA CAT GC-MGB-3'
ARNT codon 189	GTG to GTC, silent mutation
Forward primer	5' -TGC TGC CAA ACC ATT CAG ACT-3'
Reverse primer	5' -GGA ACT GAA ACA TTT GAT CTT GGA-3'
Probe: G allele	5' -VIC-CGG AGT CAG ACA CAT A-MGB-3'
Probe: C allele	5' -FAM-ACG GAG TCA GAG ACA T-MGB-3'
AhRR codon 185	CCC to GCC, Pro to Ala
Forward primer	5' -AGA CGG ATG TAA TGC ACC AGA A-3'
Reverse primer	5' -AGA GGC AGC GAT GTG TTA TGG-3'
Probe: C allele	5' -FAM-TGG GCA GCC CCC CGC C-TAMRA-3'
Probe: G allele	5' -VIC-TGG GCA GGC CCC GCC -TAMRA-3'

Table 2 Genotype and allele frequencies of AhR, ARNT, AhRR polymorphisms

Polymorphisms	Codons	Amino acids	Genotype frequencies		Allele frequencies	
			Endometriosis n (%)	Controls n (%)	Endometriosis n (%)	Controls n (%)
AhR codon 554						
G/G	AGA/AGA	Arg/Arg	24 (30.4)	22 (37.3)	G: 83 (52.5)	G: 73 (61.9)
A/G	AAA/AGA	Arg/Lys	35 (44.3)	29 (49.1)		
A/A	AAA/AAA	Lys/Lys	20 (25.3)	8 (13.6)	A: 75 (47.5)	A: 45 (38.1)
ARNT codon 189						
G/G	GTG/GTG	Val/Val	26 (32.9)	19 (32.2)	G: 92 (58.2)	G: 64 (54.2)
C/G	GTC/GTG	Val/Val	40 (50.6)	26 (44.1)		
C/C	GTC/GTC	Val/Val	13 (16.5)	14 (23.7)	C: 66 (41.8)	C: 54 (45.8)
AhRR codon 185						
C/C	CCC/CCC	Pro/Pro	20 (25.3)	27 (45.8)	C: 87 (55.1)	C: 81 (68.6)
C/G	CCC/GCC	Pro/Ala	47 (59.5)	27 (45.8)		
G/G	GCC/GCC	Ala/Ala	12 (15.2)	5 (8.4)	G: 71 (44.9)	G: 37 (31.4)

Table 3 AhR, ARNT and AhRR polymorphisms and risk of endometriosis

Polymorphism	Endometriosis n (%)	Controls n (%)	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
AhR codon 554				
G/G	24 (30.4)	22 (37.3)	1	1
A/G + A/A	55 (69.6)	37 (62.7)	1.36 (0.67-2.78)	1.65 (0.76-3.61)
ARNT codon 189				
G/G	26 (32.9)	19 (32.2)	1	1
C/G + C/C	53 (67.1)	40 (67.8)	0.97 (0.47-1.99)	0.86 (0.39-1.87)
AhRR codon 185				
C/C	20 (25.3)	27 (45.8)	1	1
C/G + G/G	59 (74.7)	32 (54.2)	2.49* (1.21-5.12)	2.53* (1.16-5.55)

[a]ORs adjusted for age and menstrual characteristics.

*P < 0.05

Table 4 AhRR codon 185 polymorphism and severity of endometriosis

Clinical stage	AhRR C/C genotype	AhRR C/G + G/G genotype	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
Controls n (%)	27 (45.8)	32 (54.2)	1	1
Stage Ⅰ-Ⅱ n (%)	8 (25.8)	23 (74.2)	2.43 (0.94-6.30)	1.78 (0.64-4.98)
Stage Ⅲ-Ⅳ n (%)	12 (25.0)	36 (75.0)	2.53* (1.10-5.81)	3.17* (1.27-7.91)

[a]ORs adjusted for age and menstrual characteristics.

*P < 0.05

P for trend: 0.02

Int. J. Cancer: **114**, 317–322 (2005)

Active and passive smoking and breast cancer risk in middle-aged Japanese women

Tomoyuki Hanaoka[1],* Seiichiro Yamamoto[2], Tomotaka Sobue[2], Satoshi Sasaki[1,3] and Shoichiro Tsugane[1] for the Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Disease Study Group

[1]*Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan*
[2]*Statistics and Cancer Control Division, National Cancer Center Research Institute, Tokyo, Japan*
[3]*National Institute of Health and Nutrition, Tokyo, Japan*

To examine the hypothesis that tobacco smoke is associated with the risk of female breast cancer, we estimated the relative risks of active and passive smoke in middle-aged Japanese women in a population-based prospective study. The cohort consisted of residents in 4 public health center areas, aged 40 to 59 years. A self-administered questionnaire survey was conducted in 1990. This analysis included 21,805 subjects, 180 of whom had developed breast cancer by December 31, 1999. When the reference was defined as never-active smokers without passive smoking, adjusted relative risks (RRs) were 1.9 (95% confidence interval [CI] = 1.0–3.6) in current active smokers, 1.2 (95% CI = 0.4–4.0) in ex-active smokers and 1.2 (95% CI = 0.8–1.6) in never-active smokers with passive smoking. The elevated risk for ever-smokers was clearly observed in premenopausal women at baseline (RR = 3.9, 95% CI = 1.5–9.9) but not in postmenopausal women (RR = 1.1, 95% CI = 0.5–2.5). In never-active smokers, the adjusted RR for passive smoking, residential or occupational/public tobacco smoke exposure was 1.1 (95% CI = 0.8–1.6). In premenopausal women, passive smoking increased the risk (RR = 2.6; 95% CI = 1.3–5.2) but not in postmenopausal women (RR = 0.7; 95% CI = 0.4–1.0). We conclude that tobacco smoking increases the risk of female breast cancer in premenopausal women.
© *2004 Wiley-Liss, Inc.*

Key words: breast neoplasms; smoking; passive smoking; cohort study

Because most established risk factors for female breast cancer cannot be modified, the etiological role of tobacco smoking has been of interest in the public health field. As shown in a recent general comment by WHO's Executive Director, the link between smoking and breast cancer has been elusive; some studies have suggested a positive link, others found no relationship and a few have suggested that smoking has protective effects.[1] A positive association has been observed in some previous case-control studies.[2–7] In contrast, little relationship has been reported by cohort studies.[8–11] Theoretically, a cohort study provides better evidence compared to a case-control study, but the limitations, *e.g.*, reference category and misclassification of smoking habits, in recent cohort studies are still under dispute.[12–15]

Tobacco smoke is well known to contain numerous possible carcinogens.[16] Although they do not directly contact mammary cells, many studies utilizing biomarkers have demonstrated that tobacco-related carcinogens reach human breast tissue.[17–19] On the other hand, antiestrogenic effects of tobacco smoke have been suggested by many published observations.[20–23] Thus, the exposure may decrease the breast cancer risk, especially in postmenopausal women.[24,25]

The objective of our study was to examine the hypothesis that tobacco smoking is associated with the risk of female breast cancer. We estimated the risks of active and passive smoking among middle-aged Japanese women in a population-based cohort study. The influence of tobacco smoke as a breast cancer risk was elucidated by menopausal status at the baseline survey of the study.

Material and methods

Study cohort

The study cohort is part of the Japan Public Health Center (JPHC)-based prospective study on cancer and cardiovascular diseases (JPHC Study, cohort I) established on January 1, 1990. The study population was defined as Japanese residents aged 40–59 years, 27,063 men and 27,435 women, in 14 administrative districts in 4 PHC areas across Japan.[26] After the initiation of the study, 37 women were found to be ineligible and were excluded, leaving 27,398 women eligible for the study. Study procedures were approved by the ethics committee of the National Cancer Center, Tokyo, Japan.

Baseline survey

A self-administered questionnaire was distributed mostly by hand and partly by mail to the subjects in 1990. They were asked about their personal and familial medical histories, smoking habit, alcohol consumption, dietary habits and other lifestyle factors. A total of 22,482 women responded to the survey (82.1% response rate). Although the date of questionnaire completion ranged from January 1990 to May 1992, 54% responded between February 1990 and March 1990. Only 4% of questionnaires were completed after October 1990. The questions on active smoking consisted of current and former smoking status, age at initiation of smoking, average number of cigarettes smoked per day and age at cessation of smoking for former smokers. Questions on passive smoking were in 2 parts: a) "Have you lived with any regular smokers?" and age at exposure ($\leq$ 20 years old, $>$ 20 years old, both), and b) " In places outside the home, *e.g.*, at work, how often are you exposed to environmental tobacco smoke $\geq$ 1 hr/day?" (almost never, 1 to 3 days/month, 1 to 4 days/week, almost everyday).

Follow-up and identification of breast cancer

We followed the subjects from recruitment until December 31, 1999. In Japan, all death certificates are submitted to a local government office and forwarded to the PHC in the area of residence. Mortality data are then sent to the Ministry of Health, Labour and Welfare and coded for inclusion in the National Vital Statistics. The registration of deaths in Japan is required by the Family Registration Law and is theoretically complete. Therefore, all deaths of the subjects were based upon death certificates from each PHC, when they remained in the original area. Changes in residence status were identified annually through the residential registry in each area. Collection of cancer incidence data and migration data was described in a previous report.[27] Briefly, on January 1, 1990, a specific cancer registry for the JPHC Study was

Grant sponsor: The Ministry of Health, Labour and Welfare of Japan.
***Correspondence to:** Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045, Japan. Fax: +81-3-3547-8578.
E-mail: thanaoka@gan2.res.ncc.go.jp
Received 15 July 2003; Accepted after revision 2 September 2004
DOI 10.1002/ijc.20709
Published online 11 November 2004 in Wiley InterScience (www.interscience.wiley.com).

established to collect cancer incidence data on the study subjects living within the study area *via* voluntary reports from local major hospitals, on-site visits to the hospitals and records from the prefecture-wide population-based cancer registry, if available (Akita and Nagano Prefectures do not have a prefecture-wide cancer registry). Cancer incidence data were collected only for subjects who were living within the study area. Site of origin and histologic type were coded using the International Classification of Disease for Oncology, second edition (ICD-O-2). By December 31, 1999, 226 new breast cancer cases had been identified. Twelve carcinoma *in situ* were not included among these breast cancer cases. A diagnosis of breast cancer was histologically confirmed in 97% of the cases. The incidence/mortality ratio in the cancer registration was 5.4, and no cases were ascertained by death certificate alone [Death Certificate Only (DCO)]. In 1.1% of cases the subjects' death certificates were used as a supplementary information source for the registry [Death Certificate Notification (DCN)]. The estimated completeness of the registration was 91.8%, which suggested that the completeness for this cohort was reasonably high.[28,29]

Migration data were obtained from residential registries. Among non-case study subjects, 1,837 (6.7%) moved out of the study area and 34 (0.1%) were lost to follow-up within the study period.

Data analysis

From the 22,482 subjects, we excluded 612 more (including 12 breast cancer cases) with a past history of cancer in any site. Consequently, after excluding still another 53 subjects who submitted incomplete information on active or passive smoking status, a total of 21,805 subjects, 180 of whom developed breast cancer, were included in this analysis. Person-years of follow-up were counted from the date of questionnaire completion until the dates of a diagnosis of breast cancer, migration out of the study areas, death or the end of the study (December 31, 1999), whichever came first.

The relative risk (RR) and 95% confidence interval (CI) were estimated by the Cox proportional hazards model, adjusting for age and area according to the SAS PHREG procedure (SAS Institute, Inc., Cary, NC). For further adjustment, we incorporated additional possible confounders into the model; education level (≥ high school and < high school), employment status (employed and unemployed), body mass index (< 22, < 25, and ≥ 25), family history of breast cancer in mother or sisters, history of past benign breast disease, age at menarche, number of births (0, ≥ 1), menopausal status (pre and post), hormone use and alcohol consumption per week (< once/week, < 250 g/week, ≥ 250 g/week). Concerning body mass index and the number of births, influence on the estimates was similar between the categorical and continuous variables. Height, weight, fruit and vegetable intake and physical activity had little influence on the estimates and thus were omitted from the adjustment in the final analysis. Breast-feeding was not incorporated in the adjustment factors because it was not included in the questionnaire. We coded current occupations recorded in an open-end column in the questionnaire according to a major occupational category (Standard Occupational Classification for Japan, the third revision of 1997, Statistic Bureau, The Ministry of Public Management). The occupational categories consisted of professionals and technicians; managers; clerks; shop and market sales workers; service workers; security workers; agricultural, forestry and fishery workers; transport and communication workers; assemblers and manual laborers; workers unclassified and unemployed. Most agricultural, forestry and fishery workers were farmers. In the analysis concerning active smoking, passive smoking was defined as a history of exposure to residential sidestream smoke in any period or exposure to sidestream smoke (almost everyday) in any occupational and/or public setting.

After excluding from the analyses 6 cases whose pathological information was uncertain, we obtained results similar to those presented.

Results

Among the 21,805 women, the prevalence of current, ex- and never-active smokers was 5.7%, 1.7% and 92.6%, respectively. Among never-active smokers, 69% reported that they had been exposed to sidestream smoke (Table I). Table II compares known risk factors and possible confounders for breast cancer among 4 categories of smoking status. These factors included characteristics reported in the literature to be risk factors, and most of them served as adjustment factors in further statistical analyses. Table III shows RRs of incidence according to active smoking. Without taking account of passive smoking in the reference category, the adjusted RR for current active smokers was 1.7 (95% CI = 1.0–3.1). When the reference condition was defined as never-active smokers without passive smoking, a 2-fold risk was observed among current active smokers (adjusted RR = 1.9; 95% CI = 1.0–3.6). Stratified analyses by employment status showed the following adjusted RRs; 1.0 (95% CI = 0.5–2.0) for unemployed women with passive smoking, 0.8 (95% CI = 0.2–3.9) for unemployed women with active smoking, 1.2 (95% CI = 0.8–1.9) for employed women with passive smoking and 2.3 (95% CI = 1.1–4.8) for employed women with active smoking. After omitting the first 3 years after the study baseline to exclude possibly ill subjects, we observed similar results (data not shown).

In premenopausal women at baseline, ever-active smokers showed a 4-fold increased risk (adjusted RR = 3.9; 95% CI = 1.5–9.9); never-active smokers with passive smoking also exhibited a significantly increased risk (adjusted RR = 2.6; 95% CI = 1.3–5.2)compared to never-active smokers without passive smoking. Stratified analyses by employment status showed increased risk for active and passive smoking in both unemployed and employed women; adjusted RR = 4.4 (95% CI = 0.6–34.6) for unemployed women with passive smoking; 7.9 (95% CI = 0.7–90.8) for unemployed women with ever-active smoking, 2.3 (95% CI = 1.1–4.9) for employed women with passive smoking and 3.3 (95% CI = 1.2–9.4) for employed women with ever-active smoking.

In postmenopausal women at baseline, no significant increased risk was observed for ever-active smokers (adjusted RR = 1.1; 95% CI = 0.5–2.5). Stratified analyses by employment status showed the following adjusted RRs; 0.6 (95% CI = 0.3–1.3) for unemployed women with passive smoking, 0.3 (95% CI = 0.04–2.6) unemployed women with ever-active smoking, 0.7 (95% CI = 0.4–1.2) for employed women with passive smoking and 1.5 (95% CI = 0.6–3.9) for employed women with ever-active smoking. When ex-smokers were eliminated from the statistical model because of the small number of cases and person-years, the risk of smoking remained essentially unchanged (data not shown).

TABLE I – SMOKING STATUS IN FEMALE STUDY SUBJECTS: JPHC STUDY COHORT I

Passive smoking	Active smoking		
	Never-smokers (n = 20169)	Ex-smokers (n = 374)	Current smokers (n = 1238)
Residential passive smoking (%)[1]			
Never	6175 (31.0)	79 (21.4)	234 (19.1)
Ever			
Before age 20	2231 (11.2)	54 (14.6)	225 (18.4)
After age 20	6957 (35.0)	136 (36.8)	444 (36.3)
Both	4536 (22.8)	101 (27.3)	320 (26.2)
Passive smoking in occupational and/or public settings (%)[2]			
Almost never	13626 (68.0)	199 (53.6)	553 (44.8)
1–3 days/month	1534 (7.7)	29 (7.8)	76 (6.2)
1–4 days/week	1057 (5.3)	25 (6.7)	76 (6.2)
Almost everyday	3811 (19.0)	118 (31.8)	529 (42.9)

[1]Missing and unavailable answers were omitted from the calculation; 270 in never-smokers, 4 in ex-smokers, 15 in current smokers.– [2]Missing were omitted from the calculation; 141 in never-smokers, 3 in ex-smokers, 4 in current smokers.

TABLE II - DISTRIBUTION OF KNOWN RISK FACTORS AND POSSIBLE CONFOUNDERS FOR BREAST CANCER BY SMOKING STATUS: JPHC STUDY COHORT 1

	Never-smokers				
	Without passive smoking (n = 5660)	With passive smoking (n = 14533)	Ex-smokers (n = 374)	Current smokers (n = 1238)	p for trend[1]
Age (mean)	49.9	49.6	49.1	48.6	<0.0001
Occupation, farmer (%)[2]	1281 (23.4)	3,014 (21.2)	46 (12.5)	131 (10.9)	<0.0001
Occupation, unemployed (%)[2]	2850 (52.1)	6,423 (45.2)	164 (44.6)	494 (41.2)	<0.0001
Education (> high school, %)[2]	597 (10.9)	1,746 (12.4)	68 (18.7)	140 (11.8)	0.02
Height (mean)	151.1	151.8	152.3	152.2	<0.0001
Weight (mean)	54.3	54.2	55.8	54.2	0.58
Body mass index (mean)	23.7	23.5	24.1	23.3	<0.0001
Family history of breast cancer in mother or sisters (%)[2]	18 (0.3)	90 (0.6)	3 (0.8)	5 (0.4)	0.18
History of past benign breast disease (%)[2]	455 (8.0)	1,525 (10.5)	40 (10.7)	98 (7.9)	0.08
Age at menarche (mean)	14.7	14.6	14.4	14.8	0.30
Parous women (%)[2]	4,922 (93.3)	13,063 (95.2)	307 (89.5)	1,043 (90.7)	0.04
Age at first delivery among parous women (mean)	25.0	24.9	25.5	24.5	<0.0001
Number of deliveries among parous women (mean)	2.9	2.9	2.8	2.9	0.29
Menopausal status (postmenopausal, %)[2]	3,045 (55.2)	7,734 (54.2)	189 (51.9)	602 (49.4)	<0.001
Previous and/or current hormone use (%)[2]	1,114 (21.0)	2,786 (20.4)	82 (23.0)	258 (22.1)	0.58
Alcohol consumption per week (mean grams)	79.2	115.7	164.0	239.3	<0.0001

[1]p for trend was calculated by Cochran-Mantel-Haenszel test.– [2]Missing were omitted from the calculation; 619 in occupation, 743 in education, 53 in family history of breast cancer, 53 in history of past benign breast disease, 1,369 in child birth, 473 in menopausal status and 1,369 in hormone use.

TABLE III - RELATIVE RISK OF FEMALE BREAST CANCER ACCORDING TO ACTIVE SMOKING: 10-YEAR FOLLOW-UP IN JPHC STUDY COHORT 1

Exposure	Number of case	Person-years	RR[1] (95% CI)	RR[2] (95% CI)
Pre-and post-menopausal women at baseline:				
Never-smoker	162	187,063	1.0	1.0
Ex-smoker	4	3,344	1.4 (0.5 to 3.8)	1.1 (0.4 to 3.5)
Current smoker	14	10,901	1.5 (0.9 to 2.6)	1.7 (1.0 to 3.1)
Pre-and post-menopausal women at baseline:				
Never-smoker without passive smoking	40	52,884	1.0	1.0
Never-smoker with passive smoking	122	134,178	1.2 (0.8 to 1.7)	1.1 (0.8 to 1.6)
Ex-smoker	4	3,344	1.6 (0.6 to 4.5)	1.2 (0.4 to 4.0)
Current smoker	14	10,901	1.7 (0.9 to 3.1)	1.9 (1.0 to 3.6)
Premenopausal women at baseline:				
Never-smoker without passive smoking	9	22,982	1.0	1.0
Never-smoker with passive smoking	68	60,272	2.9 (1.4 to 5.8)	2.6 (1.3 to 5.2)
Current- + ex-smoker	11	6,907	4.1 (1.7 to 9.9)	3.9 (1.5 to 9.9)
Postmenopausal women at baseline:				
Never-smoker without passive smoking	31	28,583	1.0	1.0
Never-smoker with passive smoking	52	71,602	0.7 (0.4 to 1.0)	0.6 (0.4 to 1.0)
Current- + ex-smoker	7	7,056	0.9 (0.4 to 2.1)	1.1 (0.5 to 2.5)

[1]Relative risks adjusted for public health center (4 areas) and age (4 5-year age groups).– [2]Relative risks adjusted for public health center, age, employment status (employed and unemployed), education level (≥high school and <high school), body mass index (<22, 22 ≤<25 and ≥25), family history of breast cancer in mother or sisters, history of past benign breast disease, age at menarche, number of births (0 and ≥1), menopausal status (pre and post), hormone use and alcohol consumption per week (<once/week, <250 g/week and ≥250 g/week).

Table IV shows RRs of incidence according to passive smoking status. Adjusted RR for any passive smoking was 1.1 (95% CI = 0.8–1.6). In premenopausal women at baseline, those with any passive smoking revealed a significantly increased risk (adjusted RR = 2.6; 95% CI = 1.3–5.2), and exposure to sidestream smoke in occupational and/or public settings itself showed increased risk (adjusted RR = 2.3; 95% CI = 1.4–3.8). Concerning passive smoking in occupational and/or public settings in premenopausal women, a dose-dependent increase was found (adjusted RR = 1.0 for "almost none"; 0.6 [95% CI = 0.4–2.4] for "1 to 3 days/month", 2.2 [95% CI = 1.4–3.7] for "≥ 1 days/week", p for trend 0.002). Past exposure to sidestream smoke at home did not show an increased risk. Among postmenopausal women at baseline, RRs for passive smoking were 0.7 (95% CI = 0.4–1.0), and those exposed to sidestream smoke in an occupational and/or public setting showed a marginal decreased risk (adjusted RR = 0.5; 95% CI = 0.2–1.0).

Discussion

In the present population-based prospective study of middle-aged Japanese women, an increased risk for active premenopausal smoking women was observed, especially when the reference was defined as never-active smokers without exposure to sidestream smoke. A subgroup analysis revealed that only premenopausal women at the study baseline showed increased risks from passive smoking. These findings were independent of reproductive risk factors and other potential confounders. In previous case-control studies, the risk for active and passive smoking was equivalent,[3,4,6,7] which seems to be implausible. However, the estimated risk for active smoking was larger than that for passive smoking in our study.

Breast cancer risks differ based on menopausal status.[30] Thus, the risk factors and the magnitude of their risk may be different before and after menopause. The etiological roles of endogenous

TABLE IV – RELATIVE RISK OF FEMALE BREAST CANCER ACCORDING TO PASSIVE SMOKING IN FEMALE NEVER-SMOKERS; 10-YEAR FOLLOW-UP IN JPHC STUDY COHORT 1

		Passive smoking		
	Never	(A) Past residential exposure (in any period)	(B) Occupational and/or public exposure (everyday)	(A) or (B)
All never-smokers				
Number of cases	40	114	37	122
Person-years	50,662	127,309	35,258	134,299
RR[1] (95% CI)	1.00	1.1 (0.8 to 1.5)	1.3 (0.9 to 1.8)	1.2 (0.8 to 1.7)
RR[2] (95% CI)	1.00	1.0 (0.7 to 1.4)	1.3 (0.9 to 1.9)	1.1 (0.8 to 1.6)
Premenopausal women at baseline:				
No. of cases	9	61	28	68
Person-years	22,263	56,896	17,884	60,320
RR[1] (95% CI)	1.00	1.7 (1.0 to 3.0)	2.1 (1.3 to 3.4)	2.9 (1.4 to 5.8)
RR[2] (95% CI)	1.00	1.6 (0.9 to 2.7)	2.3 (1.4 to 3.8)	2.6 (1.3 to 5.2)
Postmenopausal women at baseline:				
Number of cases	31	51	8	52
Person-years	27,345	68,364	16,625	71,674
RR[1] (95% CI)	1.00	0.7 (0.4 to 1.1)	0.5 (0.3 to 1.1)	0.6 (0.4 to 1.0)
RR[2] (95% CI)	1.00	0.7 (0.4 to 1.1)	0.4 (0.2 to 1.0)	0.7 (0.4 to 1.0)

[1]Relative risks adjusted for public health center (4 areas) and age 4 5-year age group).– [2]Relative risks adjusted for public health center, age, employment status (employed and unemployed), education level (≥high school and <high school), body mass index (<22, 22 ≤<25 and ≥25), family history of breast cancer in mother or sisters, history of past benign breast disease, age at menarche, number of births (0 and ≥4), menopausal status (pre and post), hormone use and alcohol consumption per week (<once/week, <250 g/week and ≥250 g/week).

hormones admit of no doubt, and a causal model of breast cancer suggested that hormones increased the breast cancer risk in adults by increasing cell proliferation and the number of target cells, and also heightened the risk of the retention of spontaneous somatic mutations.[31] Therefore, higher levels of estrogens in premenopausal women may act jointly with exogenous carcinogens in breast carcinogenesis. The carcinogenic effects of tobacco smoke may result from a balance between its carcinogenic and anti-estrogenic effects.[6] Therefore, premenopausal women are likely to be affected by tobacco carcinogens because their estrogen levels are higher, thereby possibly canceling out the anti-estrogenic effects of tobacco smoke.

Smoking was reported to be associated with a decrease in the incidence of endometrial neoplasia in postmenopausal women.[23] The net effect of tobacco smoke may be antiestrogenic in the endometrium. However, available evidence, excluding 1 prospective study in Japan,[32] indicates that smoking has no beneficial effects in the breast. We did not observe statistically significant beneficial effects in the present study. However, our data suggest that at least the carcinogenic effects of tobacco smoke are not present in postmenopausal women.

Active and passive smoking are influenced by socioeconomic status.[33,34] Occupation is in fact related to smoking habits especially in women; working women generally smoke more and are exposed to sidestream smoke more frequently. Indeed, smoking status differed among several occupation-related factors in this cohort. A stratified analysis by employment status revealed interesting findings. In postmenopausal women, increased risk was observed only in employed women, although the small numbers of cases in the subgroup analyses precluded firm conclusions. Their pack-years were comparable (employed 10 ± 11 and unemployed 13 ± 13). These findings suggest that there were unknown residual confounders or different smoking behavior in these 2 groups. Risks for passive smoking were not increased in either employed or unemployed postmenopausal women. However, in premenopausal women, risks for active or passive smoking were increased in both employed and unemployed women. These findings suggest that any tobacco smoke exposure elevated the risk in premenopausal women no matter what their occupation. Educational level can be a surrogate indicator of socioeconomic status and has been reported as one of the important risk factors for breast cancer. Although we incorporated employment status and educational level into our statistical models, unknown residual confounders concerning socioeconomic status might not necessarily have been excluded from our analysis.

In our study, past exposure to sidestream smoke at home showed different effects from those by the occupational/social exposure. Residential exposure was defined as "a smoker(s) who had lived with a subject", although the current occupational/social exposure was assessed semi-quantitatively by self-report. Intensity or duration of daily exposure could not be estimated for the residential exposure. Previous cohort studies in Japanese women also used the smoking status of husbands as an index of passive smoking and did not observe elevated risk.[32,35]

The limitations of previous case-control studies were that recall and selection bias would tend to produce spurious positive association.[11] On the other hand, the limitations of previous cohort studies including misclassification of exposure and reference category have also been pointed out.[12–15] However, a well-designed prospective study is known to provide persuasive evidence. Our prospective study design also has some advantages in estimating the risks of smoking. Although recall bias may exist with information concerning passive smoking in a case-control study, there was no recall bias in our study because of its prospective nature. Never-active smokers without passive smoking were assigned to the reference, allowing for more accurate classification of exposure. Nonresidential passive smoking, *i.e.*, occupational or public exposure to tobacco smoke, was taken into account in the analyses. Subgroup analyses concerning menopausal status were done because the combined analyses may dilute the risk estimation.

On the other hand, there are some admitted limitations. Because the exposure assessment was done at 1 point (at baseline), a misclassification of the exposure might have occurred, thereby diluting the effects if some smoking women had quit smoking during the follow-up period. Information on the menopausal status was obtained at baseline. Therefore, we did not examine the risks for pre- and post-menopausal cancer. The relatively small number of incidence cases precluded further subgroup analyses. Results of the subgroup analyses according to menopausal status in this report should be confirmed by continued follow-up.

Different effects of active or passive smoking regarding breast cancer risk had been shown in premenopausal and postmenopausal women.[7,36] In a recent study, the risk of breast cancer among smokers has been clearly reported to be elevated in premenopausal women.[36] Immature breast cells are suggested to have especially increased susceptibility to smoking-related carcinogens.[6] In our

study, 94% of subjects had delivered children, but the effect of smoking in strata defined by age of full-term birth could not be examined. On the other hand, in postmenopausal women, the risk of breast cancer among smokers has been reported not to be elevated.[36] These previous observations are consistent with our observations regarding both active and passive smoking. Race is also an important factor in the interpretation of our results. To our knowledge, this is the first prospective study to link active smoking to breast cancer risk in Asian women, although recent large-scale cohort studies in America did not detect any increased risk of breast cancer.[10,11] Genetic differences concerning important metabolic enzymes, for example, higher frequency of a variant allele of cytochrome P450 1A1 gene, were reported,[37] and endogenous estrogen levels and the number of estrogen receptors have been reported to differ between Japanese and Caucasians.[38,39] Thus, an association between smoking and breast cancer might appear more readily in Japanese. The incidence of breast cancer among premenopausal women (88/90,161 person-year) was almost the same as that among postmenopausal women (90/107,241 person-year), and the association observed in premenopausal women was strong. These might be why we observed an elevated risk due to tobacco smoking in the overall subjects.

In conclusion, tobacco smoking increases the risk of female breast cancer in premenopausal women. Both active and passive smoking are promising targets in the prevention of breast cancer.

Acknowlegements

The authors thank all staff members in each study area and in the central offices for their painstaking efforts to conduct the baseline survey and follow-up, and to the Iwate, Aomori and Okinawa cancer registries for providing the incidence data. The authors are grateful to Dr. S Watanabe and Dr. M Konishi who contributed so much to the initiation of the JPHC Study.

The Japan Public Health Center Study Group is composed of the members listed above as well as the following: J. Ogata, S. Baba, T. Mannami, National Center for Circulatory Diseases, Osaka; K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, Iwate Prefectural Ninohe Public Health Center, Iwate; Y. Miyajima, N. Suzuki, S. Nagasawa; Y. Furusugi, Akita Prefectural Yokote Public Health Center, Akita; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe, Nagano Prefectural Saku Public Health Center, Nagano; Y. Kishimoto, E. Takara, M. Kinjo, T. Fukuyama, Okinawa Prefectural Ishikawa Public Health Center, Okinawa; S. Matsushima, S. Natsukawa, Saku General Hospital, Nagano; S. Watanabe, M. Akabane, Tokyo University of Agriculture, Tokyo; M. Konishi, Ehime University, Matsuyama; S. Tominaga, Aichi Cancer Center Research Institute, Nagoya; M. Iida, S. Sato, Center for Adult Diseases, Osaka; the late M. Yamaguchi and Y. Matsumura, National Institute of Health and Nutrition, Tokyo; Y. Tsubono, Tohoku University, Miyagi; H. Iso, Tsukuba University, Ibaragi; H. Sugimura, Hamamatsu University, Shizuoka; M. Kabuto, National Institute for Environmental Studies, Ibaragi.

References

1. Crabb C. Is breast cancer linked to smoking? Bull World Health Organ 2003;81:74–4.
2. Wells AJ. Breast cancer, cigarette smoking, and passive smoking. Am J Epidemiol 1991;133:208–10.
3. Smith SJ, Deacon JM, Chilvers CE. Alcohol, smoking, passive smoking and caffeine in relation to breast cancer risk in young women: UK National Case-Control Study Group. Br J Cancer 1994;70:112–9.
4. Morabia A, Bernstein M, Heritier S, Khatchatrian N. Relation of breast cancer with passive and active exposure to tobacco smoke. Am J Epidemiol 1996;143:918–28.
5. Millikan RC, Pittman GS, Newman B, Tse CK, Selmin O, Rockhill B, Savitz D, Moorman PG, Bell DA. Cigarette smoking, N-acetyltransferases 1 and 2, and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:371–8.
6. Lash TL, Aschengrau A. Active and passive cigarette smoking and the occurrence of breast cancer. Am J Epidemiol 1999;149:5–12.
7. Johnson KC, Hu J, Mao Y. Passive and active smoking and breast cancer risk in Canada, 1994–97. The Canadian Cancer Registries Epidemiology Research Group. Cancer Causes Control 2000;11:211–21.
8. London SJ, Colditz GA, Stampfer MJ, Willett WC, Rosner BA, Speizer FE. Prospective study of smoking and the risk of breast cancer. J Natl Cancer Inst 1989;81:1625–31.
9. Jee SH, Ohrr H, Kim IS. Effects of husbands' smoking on the incidence of lung cancer in Korean women. Int J Epidemiol 1999;28:824–8.
10. Wartenberg D, Calle EE, Thun MJ, Heath CW, Jr., Lally C, Woodruff T. Passive smoking exposure and female breast cancer mortality. J Natl Cancer Inst 2000;92:1666–73.
11. Egan KM, Stampfer MJ, Hunter D, Hankinson S, Rosner BA, Holmes M, Willett WC, Colditz GA. Active and passive smoking in breast cancer: prospective results from the Nurses' Health Study. Epidemiology 2002;13:138–45.
12. Morabia A. Active and passive smoking in breast cancer. Epidemiol 2000;13:744–5.
13. Johnson KC, Wells AJ. Active and passive smoking in breast cancer. Epidemiol 2000;13:745–6.
14. Wells AJ. Re: Passive smoking exposure and female breast cancer mortality. J Natl Cancer Inst 2001;93:717–9; author reply 20–1.
15. Johnson KC. Re: Passive smoking exposure and female breast cancer mortality. J Natl Cancer Inst 2001;93:719–20; author reply 20–1.
16. International Agency for Research on Cancer. Tobacco smoking. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans, vol. 38. Lyon: IARC, 1986.
17. Petrakis NL, Maack CA, Lee RE, Lyon M. Mutagenic activity in nipple aspirates of human breast fluid. Cancer Res 1980;40:188–9.
18. Li D, Wang M, Firozi PF, Chang P, Zhang W, Baer-Dubowska W, Moorthy B, Vulimiri SV, Goth-Goldstein R, Weyand EH, DiGiovanni J. Characterization of a major aromatic DNA adduct detected in human breast tissues. Environ Mol Mutagen 2002;39:193–200.
19. Firozi PF, Bondy ML, Sahin AA, Chang P, Lukmanji F, Singletary ES, Hassan MM, Li D. Aromatic DNA adducts and polymorphisms of CYP1A1, NAT2, and GSTM1 in breast cancer. Carcinogenesis 2002;23:301–6.
20. McKinlay SM, Bifano NL, McKinlay JB. Smoking and age at menopause in women. Ann Intern Med 1985;103:350–6.
21. Ross RK, Pike MC, Vessey MP, Bull D, Yeates D, Casagrande JT. Risk factors for uterine fibroids: reduced risk associated with oral contraceptives. Br Med J (Clin Res Ed) 1986;293:359–62.
22. Parazzini F, La Vecchia C, Negri E, Cecchetti G, Fedele L. Epidemiologic characteristics of women with uterine fibroids: a case-control study. Obstet Gynecol 1988;72:853–7.
23. Brinton LA, Barrett RJ, Berman ML, Mortel R, Twiggs LB, Wilbanks GD. Cigarette smoking and the risk of endometrial cancer. Am J Epidemiol 1993;137:281–91.
24. MacMahon B, Trichopoulos D, Cole P, Brown J. Cigarette smoking and urinary estrogens. N Engl J Med 1982;307:1062–5.
25. Baron JA, La Vecchia C, Levi F. The antiestrogenic effect of cigarette smoking in women. Am J Obstet Gynecol 1990;162:502–14.
26. Sasazuki S, Sasaki S, Tsugane S. Cigarette smoking, alcohol consumption and subsequent gastric cancer risk by subsite and histologic type. Int J Cancer 2002;101:560–6.
27. Yamamoto S, Sobue T, Kobayashi M, Sasaki S, Tsugane S. Soy, isoflavones, and breast cancer risk in Japan. J Natl Cancer Inst 2003;95:906–13.
28. Parkin D, Chen V, Ferlay J, Galceran J, Storm H, Whelan S. Comparability and quality control in cancer registration. IARC Technical Report No.19. Lyon: IARC, 1994.
29. International Agency for Research on Cancer. Cancer incidence in five continents, vol. VIII, IARC Scientific Publications vol. 155. Lyon: IARC, 2002.
30. Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol 2001;2:133–40.
31. Adami HO, Persson I, Ekbom A, Wolk A, Ponten J, Trichopoulos D. The aetiology and pathogenesis of human breast cancer. Mutat Res 1995;333:29–35.
32. Nishino Y, Tsubono Y, Tsuji I, Komatsu S, Kanemura S, Nakatsuka H, Fukao A, Satoh H, Hisamichi S. Passive smoking at home and cancer risk: a population-based prospective study in Japanese nonsmoking women. Cancer Causes Control 2001;12:797–802.
33. Tseng M, Yeatts K, Millikan R, Newman B. Area-level characteristics and smoking in women. Am J Public Health 2001;91:1847–50.
34. Stamatakis KA, Brownson RC, Luke DA. Risk factors for exposure

to environmental tobacco smoke among ethnically diverse women in the United States. J Womens Health Gend Based Med 2002;11: 45–51.
35. Hirayama T. Cancer mortality in nonsmoking women with smoking husbands based on a large-scale cohort study in Japan. Prev Med 1984;13:680–90.
36. Band P, Le N, Fang R, Deschamps M. Carcinogenic and endocrine disrupting effects of cigarette smoke and risk of breast cancer. Lancet 2002;360:1044.
37. Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, Baranova H, Bathum L, Benhamou S, Boffetta P, Bouchardy C, Breskvar K, et al. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 2001;10:1239–48.
38. Shimizu H, Ross RK, Bernstein L, Pike MC, Henderson BE. Serum oestrogen levels in postmenopausal women: comparison of American whites and Japanese in Japan. Br J Cancer 1990;62:451–3.
39. Nomura Y, Kobayashi S, Takatani O, Sugano H, Matsumoto K, McGuire WL. Estrogen receptor and endocrine responsiveness in Japanese versus American breast cancer patients. Cancer Res 1977; 37:106–10.