Our results also indicated that smoking might affect the mRNA level to some extent, because a statistically significant correlation was found between urinary cotinine and *CYP1B1* mRNA. Diluted mainstream cigarette smoke condensates have been found to bind to and transcriptionally activate the AhR and other nuclear receptors (Meek and Finch, 1999). Therefore, an increased *CYP1B1* mRNA level caused by smoking is reasonable. It has been suggested that the affinity of compounds in tobacco smoke for AhR could not be explained by only BaP and other PAHs (Lofroth and Rannug, 1988). A type of dioxin, polychlorinated dibenzo-*p*-dioxin, was detected in tobacco smoke (Muto and Takizawa, 1989), and a remarkable involvement of *CYP1B1* in the metabolic activation of 5-methylchrysene, one of the stronger carcinogens in tobacco smoke, was observed (Shimada et al., 1996). However, Dassi et al. (Dassi et al., 1998) and Spencer et al. (Spencer et al., 1999) estimated *CYP1B1* mRNA levels in blood monocytes from smokers and non-smokers, but found no difference between the two groups. The reason why our results differed from their observations is not clear, but the race of the subjects and combined exposure with coke oven emissions might affect the relationship between urinary cotinine and *CYP1B1* mRNA in the present study.

Only in subjects with the *CYP1B1 Leu/Leu* type (wild type) did we observe a statistically significant relationship between *CYP1B1* mRNA and 1-OHP. *CYP1B1* is known to have four polymorphisms: *Arg*48*Gly* (exon 2), *Ala*119*Ser* (exon 2), *Leu*432*Val* (exon 3) and *Asn*453*Ser* (exon 3). The proportions of these variant alleles among Japanese was reported to be 14%, 14%, 22% and 0%, respectively (Inoue et al., 2000). Among these polymorphisms, the *Leu*432*Val* has been the most studied, and the prevalence of the *Val* allele in the present study was comparable with those in the previous reports among Chinese (Linxian, 17%) (Tang et al., 2000). Alterations in the transcription by this polymorphism have not been reported. However, an in vitro study showed that the *Val* allele had stronger enzyme activity than the *Leu* allele (Watanabe et al., 2000), and another report suggested that the *Val* allele changed the enzyme function (Bailey et al., 1998).

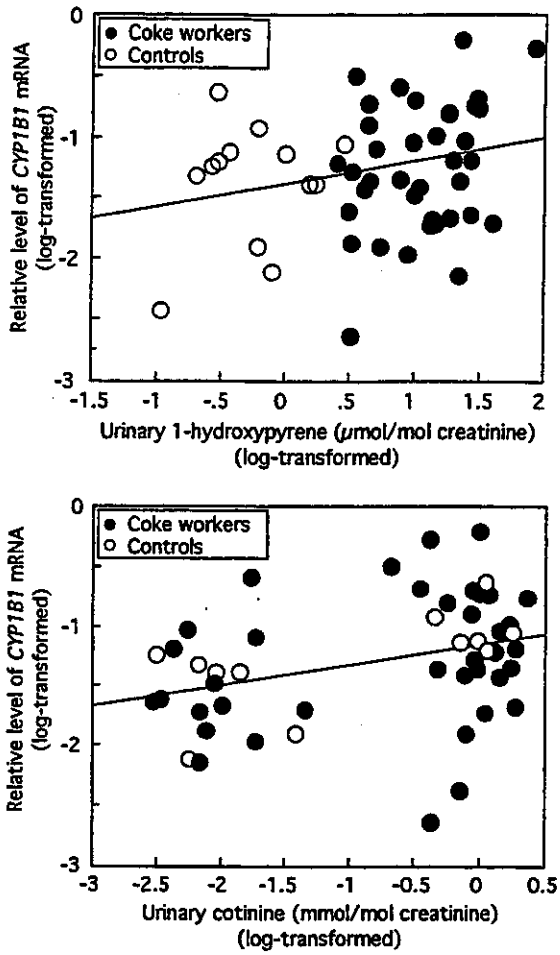

Fig. 2. Correlation between relative levels of *CYP1B1* mRNA and indices of internal exposure; Urinary 1-hydroxypyrene levels (μmol/mol creatinine, $R=0.22$, $P=0.13$, $n=50$), Urinary cotinine levels (mmol/mol creatinine, $R=0.22$, $P=0.13$, $n=50$). All values were log10-transformed.

In conclusion, our preliminary study suggests that PAH exposure in coke ovens and smoking maybe associated with *CYP1B1* mRNA levels in peripheral blood cells although mRNA is generally unstable and could be expressed following exposure to other agents. Further, study is needed to validate the *CYP1B1* mRNA in WBCs as a biomarker of environmental exposure to PAHs.

Acknowledgments

We are grateful to Hiroko Hashimoto and Tomoko Tokutake for their technical assistance. This

work was supported in part by a Grant-in-Aid for the Second Term Comprehensive 10-Year Strategy for Cancer Control, and for Research on Environmental Health from the Ministry of Health and Welfare of Japan, and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

References

Bailey LR, Roodi N, Dupont WD, Parl FF. Association of cytochrome P450 1B1 (*CYP1B1*) polymorphism with steroid receptor status in breast cancer. Cancer Res 1998;58:5038–5041.

Cosma GN, Toniolo P, Currie D, Pasternack BS, Garte SJ. Expression of the *CYP1A1* gene in peripheral lymphocytes as a marker of exposure to creosote in railroad workers. Cancer Epidemiol Biomarkers Prev 1992;1:137–142.

Dassi C, Signorini S, Gerthoux P, Cazzaniga M, Brambilla P. Cytochrome P450 1B1 mRNA measured in blood mononuclear cells by quantitative reverse transcription-PCR. Clin Chem 1998;44:2416–2421.

Fritsche E, Bruning T, Jonkmanns C, Ko Y, Bolt HM, Abel J. Detection of cytochrome P450 1B1 Bfr I polymorphism: genotype distribution in healthy German individuals and in patients with colorectal carcinoma. Pharmacogenetics 1999;9:405–408.

Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. Genome Res 1996;6:995–1001.

Hara K, Hanaoka T, Yamano Y, Itani T. Urinary 1-hydroxypyrene levels of garbage collectors with low-level exposure to polycyclic aromatic hydrocarbons. Sci Total Environ 1997;199:159–164.

Hayashi S, Watanabe J, Nakachi K, Eguchi H, Gotoh O, Kawajiri K. Interindividual difference in expression of human Ah receptor and related P450 genes. Carcinogenesis 1994;15:801–806.

Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. Genome Res 1996;6:986–994.

Horstmann M. Simple high-performance liquid chromatographic method for rapid determination of nicotine and cotinine in urine. J Chromatogr 1985;344:391–396.

Inoue K, Asao T, Shimada T. Ethnic-related differences in the frequency distribution of genetic polymorphisms in the *CYP1A1* and *CYP1B1* genes in Japanese and Caucasian populations. Xenobiotica 2000;30:285–295.

Lofroth G, Rannug A. Ah receptor ligands in tobacco smoke. Toxicol Lett 1988;42:131–136.

Meek MD, Finch GL. Diluted mainstream cigarette smoke condensates activate estrogen receptor and aryl hydrocarbon receptor-mediated gene transcription. Environ Res 1999;80:9–17.

Muto H, Takizawa Y. Dioxins in cigarette smoke. Arch Environ Health 1989;44:171–174.

Pan G, Hanaoka T, Yamano Y, Hara K, Ichiba M, Wang Y, Zhang J, Feng Y, Shujuan Z, Guan D, Gao G, Liu N, Takahashi K. A study of multiple biomarkers in coke oven workers a cross-sectional study in China. Carcinogenesis 1998;19:1963–1968.

Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP, Sutter TR. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. Cancer Res 1996;56:2979–2984.

Spencer DL, Masten SA, Lanier KM, Yang X, Grassman JA, Miller CR, Sutter TR, Lucier GW, Walker NJ. Quantitative analysis of constitutive and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced cytochrome P450 1B1 expression in human lymphocytes. Cancer Epidemiol Biomarkers Prev 1999;8:139–146.

Sutter TR, Tang YM, Hayes CL, Wo YY, Jabs EW, Li X, Yin H, Cody CW, Greenlee WF. Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. J Biol Chem 1994;269:13092–13099.

Tang YM, Green BL, Chen GF, Thompson PA, Lang NP, Shinde A, Lin DX, Tan W, Lyn-Cook BD, Hammons GJ, Kadlubar FF. Human *CYP1B1 Leu*432*Val* gene polymorphism: ethnic distribution in African-Americans, Caucasians and Chinese; oestradiol hydroxylase activity; and distribution in prostate cancer cases and controls. Pharmacogenetics 2000;10:761–766.

Tang YM, Wo YYP, Stewart J, Hawkins AL, Griffin CA, Sutter TR, Greenlee WF. Isolation and characterization of the human cytochrome P450 *CYP1B1* gene. J Biol Chem 1996;271:28324–28330.

Vanden Heuvel JP, Clark GC, Thompson CL, McCoy Z, Miller CR, Lucier GW, Bell DA. *CYP1A1* mRNA levels as a human exposure biomarker: use of quantitative polymerase chain reaction to measure *CYP1A1* expression in human peripheral blood lymphocytes. Carcinogenesis 1993;14:2003–2006.

Watanabe J, Shimada T, Gillam EM, Ikuta T, Suemasu K, Higashi Y, Gotoh O, Kawajiri K. Association of *CYP1B1* genetic polymorphism with incidence to breast and lung cancer. Pharmacogenetics 2000;10:25–33.

Willey JC, Coy EL, Frampton MW, Torres A, Apostolakos MJ, Hoehn G, Schuermann WH, Thilly WG, Olson DE, Hammersley JR, Crespi CL, Utell MJ. Quantitative RT-PCR measurement of cytochromes p450 1A1, 1B1, and 2B7, microsomal epoxide hydrolase, and NADPH oxidoreductase expression in lung cells of smokers and non-smokers. Am J Respir Cell Mol Biol 1997;17:114–124.

Xu Z, Brown LM, Pan GW, Liu TF, Gao GS, Stone BJ, Cao RM, Guan DX, Sheng JH, Yan ZS, Dosemeci M, Fraumeni JF, Blot WJ. Cancer risks among iron and steel workers in Anshan, China, Part II: Case-control studies of lung and stomach cancer. Am J Ind Med 1996;30:7–15.

Biotechnology Letters **25**: 509–512, 2003.

Chip ligating human genomic DNA serves as storage material and template for polymerase chain reaction

T. Hanaoka[1,*], O. Takai[2], K. Takahashi[2] & S. Tsugane[1]
[1]*Epidemiology and Biostatistics Division, National Cancer Center Research Institute East, Chiba, Japan*
[2]*Clinical Biochemistry, Department of Nursing, Faculty of Health Science, Hiroshima International University, Japan*
**Author for correspondence (Fax: +81-471-34-6862; E-mail: thanaoka@east.ncc.go.jp)*

Received 17 December 2002; Revisions requested 14 January 2003; Revisions received 29 January 2003; Accepted 29 January 2003

Key words: DNA chip, polymerase chain reaction, restriction enzyme

Abstract

A chip was developed to store DNA for medical research. The optional restriction site fixed on the chip can randomly ligate with whole human genomic DNA treated by the corresponding restriction enzyme. PCR can then use the chip as template DNA. Moreover, a chip fixing two restriction sites (e.g. *Eco*RI and *Hin*dIII) showed the amplification by PCR for any location of genomic DNA. Repetitive PCRs have confirmed that a DNA chip can be stored by at −4 °C for 2 years.

Introduction

DNA storage methods have often been discussed as a basic and increasingly important issue among medical researchers, especially epidemiologists (Austin *et al.* 1996, Steinberg *et al.* 1997, Ellsworth & Manolio 1999). The amount of DNA collected from study participants is usually limited and has restricted repetitive usage. Immortalization of cell lines provides a way to reproduce DNA, but requires specific conditions (Austin *et al.* 1996) and is not appropriate for studies in fields which use a large number of samples such as genetic epidemiology. The purpose of this study was to confirm that a chip ligating whole human genomic DNA can serve as template DNA for polymerase chain reactions (PCRs). The effect of its refrigeration on repetitive PCRs is also reported.

Materials and methods

Chemicals

A DNA isolation kit, restriction enzymes, T4 DNA Ligase was purchased from Takara Biochemicals (Osaka, Japan), Taq DNA polymerase from Applied Biosystems (CA), and Taq antibody from Toyobo (Tokyo, Japan). All other chemicals used were of analytical grade and obtained commercially.

Material

A chip (3 mm × 3 mm × 0.66 mm) provided from Nippon Parkerizing Hiroshima Co. (Hiroshima, Japan) was coated by a diamond on one side because of its high thermoconductivity. Preparation of the chip is briefly depicted in Figure 1. Naked restriction sites on the chip can ligate with various lengths of DNA fragments treated with the corresponding restriction enzyme. In this study, we fixed three type of restriction sites on the surface; *Eco*RI, *Hin*dIII and *Eco*RI + *Hin*dIII. The DNA ligation capacity was estimated as 0.2 pmol/mm^2.

Samples

Peripheral venous blood was collected in an Na_2-EDTA tube from healthy volunteers who gave their informed consent, and all samples was anonymized. Total DNA was isolated from whole blood using a

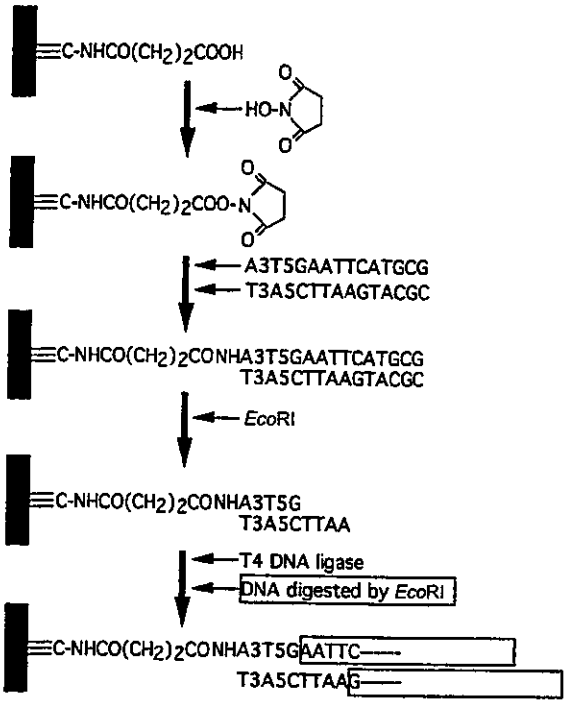

Fig. 1. Schematic representation for preparation of the chip. Each carboxyl radical fixed on the surface of the chip is esterified by *N*-hydroxysuccinimide and combined with an optional oligonucleotide including an optional restriction site. This oligonucleotide is treated with the same enzyme (in this schema, *Eco*RI), then restriction sites appear on the chip. A naked *Eco*RI site on the chip can be hybridized with various lengths of DNA fragments treated with *Eco*RI. Three hundred ng of the digested DNA was mixed with the chip prepared, 35 Unit of T4 DNA ligase and 2 μl of a reaction buffer, and the total volume in the reaction was adjusted to 50 μl by distilled H_2O, and then incubated at 16 °C for 2 h.

commercial kit. The DNA sample was then digested by, *Eco*RI or *Hin*dIII restriction enzymes.

Ligation with DNA

Digested DNA, 300 ng, was ligated with the chip by T4 DNA Ligase. The chip was then washed by distilled H_2O and stored in TE buffer at −4 °C until further experiments.

Polymerase chain reaction (PCR)

PCRs were done in a tube with the prepared DNA chip as a template. After two PCR cycles, the PCR solution in the tube was removed to a new reaction tube, and the PCR was continued for the 3rd to the 40th cycle without the chip. The chip was then removed from the former tube and stored in a stock tube with TE buffer at −4 °C after washing by distilled H_2O. For PCR of L-*myc* gene intron 2 containing an *Eco*RI site (Kawashima *et al.* 1988), new primer sets were designed, and another primer set published previously was also used (Shibuta *et al.* 1998). For PCR of other genes, primer sets used were published previously; Cytochrome P450 1A1 gene (*CYP1A1*) (Oyama *et al.* 1995) and glutathione *S*-transferase M1 gene (*GSTM1*) (Groppi *et al.* 1991).

Results and discussion

The L-*myc* gene was amplified using raw DNA sample (Figure 2a, lane 1), and, after digestion by *Eco*RI, the product was confirmed to show a digested fragment (Figure 2a, lane 2). However, the L-*myc* was not amplified using *Eco*RI site-fixed chip (Figure 2a, lane 3) because the sequence contained *Eco*RI sites, and all DNA fragments ligated on the chip had no *Eco*RI sites. This phenomenon was reproduced by an experiment using raw DNA sample digested by *Eco*RI (Figure 2a, lane 4). These observations suggested that two different restriction sites should be fixed on a chip when any location of DNA had to be amplified.

Second, we prepared a *Hin*dIII site-fixed chip and simultaneously used an *Eco*RI site- and *Hin*dIII site-fixed chip in one PCR tube. Successful amplification of the L-*myc* fragment was observed (Figure 2b-1).

Third, we fixed two restriction sites, *Eco*RI and *Hin*dIII, on the surface of a single chip. DNA, 150 ng, was separately digested by *Eco*RI and *Hin*dIII, and ligated with one chip. The PCR products of the L-*myc* were seen in Figure 2b-2.

In the above experiment, the product was observed up to the 50th experiment, after which observation proved difficult. The decrease in PCR product after repetitive usage of one specific location of one gene was a limitation of the chip. According to our experience, the hot-start PCR method had improved the number of PCR experiment repetitions. The reason why the PCR amplification is limited may be explained as follows; the three-dimensional structure of DNA fragments containing the amplified location on the chip is changed, and the interaction between the neighboring DNA fragments further disturb PCR at the same location. However, such speculation has not been validated by any visualization. A 300 ng DNA sample was required to make one chip; thus, one PCR experiment required approx. 6 ng DNA sample if limited to the 50th experiment. However, in practical use, the PCR of one specific location was not repeated

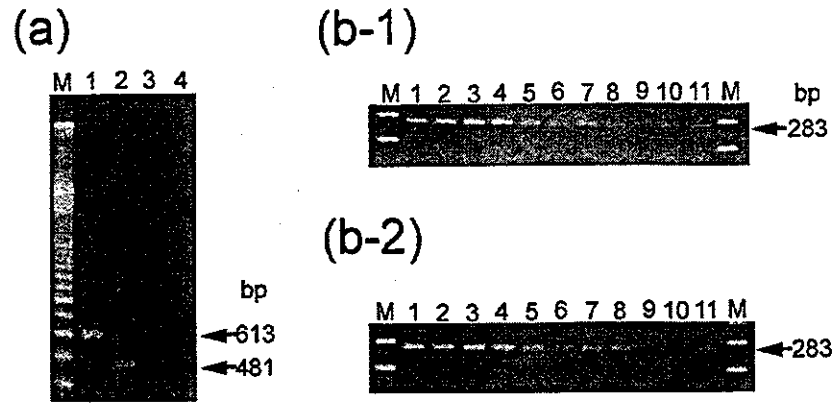

Fig. 2. Results of PCRs. (a) PCRs of one location of L-*myc* containing *Eco*RI site using the *Eco*RI site-fixed chip. Lane 1: the product (613 bp) using a raw DNA sample as a template; lane 2: a fragment (481 bp) produced from the product (613 bp) by *Eco*RI digestion; lane 3: no product using the chip; and lane 4: no product using a raw DNA sample treated with *Eco*RI. M: a part of 100 bp ladder marker. A PCR reaction was done in a tube with the DNA chip as a template plus 1.25 Unit of Taq DNA polymerase, 5 μl of 2 mM dNTP mixture, 1 μl of each 10 μM sense and anti-sense primer and 5 μl of PCR buffer, with the total volume in the reaction adjusted to 50 μl by distilled H_2O, The polymerase was incubated at room temperature for 5 min with Taq antibody before the reaction. The PCR conditions were as follows: (i) 94 °C for 3 min; (ii) 40 cycles of 94 °C for 30 s, 66 °C for 1 min and 72 °C for 30 s; and (iii) 72 °C for 30 s. After two reaction cycles, the PCR solution in the tube was removed to a new reaction tube, and the PCR reaction was continued for the 3rd to the 40th cycle without the chip. Specific primer sets were described elsewhere. (b-1) PCRs of one location of L-*myc* containing *Eco*RI site (187 bp) using both *Eco*RI site- and *Hin*dIII site-fixed chips simultaneously; (b-2) using a single chip with two restriction sites, *Eco*RI and *Hin*dIII. Numbers on the lanes of ethidium bromide-stained gel indicate the 1st, 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th and 50th PCR experiment. M: a part of 100 bp ladder marker. Specific primer sets for L-*myc* were designed as follows: 5′-CCTGAACCTGTTTGTGAGCTTC-3′ and 5′-AAGCTTGAGCCCCTTTGTCA-3′.

many times for the same sample. We observed that random usage of different primers of different genes extended the chip life up to over the 75th experiment (data not shown). Thus, the degradation of the ligated DNA fragments on the chip is unlikely to occur and would not be the reason for the limited repetition of PCR at one location of a given gene.

To confirm PCRs of other genes, *GSTM1* and *CYP1A1* exon 7 were selected for experiments. L-*myc* is located on chromosome 1p32, and *GSTM1* on the same chromosome but at a different location (chromosome 1p13.3), *CYP1A1* on a different chromosome (chromosome 15q22-q24). Both *GSTM1* and *CYP1A1* were successfully amplified using *Eco*RI site-fixed chips. Moreover, the PCR product of the *CYP1A1* successfully served for the *Hin*cII RFLP analysis as a test (data not shown). These findings may support applications to amplicons of all locations in the genome.

DNA specimens are generally stored as a solution or in the form of evaporated pellets. However, this chip can provide a means of safe storage of DNA samples because an identification number can be typed on its reverse and the DNA can be stored as a solid material. We have confirmed that DNA chips stored at −4 °C for 24 months have not affected PCRs to date. Limitation of the preservation period is under investigation. In conclusion, these experiments suggest a new preservation method. Prospective experiments will be necessary to confirm the potential preservation period.

Acknowledgements

The authors wish to thank Ms Hiroko Hashimoto for her technical assistance. This work was supported in part by the Grant-in-Aid for Cancer Research, for the Second Term Comprehensive 10-year Strategy for Cancer Control and for Research on Environmental Health from the Ministry of Health, Labor and Welfare of Japan, and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Austin MA, Ordovas JM, Eckfeldt JH, Tracy R, Boerwinkle E, Lalouel JM, Printz M (1996) Guidelines of the National Heart, Lung, and Blood Institute Working Group on Blood Drawing, Processing, and Storage for Genetic Studies. *Am. J. Epidemiol.* 144: 437–441.

Ellsworth DL, Manolio TA (1999) The emerging importance of genetics in epidemiologic research. I. Basic concepts in human genetics and laboratory technology. *Ann. Epidemiol.* 9: 1–16.

Groppi A, Coutelle C, Fleury B, Iron A, Begueret J, Couzigou P (1991) Glutathione *S*-transferase class mu in French alcoholic cirrhotic patients. *Hum. Genet.* **87**: 628–630.

Kawashima K, Shikama H, Imoto K, Izawa M, Naruke T, Okabayashi K, Nishimura S (1988) Close correlation between restriction fragment length polymorphism of the L-MYC gene and metastasis of human lung cancer to the lymph nodes and other organs. *Proc. Natl. Acad. Sci. USA* **85**: 2353–2356.

Oyama T, Mitsudomi T, Kawamoto T, Ogami A, Osaki T, Kodama Y, Yasumoto K (1995) Detection of CYP1A1 gene polymorphism using designed RFLP and distributions of CYP1A1 genotypes in Japanese. *Int. Arch. Occup. Environ. Health* **67**: 253–256.

Shibuta K, Mori M, Haraguchi M, Yoshikawa K, Ueo H, Akiyoshi T (1998) Association between restriction fragment length polymorphism of the L-*myc* gene and susceptibility to gastric cancer. *Brit. J. Surg.* **85**: 681–684.

Steinberg KK, Sanderlin KC, Ou CY, Hannon WH, McQuillan GM, Sampson EJ (1997) DNA banking in epidemiologic studies. *Epidemiol. Rev.* **19**: 156–162.

Soy, Isoflavones, and Breast Cancer Risk in Japan

Seiichiro Yamamoto, Tomotaka Sobue, Minatsu Kobayashi, Satoshi Sasaki, Shoichiro Tsugane

For the Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases (JPHC Study) Group

Background: **Although isoflavones, such as those found in soy, have been shown to inhibit breast cancer in laboratory studies, associations between consumption of isoflavone-containing foods and breast cancer risk have been inconsistent in epidemiologic studies. We evaluated the relationship between isoflavone consumption and breast cancer risk among women in the Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases (JPHC Study).** ***Methods:*** **In January 1990, 21 852 Japanese female residents (aged 40–59 years) from four public health center areas completed a self-administered questionnaire, which included items about the frequency of soy consumption. Through December 1999 and 209 354 person-years of follow-up, 179 women were diagnosed with breast cancer. Cox proportional hazards regression was used to estimate the relative risks (RRs) and 95% confidence intervals (CIs) for breast cancer in relation to consumption of miso soup, soyfoods, and estimated isoflavones. All statistical tests were two-sided.** ***Results:*** **Consumption of miso soup and isoflavones, but not of soyfoods, was inversely associated with the risk of breast cancer. The associations did not change substantially after adjustment for potential confounders, including reproductive history, family history, smoking, and other dietary factors. Compared with those in the lowest quartile of isoflavone intake, the adjusted RRs for breast cancer for women in the second, third, and highest quartiles were 0.76 (95% CI = 0.47 to 1.2), 0.90 (95% CI = 0.56 to 1.5), and 0.46 (95% CI = 0.25 to 0.84), respectively (P_{trend} = .043). The inverse association was stronger in postmenopausal women (P_{trend} = .006).** ***Conclusion:*** **In a population-based, prospective cohort study in Japan, frequent miso soup and isoflavone consumption was associated with a reduced risk of breast cancer. [J Natl Cancer Inst 2003;95:906–13]**

Soy is widely consumed in Asian countries, where the incidence of breast cancer is lower than that in Western countries *(1)*. In Japan, soy is consumed in various forms, including dried or green soybeans, tofu (soybean curd), natto (fermented soybeans), miso (fermented soybean paste), okara (tofu lees), soybean sprouts, soymilk, yuba (soy milk skin), kinako (soy flour), and soy sauce. Soy is a primary source of isoflavones, a group of phytoestrogens that have been hypothesized to reduce the risk of breast cancer. Genistein, daidzein, and their corresponding glucosides account for the major portion of isoflavones and have been the focus of numerous studies. Several experimental studies have shown that soy or isoflavones have anticarcinogenic effects on hormone-related cancers and that these effects may be related to their estrogenic, antiestrogenic, or other activities *(2–5)*. Other possible anticarcinogenic mechanisms associated with soy or isoflavones include inhibiting protein tyrosine kinases or other enzymes that interfere with cell growth and survival; stimulating sex hormone-binding globulin production, thereby decreasing the amount of free and active hormone in the blood; protecting DNA from damage via antioxidant effects; and inhibiting angiogenesis *(2–5)*.

By contrast with results from experimental studies, results from epidemiologic studies that assessed associations between soy or isoflavone consumption and breast cancer risk have varied. To date, 13 studies have evaluated associations between soy or isoflavone consumption and breast cancer risk (Table 1). Among studies that reported results for premenopausal women, four case–control studies conducted among Singapore Chinese *(6)*, Japanese *(7)*, Asian American *(8)*, and Chinese *(9)* populations found statistically significant inverse associations between soy consumption and breast cancer risk. However, one prospective American study *(10)* and four case–control studies conducted among Chinese *(11)*, American and Canadian *(12)*, non-Asian American *(13)*, and Asian American *(14)* populations found no such association. Among studies that reported results for postmenopausal women, two case–control studies conducted among Chinese *(9)* and Asian American *(14)* populations found statistically significant inverse associations, whereas two prospective American studies *(10,15)* and five case–control studies conducted among Singapore Chinese *(6)*, Japanese *(7)*, Chinese *(11)*, Asian American *(8)*, and non-Asian American *(13)* populations found no such associations. Two other prospective studies and one case–control study conducted among Japanese populations *(16–18)* reported combined results for pre- and postmenopausal women and found no statistically significant association between soy consumption and breast cancer risk. Thus, results were equivocal, even in studies using the same study design, geographic location, and menopausal status.

Because Japanese women have a low incidence of breast cancer *(1)* and a high intake of isoflavones, approximately 700 times that of U.S. Caucasians *(19)*, the Japanese population is an ideal setting for determining whether an association exists. To examine the possible association between consumption of soy and isoflavones and the risk of breast cancer, we conducted a population-based, prospective cohort study in Japan.

Affiliations of authors: S. Yamamoto, T. Sobue, Cancer Information and Epidemiology Division, National Cancer Center Research Institute, Tokyo, Japan; M. Kobayashi, S. Sasaki, S. Tsugane, Epidemiology and Biostatistics Division, National Cancer Center Research Institute, East, Kashiwa, Japan.

Correspondence to: Seiichiro Yamamoto, Ph.D., Cancer Information and Epidemiology Division, National Cancer Center Research Institute, 5–1-1, Tsukiji, Chuo-ku, Tokyo, Japan (e-mail: siyamamo@ncc.go.jp).

See "Appendix" for names and affiliations of members of the JPHC Study Group.

See "Notes" following "References."

Journal of the National Cancer Institute, Vol. 95, No. 12,

Table 1. Summary of epidemiological studies investigating soy or isoflavone comsumption and breast cancer

Author, year of publication (reference)	Population	No. of subjects	Exposure comparison category	Menopausal status	Relative risk (95% confidence intervals)
Cohort study					
Hirayama 1990 *(16)*	Japanese	241 cases 2 140 369 person-years	Miso soup: daily vs. non-daily	Combined	0.85 (0.68 to 1.06)
Greenstein et al. 1996 *(15)*	American	1018 cases 34 388 women	Soy or tofu: consumers vs. nonconsumers	Post	0.76 (0.50 to 1.18)
Key et al. 1999 *(17)*	Japanese	427 cases 488 989 person-years	Miso soup: ≥5 vs. ≤1 time/wk	Combined	0.87 (0.68 to 1.12)
Horn-Ross et al. 2002 *(10)*	American	711 cases 222 249 person-years	Genistein: fifth vs. first quintile	Combined*	1.0 (0.7 to 1.3)
Case–control study					
Hirohata et al. 1985 *(18)*	Japanese	212 cases 212 controls	Fat from soybean products: mean intake	Combined	Not significant
Lee et al. 1992 *(6)*	Singapore Chinese	200 cases 420 controls	Total soya products: ≥55.0 vs. <20.3 g/day	Pre Post	0.4 (0.2 to 0.9) 1.1 (0.5 to 2.3)
Hirose et al. 1995 *(7)*	Japanese	1186 cases 23 163 controls	Tofu: >3 vs. ≤3 times/wk	Pre Post	0.81 (0.65 to 0.99) 1.17 (0.92 to 1.49)
Yuan et al. 1995 *(11)*	Chinese	834 cases 834 controls	Soy protein: per 18 g/day	Combined*	1.0 (0.7 to 1.4)
Wu et al. 1996 *(8)*	Asian-American	597 cases 966 controls	Tofu: per 1 time/wk	Pre Post	0.84 (0.70 to 0.99) 0.86 (0.66 to 1.13)
Witte et al. 1997 *(12)*	American and Canadian	140 cases 222 controls	Tofu or soybeans: 1 serving/wk vs. none	Pre	0.5 (0.2 to 1.1)
Dai et al. 2001 *(9)*	Chinese	1459 cases 1556 controls	Soy protein: >139.1 vs. ≤18.6 g/wk	Combined*	0.66 (0.46 to 0.95)
Horn-Ross et al. 2001 *(13)*	Non-Asian American	1326 cases 1657 controls	Isoflavones: per 1000 μg/day	Pre Post	1.00 (0.98 to 1.02) 0.99 (0.97 to 1.01)
Wu et al. 2002 *(14)*	Asian-American	501 cases 594 controls	Isoflavones: >12.68 vs. ≤1.79 mg/1000 kcal	Pre Post	0.60; $P>.05$ 0.39; $P<.05$

*Results were not substantially different when analyzed separately by menopausal status.

Subjects and Methods

Study Cohort

The study cohort is a part of the Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases (JPHC Study) *(20–23)*. A detailed design of the study has been presented elsewhere *(24)*. The JPHC Study Cohort I was established on January 1, 1990, and includes 27 435 women and 27 063 men aged 40–59 years who registered their address in one of 14 administrative districts supervised by five public health centers (Ninohe Public Health Center in Iwate Prefecture, Yokote Public Health Center in Akita Prefecture, Saku Public Health Center in Nagano Prefecture, Ishikawa Public Health Center in Okinawa Prefecture, and Katsushika Public Health Center in the Tokyo metropolitan area) *(25)*. The subjects from the Katsushika Public Health Center were not included in the analysis because incidence data were not collected. This study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Exposure Data

The study period was from January 1, 1990, through December 31, 1999. In 1990, a baseline survey was conducted in which the study participants completed a self-administered questionnaire. Participants answered questions regarding smoking status; habitual intake of foods and beverages, including alcohol; physical activity; personal and family history of diseases; occupation; educational level; personality; and reproductive history. Thirty-eight questions concerned the consumption of foods and nonalcoholic beverages, of which two items dealt specifically with consumption of soy and isoflavones. One item asked about consumption of "miso soup" and the other about consumption of "soybeans, tofu, deep-fried tofu, and natto," which are foods that have soybeans as the major ingredient. We refer to the latter items as "soyfoods" throughout this article.

The frequency of miso soup consumption was divided into four categories: almost never, one or two bowls per week, three or four bowls per week, and almost daily. Study participants who answered "almost daily" were then asked an additional question regarding the average number of bowls of miso soup consumed per day. The frequency of soyfood intake was divided into four categories: almost never, one or two times per week, three or four times per week, and almost daily. Isoflavone intake was calculated using these two items, with a portion size of 100 mL for miso soup (10 g of miso paste, 2.9 mg of genistein) and 55 g for soyfoods (17.3 mg of genistein). Among the isoflavones, we reported all the results for genistein as "isoflavones" because the intake estimates of genistein, daidzein, and total isoflavone (defined as the sum of genistein and daidzein intake) were highly correlated and the relative risk (RR) estimates for them were similar. This similarity among estimates came from the fact that all three were calculated using answers to questions about miso soup and soyfood consumption. The portion size and isoflavone content were estimated from the validation study *(19,26)*. In the validation study, 247 subjects had provided 28-day dietary records and blood and urine samples between 1994 and 1995. The validation study showed that miso soup and soyfoods contributed more than 80% of the total isoflavone intake. The Spearman's correlation coefficients between the frequency of miso soup and soyfood consumption from the questionnaire and that from dietary records were 0.54 and 0.49, respectively, and between isoflavone intake estimated from the questionnaire and that from dietary records was 0.54. For reproducibility of estimation from the questionnaire, the correlation coefficients

for the frequency of consumption of miso soup and of soyfoods, and estimated isoflavone intake between two questionnaires administered 5 years apart were 0.70, 0.53, and 0.61, respectively.

The questionnaire included an item regarding consumption of "vegetables other than yellow and green vegetables such as Chinese cabbage, radish, tomato, cucumber, and so on"; this item includes soybean sprouts, which also contain isoflavones. Isoflavone intake from this item is negligible (0.6% of total isoflavone intake) but was included in the analysis.

Follow-Up

On January 1, 1990, a specific cancer registry for the JPHC Study was 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 prefecturewide population-based cancer registry, if available (Akita prefecture does not have a prefecturewide cancer registry).

The site of origin and histologic type of all cancers were coded using the International Classification of Diseases for Oncology, second edition (ICD-O-2) *(27)*. Among the 27 435 female study participants, 37 found to be ineligible after study entry and 12 diagnosed with breast cancer before study entry—the date of diagnosis was confirmed from death certificates—were excluded from the analysis. Of the remaining 27 386 participants, 225 participants were diagnosed with breast cancer during the study period. Eleven participants diagnosed with carcinoma *in situ* and two participants with uncertain diagnosis with regard to whether the tumor was benign or malignant were not considered as having breast cancers. A diagnosis of breast cancer was histologically confirmed for 97.4% of the patients by pathologists in local hospitals. The ratio of incidence to mortality was 5.4, and no cancer diagnoses were ascertained by death certificate alone, indicating that the completeness of cancer registration in this cohort was high.

Migration data were obtained from the residential registry. Among study participants, 1837 persons (6.7%) moved out of a study area and 34 persons (0.1%) were lost to follow-up during the study period.

Statistical Analysis

We excluded 4913 participants, of whom 33 were breast cancer patients, who did not answer the baseline questionnaire; 615 participants, of whom 13 were breast cancer patients, who answered a positive history of any cancer; and six participants, none of whom were breast cancer patients, who did not answer questions regarding dietary intake of foods and drinks. Thus, our analysis included 21 852 study participants, 179 of whom had breast cancer.

Person-years of follow-up were counted from the start of the study period (January 1, 1990) until the date of breast cancer diagnosis, the date of emigration from a study area, the date of death, or the end of the study period (December 31, 1999), whichever came first. For 34 participants who were lost to follow-up, the last confirmed date of their existence was used as the date of censoring. In total, 209 354 person-years were observed for 21 852 women. The crude incidence rate for breast cancer was calculated by dividing the number of breast cancer cases by the number of person-years. The incidence rates and the RRs of breast cancer are calculated for the categories of the frequency of miso soup consumption, soyfood consumption, and isoflavone intake in quartiles, with the lowest consumption category as the referent. Because most participants consumed miso soup daily, the frequency of miso soup consumption was categorized further into four groups using the information concerning average bowls per day as follows: not daily, one bowl per day, two bowls per day, and three or more bowls per day. Because few participants answered "almost never" to the questions on soyfood consumption, the frequency of soyfood consumption was also categorized further into three groups: less than two times per week, 3–4 times per week, and almost daily. RRs of breast cancer were estimated using the Cox proportional hazards model, with area (public health center) and 5-year age category at baseline (1990) as strata, by using the SAS PHREG procedure (version 8.02; SAS Institute, Cary, NC). This procedure allows for a different baseline hazard for each stratum. The assumptions for the Cox proportional hazards model were checked and found to hold.

In addition, adjusted RRs were also calculated. The following variables were included as potential confounders: history of benign breast diseases; family history of breast cancer in female first-degree relatives; active smoking status (never smoker, previous smoker, current smoker); passive smoking history at home; leisure-time physical activity (almost never, 1–3 times per month, 1–2 times per week, 3–4 times per week, almost daily); educational level (junior high school, high school, junior college, university, or higher); alcohol consumption (almost never, 1–3 times per month, more than once per week with less than 100 g ethanol per week, and more than once per week with more than 100 g ethanol per week); total energy intake; consumption of meat, vegetables, and fruits; age at menarche; number of pregnancies; menopausal status at the baseline questionnaire; use of exogenous female hormones (never used, previously used, currently used); height; weight; body mass index (BMI); and age at first pregnancy. Study participants were not asked about oral contraceptive use, but use among this population is rare. We did not include miso soup, soyfoods, and isoflavone consumption in the same models because of their colinearity. We performed two separate analyses: one with all participants and one after excluding 45 participants who were diagnosed with breast cancer during the first 3 years of follow-up and the seven participants whose diagnoses were not based on microscopic evidence. We excluded the 45 participants to eliminate those who may have had breast cancer but were not diagnosed when they answered the questionnaire. Linear trends were tested in the Cox models by treating the consumption categories as ordinal variables (consecutive integers). All P values are two-sided.

Results

Baseline Characteristics

At the time of the baseline survey, 2.7% of all study participants almost never consumed miso soup, 7.1% consumed one or two bowls per week, 15.4% consumed three or four bowls per week, and 74.8% consumed miso soup almost daily. Among the participants who consumed miso soup almost daily, 22.8% consumed one bowl per day, 43.1% consumed two bowls per day, and 34.1% consumed three or more bowls per day. Also at baseline, 2.2% of all participants almost never consumed soyfoods, 17.3% consumed soyfoods one or two times per week, 35.1% consumed soyfoods three or four times per week, and 45.4% consumed soyfoods almost daily.

From this information, we calculated the isoflavone consumption. The characteristics of the participants according to quartile of isoflavone consumption are shown in Table 2. Participants with higher isoflavone consumption were slightly older, had fewer pregnancies, were more likely to be postmenopausal, were younger at first pregnancy, and had slightly less education than those with lower isoflavone consumption (P_{trend}<.001 for all variables). Smoking status was associated with isoflavone consumption. Among those with higher isoflavone consumption, there were more never smokers and fewer past and current smokers than among those with lower isoflavone consumption (P_{trend}<.001). Passive smoking history was slightly less common among those in the lowest quartile of isoflavone consumption than it was among those in the other quartiles. Participants with higher isoflavone consumption had higher total energy intake; higher consumption of fish, meat, vegetables, and fruits; and lower consumption of alcohol than participants with lower isoflavone consumption (P_{trend}<.001 for all variables). Isoflavone intake was not linearly associated with a history of benign breast diseases, family history of breast cancer in female first-degree relatives, age at menarche, use of exogenous female hormones, height, weight, body mass index, or leisure time physical activity.

Miso Soup, Soyfood, and Isoflavone Intake and Breast Cancer Risk

We next determined whether there was an association between the consumption of miso soup, soyfoods, and isoflavones and risk of breast cancer. Area- and age-adjusted and fully adjusted RRs were not substantially changed. We found statistically significant inverse associations between breast cancer risk and consumption of miso soup (P_{trend} = .042) and isoflavone consumption (P_{trend} = .043) (Table 3). Compared with women in the lowest quartile of isoflavone consumption, those in the second, third, and highest quartiles of isoflavone consumption had adjusted RRs of breast cancer of 0.76 (95% CI = 0.47 to

Table 2. Characteristics of study participants at baseline according to isoflavone* consumption in the Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases Cohort I

	Isoflavone consumption				
	Lowest (N = 4690)	Second (N = 6003)	Third (N = 5643)	Highest (N = 5516)	P_{trend}†
Age, y‡	48.8 ± 5.8	49.4 ± 5.9	49.8 ± 5.9	50.5 ± 5.8	<.001
History of benign breast disease, %§	9.3	10.6	9.3	9.5	.60
Family history of breast cancer in female first-degree relatives, %	0.9	1.1	1.1	0.9	.98
Age at menarche, y	14.7 ± 1.9	14.6 ± 1.8	14.6 ± 1.8	14.7 ± 1.7	.15
No. of pregnancies	3.3 ± 1.9	3.2 ± 1.7	3.2 ± 1.7	3.1 ± 1.5	<.001
Age at first pregnancy, y	24.5 ± 3.7	24.5 ± 3.5	24.2 ± 3.5	23.7 ± 3.2	<.001
Menopausal status, %					<.001
Premenopause	52.5	48.3	44.6	38.9	
Natural menopause	39.7	43.7	46.2	51.5	
Surgical menopause	7.8	8.1	9.3	9.6	
Use of exogenous female hormones, %					.52
Never	80.1	78.9	79.4	78.8	
Past	18.1	19.3	18.9	19.6	
Current	1.9	1.9	1.7	1.7	
Height, cm	151.5 ± 5.4	151.8 ± 5.3	151.6 ± 5.3	151.6 ± 5.3	.95
Weight, kg	54.2 ± 8.2	54.3 ± 7.5	54.4 ± 7.9	54.2 ± 7.7	.76
Body mass index, kg/m^2	23.6 ± 3.4	23.5 ± 3.1	23.6 ± 3.3	23.5 ± 3.1	.62
Smoking, %					<.001
Never	88.5	92.6	92.9	95.9	
Past	2.3	2.0	1.7	0.9	
Current	9.3	5.4	5.4	3.2	
History of passive exposure to smoke at home, %	67.2	70.5	70.6	70.4	<.001
Leisure-time physical activity (>1 time/mo), %	20.4	22.7	23.0	19.4	<.001
Educational level beyond high school, %	10.4	12.2	10.7	7.8	<.001
Alcohol consumption (>100 g ethanol/wk), %	11.5	11.1	11.5	8.4	<.001
Total energy intake, kcal/day	1202.3 ± 390.0	1352.0 ± 317.1	1414.9 ± 343.3	1586.0 ± 368.5	<.001
Fish, g/day	31.1 ± 24.6	40.8 ± 26.4	47.8 ± 29.3	59.3 ± 30.9	<.001
Meat, g/day	34.7 ± 19.7	38.5 ± 20.5	39.0 ± 21.4	40.1 ± 22.7	<.001
Vegetables, g/day	139.1 ± 86.9	172.5 ± 86.0	183.2 ± 91.9	221.1 ± 84.8	<.001
Fruits, g/day	105.0 ± 107.1	126.5 ± 98.0	133.8 ± 109.0	152.2 ± 114.5	<.001
Miso soup, %					<.001
<1 time/day	62.0	22.2	22.4	0.0	
1 cup/day	14.9	22.9	29.5	0.0	
2 cups/day	21.6	40.3	24.7	40.1	
≥3 cups/day	1.5	14.6	23.4	59.9	
Soyfoods, %					<.001
<2 times/wk	76.7	10.3	0.4	0.1	
3–4 times/wk	23.3	86.9	23.0	1.3	
Almost every day	0.0	2.8	76.6	98.7	
Isoflavone, mg/day	6.9 ± 2.6	13.0 ± 2.1	20.0 ± 2.1	25.3 ± 2.2	<.001

*All results for genistein are reported as isoflavones because the intake estimates for genistein, daidzein, and total isoflavone were highly correlated.
†All *P* values are two-sided.
‡Values are reported as means with standard deviations.
§Because of rounding, not all percentages add to 100.

Table 3. Relative risk (RR) and 95% confidence intervals (CIs) of breast cancer according to miso soup, soyfood, and isoflavone* consumption in the Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases Cohort I

	No. of cases	Person-years of follow-up	Incidence rate per 100 000	Area- and age-adjusted		Multivariable†	
				RR (95% CI)	P_{trend}‡	RR (95% CI)	P_{trend}
Miso soup							
<1 cup/day	51	51 859	98.3	1	.005	1	.042
1 cup/day	39	35 560	109.7	1.1 (0.71 to 1.6)		1.1 (0.67 to 1.7)	
2 cups/day	58	67 764	85.6	0.80 (0.54 to 1.2)		0.74 (0.46 to 1.2)	
≥3 cups/day	31	54 171	57.2	0.51 (0.32 to 0.83)		0.60 (0.34 to 1.1)	
Soyfoods							
<2 times/wk	38	40 356	94.2	1	.49	1	.44
3–4 times/wk	60	73 434	81.7	0.86 (0.57 to 1.3)		0.83 (0.52 to 1.3)	
Almost daily	81	95 564	84.8	0.85 (0.57 to 1.3)		0.81 (0.49 to 1.3)	
Isoflavone quartile							
Lowest	44	44 361	99.2	1	.025	1	.043
Second	50	57 457	87.0	0.85 (0.56 to 1.3)		0.76 (0.47 to 1.2)	
Third	52	54 006	96.3	0.90 (0.60 to 1.4)		0.90 (0.56 to 1.5)	
Highest	33	53 530	61.6	0.54 (0.33 to 0.87)		0.46 (0.25 to 0.84)	

*All results for genistein are reported as isoflavone because the intake estimates for genistein, daidzein, and total isoflavone were highly correlated.

†Adjusted by area; age; age at menarche; number of pregnancies; menopausal status; age at first pregnancy; active and passive smoking; alcohol consumption; leisure-time physical activity; educational level; total energy; and meat, fish, vegetable, and fruit consumption.

‡All P values are two-sided.

1.2), 0.90 (95% CI = 0.56 to 1.5), and 0.46 (95% CI = 0.25 to 0.84), respectively. There was no statistically significant association between consumption of soyfoods and breast cancer risk (P_{trend} = .44). Crude incidence rates varied from 57.2 to 109.7 per 100 000 person-years for miso soup consumption categories, which was a larger variation than those for soyfood categories (81.7 to 94.2 per 100 000 person-years) and those for isoflavone intake quartiles (61.6 to 99.2 per 100 000 person-years).

Breast Cancer Risk by Menopausal Status

We next stratified the data according to menopausal status at the time of the baseline questionnaire and examined associations between miso soup, soyfood, or isoflavone consumption and breast cancer risk, using participants in the lowest consumption category as the referent group. Among premenopausal and postmenopausal women considered separately, miso soup consumption was inversely associated with breast cancer risk. Consumption of soyfoods was inversely associated with breast cancer risk among postmenopausal women (P_{trend} = .031). This inverse association led to a stronger inverse association found in postmenopausal women for isoflavone consumption (P_{trend} = .006) than in premenopausal women (Table 4). Crude incidence rates varied from 49.1 to 123.6 per 100 000 person-years among isoflavone quartiles for premonopausal women, which was a larger variation than that for postmenopausal women.

Sensitivity Analysis

We next excluded the 45 participants who were diagnosed with breast cancer during the first 3 years of follow-up and the seven participants whose diagnoses were not made on the basis of histologic evidence. None of the results changed substantially.

The results for the multivariable analysis in Tables 3 and 4 show the RRs after adjusting for possible confounding variables that were associated with isoflavone consumption (P<.20 for all associations). None of the results substantially changed by using other multivariable models, such as those including all the potential confounding variables listed in Table 2.

Table 4. Relative risk (RR) and 95% confidence intervals (CIs) of breast cancer according to isoflavone* consumption by baseline menopausal status in the Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases Cohort I

Isoflavone quartile	No. of cases	Person-years of follow-up	Incidence rate per 100 000	Area- and age-adjusted		Multivariable†	
				RR (95% CI)	P_{trend}‡	RR (95% CI)	P_{trend}
Premenopausal women							
Lowest	21	22 676	92.6	1	.20	1	.97
Second	29	27 129	106.9	1.1 (0.63 to 2.0)		1.0 (0.50 to 2.0)	
Third	29	23 467	123.6	1.3 (0.71 to 2.3)		1.6 (0.79 to 3.1)	
Highest	10	20 356	49.1	0.48 (0.22 to 1.1)		0.66 (0.25 to 1.7)	
Postmenopausal women							
Lowest	22	20 644	106.6	1	.037	1	.006
Second	21	29 168	72.0	0.64 (0.35 to 1.2)		0.58 (0.29 to 1.1)	
Third	23	29 630	77.6	0.64 (0.35 to 1.2)		0.50 (0.25 to 1.0)	
Highest	21	32 195	65.2	0.47 (0.25 to 0.90)		0.32 (0.14 to 0.71)	

*All results for genistein are reported as isoflavone because the intake estimates for genistein, daidzein, and total isoflavone were highly correlated.

†Adjusted by area; age; age at menarche; number of pregnancies; age at first pregnancy; active and passive smoking; alcohol consumption; leisure-time physical activity; educational level; total energy; and meat, fish, vegetable, and fruit consumption.

‡All P values are two-sided.

DISCUSSION

Our cohort study found a statistically significant inverse association between miso soup or isoflavone intake and risk of breast cancer in Japanese women. Women with the highest intake of isoflavone (as genistein, 25.3 mg/day) or those with the highest consumption of miso soup (three or more bowls/day) had approximately half the risk of breast cancer as women with the lowest intake of isoflavone (as genistein, 6.9 mg/day) or those with the least consumption of miso soup (less than once a day), respectively. We found no association between consumption of soyfoods and breast cancer risk.

The inverse association between isoflavone intake and breast cancer risk found in our epidemiologic study is consistent with results from ecologic and experimental studies *(2–5)*. Lack of association between soy or isoflavone consumption and breast cancer risk in previous epidemiologic studies may be the result of recall bias in case–control studies, errors in exposure measurements, or small exposure variation in Western subjects.

Our study had several methodologic advantages over previous studies. First, we evaluated the association in a prospective cohort study of participants enrolled from the general population. A prospective study is free from recall bias, and the results from the general population are more applicable than those from a specific population, such as an occupational cohort or a cohort of a population with a specific characteristic. Second, we determined the risk for breast cancer incidence rather than breast cancer deaths using highly precise cancer incidence data. Incidence is a more direct measure of breast cancer risk than death because time to breast cancer death is greatly influenced by the treatment received. Third, we estimated isoflavone intake as well as soy intake by using a validated questionnaire. We could therefore investigate the association between breast cancer and isoflavone intake separately from other contents in soy. Fourth, we determined that there was a large variation in isoflavone consumption among participants. Associations between disease and exposure can be detected more easily when exposure has wide variability. Indeed, median isoflavone intake among all the study participants was seven times higher than that among Chinese in Singapore, 15 times higher than that among U.S. non-Asian (African American, Latina, and white) women, and 700 times higher than that among U.S. Caucasians *(28–30)*. Fifth, our cohort was established in 1990, thus reflecting present-day lifestyles and dietary habits and a large variation in the distribution of possible risk factors. Although consumption of isoflavones was high throughout Japan before World War II, the variation in intake levels is increasing with increasing westernization of the Japanese population. In addition, other risk factors for breast cancer, such as earlier age at menarche and high fat and meat consumption *(31)* are also becoming more common among the Japanese. This difference in the distribution of possible risk factors may explain the difference between our results and those of Japanese cohorts that were established in the 1960s and 1970s *(16,17)*.

The highest incidence (99.2/100 000) of breast cancer in the present study was observed among women in the lowest quartile of isoflavone consumption. This incidence rate is lower than that among corresponding age groups (40–69 years) in Western populations *(1)*. Women in the lowest quartile of isoflavone consumption consumed approximately 6.9 mg/day of genistein, which is still 250 times more than the daily amount consumed by U.S. Caucasian women but only several times more than that consumed by U.S. non-Asian women. One hypothesis drawn from this comparison is that there may be a potential dose–response relationship, even among those with lower intake levels of isoflavones observed among Western cohorts, although it may be difficult to detect because of small variations.

Our study has several limitations. First, the number of breast cancer cases was small. Possible associations between breast cancer risk and soyfoods that were not statistically significant in our study may be detected among larger sample sizes. For miso soup and isoflavones, although our study found a statistically significant association with breast cancer, more precise RR estimates can be derived from larger sample sizes. Second, our food-frequency questionnaire included only two items concerning soybean-ingredient foods (i.e., miso soup and soyfoods), making it impossible to investigate the difference in effects between types of soybean-ingredient foods. Third, although we adjusted for the consumption of dietary items other than soy as much as possible, we cannot exclude the possibility of residual confounding by other dietary characteristics.

The inverse association between isoflavone intake and breast cancer risk was stronger in postmenopausal women than premenopausal women. Although this result is somewhat surprising in that it is not consistent with previous epidemiologic studies (Table 1), experimental studies have shown effects of soy or isoflavones on hormone levels in postmenopausal women *(32–34)*. This stronger inverse association observed in postmenopausal women may explain the larger discrepancy in breast cancer incidence between Japan and Western countries in older age groups than in younger age groups *(1)*. Although components of soy are known to affect menstrual cycles in different ways *(35–40)* and late menopause is known to be associated with breast cancer risk *(31)*, the link between soy, menopause, and breast cancer remains to be elucidated. Because a proportion of the premenopausal women at the baseline survey may have experienced menopause during the study period, our results for premenopausal women may be contaminated with those for the postmenopausal women. Regardless, our data suggest that menopausal status would be a confounder, an intermediate variable, or an effect modifier when analyzing the association between intake of isoflavones and breast cancer risk and should be taken into account in any analysis. In addition, the fact that the proportion of hormone receptor-positive breast cancers increases with age among U.S. women *(41)* may also explain the discrepancy between Japanese and Western countries in incidence among older age groups. This is consistent with evidence that the anticarcinogenic effect of isoflavones comes from the antiestrogenic or estrogenic activity through their affinity to estrogen receptors *(42)*.

Monotonic inverse relationships were observed between breast cancer risk and miso soup consumption but were not clearly observed in soyfood and isoflavone consumption. This non-monotonicity may be a result of the relatively low validity of intake estimates from soyfood because they consist of four different types of soybean-ingredient foods (soybeans, tofu, deep-fried tofu, and natto). Indeed, the difference between subjects in the second and third quartiles of isoflavone intake was due mainly to a difference in the consumption frequency of soyfoods (Table 2). The difference in results for soyfoods and isoflavones may also be due to the composite nature of the question on soyfoods. More valid estimates of soyfood intake

might reveal monotonic relationships between soyfood and isoflavone intake and breast cancer risk.

A reduced breast cancer risk was also associated with other Japanese eating habits including eating more rice, pickles, vegetables, fish, and less bread and butter (Yamamoto S: unpublished data). Among these, some (rice, pickles, vegetables, and fish) are commonly served with miso soup and soyfoods, whereas others (bread and butter) are less commonly served with them. Japanese eating habits, food items, or lifestyle might also help to explain the low risk of breast cancer among this population. Soy, however, showed the strongest associations after adjustment for other foods and lifestyles and, on the basis of experimental data, provides the most biologically plausible explanation for reducing breast cancer risk. The relationship between soy or isoflavone intake and breast cancer risk should be further investigated by collecting more cases, by more sophisticated analyses, and by using a more intensive food-frequency questionnaire such as that used in the 5-year follow-up survey of our cohort *(19)*. This further investigation may clarify different roles for various soybean-ingredient foods on breast cancer risk, which was suggested by our stratified analysis by menopausal status in which a statistically significant relationship was observed between soyfoods and breast cancer risk for postmenopausal women. We plan to conduct a series of nested case–control studies using stored blood samples collected at the baseline survey and the 5-year follow-up survey *(43)* to elucidate the association between levels of serum isoflavones and the risk of breast cancer *(44)*.

In conclusion, in a prospective cohort study in Japan, we found that frequent miso soup and isoflavone consumption reduced the risk of breast cancer. We found no evidence for such an association for intake of soyfoods.

Appendix

Members of the Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases (JPHC Study) Group: S. Tsugane, S. Sasaki, T. Sobue, National Cancer Center Research Institute, Kashiwa and Tokyo; J. Ogata, S. Baba, National Cardiovascular Center, Suita; K. Miyakawa, F. Saito, A. Koizumi, Iwate Prefectural Ninohe Public Health Center, Ninohe; Y. Miyajima, N. Suzuki, S. Nagasawa; Akita Prefectural Yokote Public Health Center, Yokote; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, Nagano Prefectural Saku Public Health Center, Saku; Y. Kishimoto, E. Takara, M. Kinjo, T. Fukuyama, Okinawa Prefectural Ishikawa Public Health Center, Ishikawa; S. Matsushima, S. Natsukawa, Saku General Hospital, Usuda; 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, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka; the late M. Yamaguchi, Y. Matsumura, National Institute of Health and Nutrition, Tokyo; Y. Tsubono, Tohoku University, Sendai; H. Iso, Tsukuba University, Tsukuba; H. Sugimura, Hamamatsu University, Hamamatsu; M. Kabuto, National Institute for Environmental Studies, Tsukuba.

References

(1) Parkin, DM, Whelan SL, Ferlay J, Raymond L, Young J, editors. Cancer incidence in five continents. Vol. VII. Lyon (France): IARC Sci Publ 1997;143.

(2) Adlercreutz H, Mazur W. Phyto-oestrogens and Western diseases. Ann Med 1997;29:95–120.

(3) Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A. Phyto-oestrogens: where are we now? Br J Nutr 1998;79:393–406.

(4) Adlercreutz CH, Goldin BR, Gorbach SL, Höckerstedt KA, Watanabe S, Hämäläinen EK, et al. Soybean phytoestrogen intake and cancer risk [published erratum appears in J Nutr 1960;125]. J Nutr 1995;125:757S–70S.

(5) Messina MJ, Persky V, Setchell KD, Barnes S. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr Cancer 1994;21:113–31.

(6) Lee HP, Gourley L, Duffy SW, Esteve J, Lee J, Day NE. Risk factors for breast cancer by age and menopausal status: a case-control study in Singapore. Cancer Causes Control 1992;3:313–22.

(7) Hirose K, Tajima K, Hamajima N, Inoue M, Takezaki T, Kuroishi T, et al. A large-scale, hospital-based case-control study of risk factors of breast cancer according to menopausal status. Jpn J Cancer Res 1995;86:146–54.

(8) Wu AH, Ziegler RG, Horn-Ross PL, Nomura AM, West DW, Kolonel LN, et al. Tofu and risk of breast cancer in Asian-Americans. Cancer Epidemiol Biomarkers Prev 1996;5:901–6.

(9) Dai Q, Shu XO, Jin F, Potter JD, Kushi LH, Teas J, et al. Population-based case-control study of soyfood intake and breast cancer risk in Shanghai. Br J Cancer 2001;85:372–8.

(10) Horn-Ross PL, Hoggatt KJ, West DW, Krone MR, Stewart SL, Anton-Culver H, et al. Recent diet and breast cancer risk: the California Teachers Study (USA). Cancer Causes Control 2002;13:407–15.

(11) Yuan JM, Wang QS, Ross RK, Henderson BE, Yu MC. Diet and breast cancer in Shanghai and Tianjin, China. Br J Cancer 1995;71:1353–8.

(12) Witte JS, Ursin G, Siemiatycki J, Thompson WD, Paganini-Hill A, Haile RW. Diet and premenopausal bilateral breast cancer: a case-control study. Breast Cancer Res Treat 1997;42:243–51.

(13) Horn-Ross PL, John EM, Lee M, Stewart SL, Koo J, Sakoda LC, et al. Phytoestrogen consumption and breast cancer risk in multiethnic population. The Bay Area Cancer Study. Am J Epidemiol 2001;154:434–41.

(14) Wu AH, Wan P, Hankin J, Tseng CC, Yu MC, Pike MC. Adolescent and adult soy intake and risk of breast cancer in Asian-Americans. Carcinogenesis 2002;23:1491–6.

(15) Greenstein J, Kushi L, Zheng W, Fee R, Campbell D, Sellers T, et al. Risk of breast cancer associated with intake of specific foods and food groups. Am J Epidemiol 1996;143:S36.

(16) Hirayama T. Life-style and mortality: a large-scale census-based cohort study in Japan. In: Wahrendorf J, editor. I: Contributions to epidemiology and biostatistics. Vol. 6. Basel (Switzerland): Karger; 1990.

(17) Key TJ, Sharp GB, Appleby PN, Beral V, Goodman MT, Soda M, et al. Soya foods and breast cancer risk: a prospective study in Hiroshima and Nagasaki, Japan. Br J Cancer 1999;81:1248–56.

(18) Hirohata T, Shigematsu T, Nomura AM, Nomura Y, Horie A, Hirohata I. Occurrence of breast cancer in relation to diet and reproductive history: a case-control study in Fukuoka, Japan. Natl Cancer Inst Monogr 1985;69:187–90.

(19) Yamamoto S, Sobue T, Sasaki S, Kobayashi M, Arai Y, Uehara M, et al. Validity and reproducibility of a self-administered food-frequency questionnaire to assess isoflavone intake in a Japanese population in comparison with dietary records and blood and urine isoflavones. J Nutr 2001;131:2741–7.

(20) Tsugane S, Fahey MT, Sasaki S, Baba S. Alcohol consumption and all-cause and cancer mortality among middle-aged Japanese men: seven-year follow-up of the JPHC study cohort I. Japan Public Health Center. Am J Epidemiol 1999;150:1201–7.

(21) Hara M, Sasaki S, Sobue T, Yamamoto S, Tsugane S. Comparison of cause-specific mortality between respondents and non-respondents in a population-based prospective study: ten-year follow-up of JPHC Cohort I. Japan Public Health Center. J Clin Epidemiol 2002;55:150–6.

(22) Hara M, Sobue T, Sasaki S, Tsugane S. Smoking and risk of premature death among middle-aged Japanese: ten-year follow-up of the Japan Public Center-based prospective study on cancer and cardiovascular diseases (JPHC Study) cohort I. Jpn J Cancer Res 2002;93:6–14.

(23) Sobue T, Yamamoto S, Hara M, Sasazuki S, Sasaki S, Tsugane S. Cigarette smoking and subsequent risk of lung cancer by histologic type in middle-aged Japanese men and women: the JPHC Study. Int J Cancer 2002;99:245–51.

(24) Watanabe S, Tsugane S, Sobue T, Konishi M, Baba S. Study design and organization of the JPHC study. Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol 2001;11:S3–7.

(25) Yamamoto S, Watanabe S. Geographic characteristics and mortality pro-

files in the JPHC Study. Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol 2001;11(6 Suppl):S8–23.
(26) Tsubono Y, Kobayashi M, Sasaki S, Tsugane S. Validity and reproducibility of a self-administered food frequency questionnaire used in the baseline survey of the JPHC Study Cohort I. J Epidemiol 2003;13:S125–33.
(27) World Health Organization (WHO). International classification of diseases for oncology, 2nd edition. Geneva (Switzerland): WHO; 1990.
(28) Seow A, Shi CY, Franke AA, Hankin JH, Lee HP, Yu MC. Isoflavonoid levels in spot urine are associated with frequency of dietary soy intake in a population-based sample of middle-aged and older Chinese in Singapore. Cancer Epidemiol Biomarkers Prev 1998;7:135–40.
(29) Horn-Ross PL, Lee M, John EM, Koo J. Sources of phytoestrogen exposure among non-Asian women in California, USA. Cancer Causes Control 2000;11:299–302.
(30) Strom SS, Yamamura Y, Duphorne CM, Spitz MR, Babaian RJ, Pillow PC, et al. Phytoestrogen intake and prostate cancer: a case-control study using a new database. Nutr Cancer 1999;33:20–5.
(31) American Institute for Cancer Research (AICR)/World Cancer Research Fund. Food, nutrition and the prevention of cancer: a global perspective. Breast. Washington (DC): AICR; 1997. p. 252–87.
(32) Duncan AM, Underhill KE, Xu X, Lavalleur J, Phipps WR, Kurzer MS. Modest hormonal effects of soy isoflavones in postmenopausal women. J Clin Endocrinol Metab 1999;84:3479–84.
(33) Pino AM, Valladares LE, Palma MA, Mancilla AM, Yanez M, Albala C. Dietary isoflavones affect sex hormone-binding globulin levels in postmenopausal women. J Clin Endocrinol Metab 2000;85:2797–800.
(34) Xu X, Duncan AM, Wangen KE, Kurzer MS. Soy consumption alters endogenous estrogen metabolism in postmenopausal women. Cancer Epidemiol Biomarkers Prev 2000;9:781–6.
(35) Cassidy A, Bingham S, Setchell KD. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr 1994;60:333–40.
(36) Cassidy A, Bingham S, Setchell K. Biological effects of isoflavones in young women: importance of the chemical composition of soybean products. Br J Nutr 1995;74:587–601.
(37) Lu LJ, Anderson KE, Grady JJ, Nagamani M. Effects of soya consumption for one month on steroid hormones in premenopausal women: implications for breast cancer risk reduction. Cancer Epidemiol Biomarkers Prev 1996; 5:63–70.
(38) Nagata C, Takatsuka N, Inaba S, Kawakami N, Shimizu H. Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. J Natl Cancer Inst 1998;90:1830–5.
(39) Duncan AM, Merz BE, Xu X, Nagal TC, Phipps WR, Kurzer MS. Soy isoflavones exert modest hormonal effects in premenopausal women. J Clin Endocrinol Metab 1999:84;192–7.
(40) Lu LJ, Anderson KE, Grady JJ, Kohen F, Nagamani M. Decreased ovarian hormones during a soya diet: implications for breast cancer prevention. Cancer Res 2000;60:4112–21.
(41) Li CI, Darling JR, Malone KE. Incidence of invasive breast cancer by hormone receptor status from 1992 to 1998. J Clin Oncol 2003;21:28–34.
(42) Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998;139:4252–63.
(43) Willett W. Future research directions. In: Willett W, editor. Nutritional epidemiology. 2nd ed. New York (NY): Oxford University Press; 1998. p.486.
(44) Ingram D, Sanders K, Kolybaba M, Lopez D. Case-control study of phytoestrogens and breast cancer. Lancet 1997;350;990–4.

NOTES

Present address: Dr. S. Sasaki, National Institute of Health and Nutrition, Tokyo, Japan.

Supported by Grants-in-Aid for Cancer Research (13S-2) and by the Research on Food and Chemical Safety Health and Labour Sciences Research Grant (14120701), both from the Ministry of Health, Labour and Welfare of Japan.

We thank all staff members in each study area and in the central offices for their efforts in conducting the baseline survey and follow-up. We also wish to thank the Iwate, Aomori, and Okinawa cancer registries for providing incidence data.

Manuscript received October 7, 2002; revised April 2, 2003; accepted April 15, 2003.

〔総　説〕

内分泌撹乱物質と子宮内膜症との関連性について

土谷　雅紀, 今井　博久, 中尾　裕之, 黒田　嘉紀, 加藤　貴彦
宮崎医科大学　医学部　公衆衛生学講座

要　　旨: 近年, 環境汚染と人の健康問題との関連性が大きく懸念されている. 内分泌撹乱物質は生体内で本来機能するホルモン作用を阻害・模倣する外因性の化学物質であり, ダイオキシン類などがその例として知られている. 子宮内膜症と診断される女性の数は増加しているとされ, 生殖年齢にある女性の約7−10%が子宮内膜症であると推定される. 最近では子宮内膜症と内分泌撹乱物質, 特にダイオキシン類との関連性が大きな話題となっている. 子宮内膜症の発生・進展機序は未だ詳しく解明されておらず, 有効な予防法も確立されていない. 現在さまざまな方法によって内分泌撹乱物質の作用機序や影響を解明する研究が進んでいる. 子宮内膜症の発症には遺伝要因, 環境要因などが相互に関係しており, 遺伝子多型と環境要因の関連性が注目されているが未だその全容は明らかではない. これらの新しい研究が子宮内膜症の病態解明に貢献することが期待されている.

キーワード: 内分泌撹乱物質, ダイオキシン類, 子宮内膜症, 環境要因, 遺伝子多型.

(2003年6月13日　受付, 2003年7月11日　受理)

はじめに

近年, 環境汚染と人の健康問題との関連性が大きく懸念されている. 日常生活において身のまわりには多種多様な化学物質が存在しており, それらから完全に逃れることはもはや不可能である.

シーア・コルボーン著の「奪われし未来」は環境汚染が野生動物の生殖障害など様々な問題を引き起こしていることを示し, さらに人類の未来への道行きを「無視界飛行」と例えて警告している [1]. 「奪われし未来」の出版以降, 内分泌撹乱物質(いわゆる環境ホルモン)の存在は広く世間に知られるようになり, 現在, 数多くの基礎的・臨床的研究ならびに健康影響への調査が行われている.

本稿では内分泌撹乱物質について現在までに得られている知見を概説し, さらに内分泌撹乱物質による健康障害の例として子宮内膜症を取り上げ, これまでの知見を整理した.

内分泌撹乱物質とは

1. 内分泌撹乱物質の定義

内分泌撹乱物質は「動物の生体内に取り込まれた場合に, 本来その生体内で営まれている正常なホルモン作用に影響を与える外因性の物質」と定義される [2].

内分泌撹乱物質の候補としてあげられてい

る化学物質は多岐にわたり[3], 有機塩素系化合物(ダイオキシン類, PCBなど), 農薬・殺虫剤(DDTなど), 芳香族化合物(ビスフェノールAなど), 金属化合物(有機スズなど), その他の化学物質(植物エストロゲンや合成エストロゲンなど)のように分類することができる(Table 1).

2. 内分泌撹乱物質の作用機序

ダイオキシン類は芳香族炭化水素レセプター(Arylhydrocarbon receptor, AhR)を, その他の多くの内分泌撹乱物質はエストロゲンレセプターを介してその作用を発現するとされる.

ダイオキシン類は細胞内のAhRと複合体を形成した後, 核内に移行する. 核内ではAh receptor nuclear translocator(Arnt)とヘテロダイマーを形成し, 応答遺伝子上流のXenobiotic responsive element(XRE)に結合して転写を促進する. この機構によってcytochrome P450(CYP)1A1, 1B1などの遺伝子が活性化される. 最近ではAhR repressor(AhRR)の存在も報告されている(Fig. 1, 2).

内分泌撹乱物質の作用の特徴としてエストロゲン類似作用を示すものが多いこと, 生体内での蓄積性が高い物質が多いことがあげられる. また従来では影響がないと考えられていた低用量でも作用を示し, その用量より多くても少なくても効果が弱くなる低用量効果

Table 1. 主要な内分泌撹乱物質

種類	説明
ダイオキシン類	ポリ塩化ジベンゾパラジオキシン, ポリ塩化ジベンゾフランの異性体の総称. ダイオキシン類の毒性は異性体によって大きく異なり, 2,3,7,8-tetrachlorodibenzo-p-dioxin(2,3,7,8-TCDD)の毒性が最も強い. 主に廃棄物の焼却過程で非意図的に生成される.
ポリ塩化ビフェニール(PCB)	コンデンサーなどの熱媒体として広く使用されていたが, 1972年に生産中止となっている. 現在でも生体や環境中から検出されている.
ジクロロジフェニルトリクロロエタン(DDT)	農薬, 殺虫剤として大量に使用されていたが, 1981年に生産中止となっている. 現在でも生体や環境中から検出されている.
ビスフェノールA	エポキシ樹脂の原料・酸化防止剤として年間数十万トン使用されている.
フタル酸エステル	塩化ビニルなど, プラスチックの可塑剤として広く使用されている.
有機スズ	船底や魚網の防除剤として使用されていた.
ジエチルスチルベストロール(DES)	流産予防薬として使用された合成エストロゲン製剤. 女児の膣癌の発生が次世代影響として明らかになっている.

ダイオキシン類, 有機スズ以外はエストロゲンレセプターを介して作用する

を有するとされる.

3. 内分泌撹乱物質による健康障害

内分泌撹乱物質にはさまざまな作用があり,生殖,神経,免疫系など生体の多くの機能に影響を及ぼす可能性が指摘されている. 内分泌撹乱物質による健康障害として乳癌の増加や精子数の減少,ある種の先天異常や発育障害の発生などが報告されている(Table 2).

子宮内膜症とは

1. 子宮内膜症とはどのような疾患か

子宮内膜症は「子宮内膜組織あるいはそれに類似した組織が子宮以外の部位で発生・発育する疾患」と定義される.子宮内膜症はエストロゲン依存性の疾患であり,月経と同様に出血や増殖を繰り返しながら除々に症状が進行していくと考えられている.

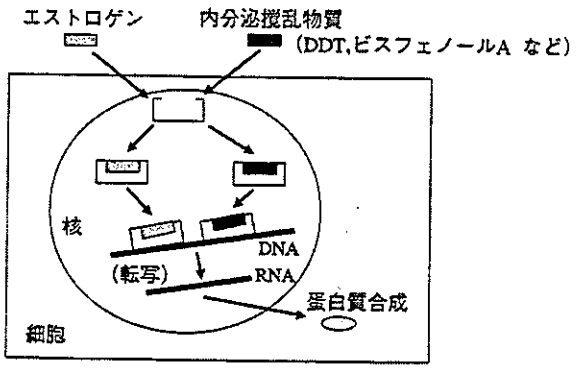

Fig. 1. 内分泌撹乱物質のエストロゲンレセプターを介する作用機序.

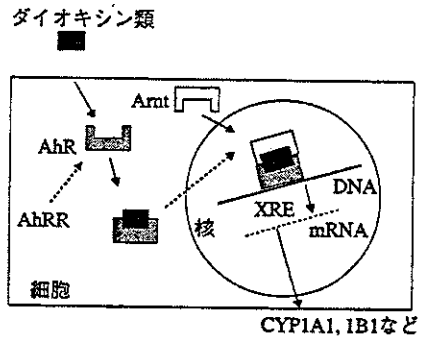

Fig. 2. 内分泌撹乱物質(ダイオキシン類)のAhRを介する作用機序.

Table 2. 内分泌撹乱物質による健康障害

生殖系への影響	神経系への影響	免疫系への影響	その他
子宮内膜症	行動異常 [9]	免疫異常 [13]	先天異常(尿道下裂など) [15]
乳癌,子宮体癌,卵巣癌,前立腺癌,精巣癌の増加 [4−6]	発達障害 [10]	アレルギー疾患 [14]	思春期の早発化 [16]
精子数の減少 [7]	精神発達遅延 [11]		
不妊症 [8]	知能障害,IQ低下 [12]		

現時点では一定の結論は得られておらず,今後も詳しい調査が必要である

子宮内膜症は疼痛と不妊を主症状とする疾患であり,疼痛には月経困難症,下腹痛,腰痛,性交痛などがある.少子化に代表される女性のライフスタイルの変容,初経年齢の低下,腹腔鏡の普及といった様々な要因によって子宮内膜症と診断される女性の数は近年増加しているとされる [17−19].

子宮内膜症は生殖年齢にある女性の約7−10%程度にみられ[20−22],女性のプライマリケアの観点からも重点的に取り組むべき課題であるが,その発生・進展機序は未だ詳しく解明されていない.

2.子宮内膜症の発生機序

子宮内膜症は骨盤腔外にも発生し,その発生部位の違いによって発生機序が異なる可能性が指摘されている.骨盤腹膜における子宮内膜症の発生機序は2つの学説に総括され,その1つは月経血と共に子宮内膜組織が卵管を介して腹腔内に逆流し,それが生着・増殖するという子宮内膜移植説である.もう1つは卵巣や腹膜の上皮が何らかの原因によって子宮内膜症組織に化生するという体腔上皮化生説である [23].

月経血の逆流は大部分の女性で認められるいわゆる「生理的な」現象であり,一部の女性だけに子宮内膜症が発症する原因は不明である.子宮内膜症の発生・進展には内分泌学的な要因以外にも,免疫,遺伝,環境要因といった様々な因子が関係しており,問題解決を困難なものとしている.

内分泌撹乱物質と子宮内膜症

1.環境要因と子宮内膜症

子宮内膜症はエストロゲン依存性の疾患であり,子宮内膜症の発症・増悪にエストロゲン様作用をもつ内分泌撹乱物質が関与している可能性が推測されている.

Table 3. ダイオキシン類と子宮内膜症に関する研究

報告者	年次	研究報告	文献
汚染状況			
Mayani	1997	子宮内膜症患者における血中 TCDD 濃度の調査	[24]
Eskenazi	2000	ダイオキシン類汚染地域における女性の健康調査	[25]
発生・増悪			
Rier	1993	TCDD 投与によって用量反応的に子宮内膜症が発症	[26]
Rier	1995	TCDD 投与によるサイトカイン異常と子宮内膜症の関連性	[27]
Johnson	1997	ダイオキシン類による子宮内膜症組織の発育	[28]
Cummings	1999	TCDD の子宮内曝露による仔の子宮内膜症発症	[29]
作用機構			
Kuchenfoff	1999	子宮内膜組織における AhR の発現	[30]
Igarashi	1999	子宮内膜組織におけるダイオキシン関連遺伝子の発現	[31]
Bulun	2000	子宮内膜症組織におけるダイオキシン関連遺伝子の発現	[32]
Bofinger	2001	TCDD 曝露による子宮内膜での CYP1A1, 1B1遺伝子の発現	[33]
Ohtake	2003	ダイオキシン類の作用機構を分子レベルで解明	[34]

2. ダイオキシン類と子宮内膜症

分子生物学や毒性学,疫学,臨床研究などによって内分泌撹乱物質の人体への影響や作用機序を解明する研究が進んでいる.Rier らの報告以降,内分泌撹乱物質と子宮内膜症の関連性を調査した多くの研究が行われており,特にダイオキシン類について多くの報告がされている.

それらを大別すると,ダイオキシン類の汚染状況と子宮内膜症の発生率について調査したもの,ダイオキシン類によって実験的に子宮内膜症の発生・増悪を確認するもの,ダイオキシン類の作用機構に関連する遺伝子の発現をみるものなどに分類することができる(Table 3).

Rier らはアカゲザルに4年間2, 3, 7, 8-tetrachlorodibenzo-p-dioxin(2,3,7,8-TCDD)を投与し,投与終了後10年して腹腔内の子宮内膜症の発症を検討した.その結果2,3,7,8-TCDD 投与群での子宮内膜症発症率は対照群と比較して高く,しかも投与群に進行例が多かった (Table 4).

また Mayani らは子宮内膜症患者の2,3,7,8-TCDD 血中濃度を測定し,症例群では対照群と比べその血中濃度が高値であったことを報告している.

人体におけるダイオキシン類汚染状況と子宮内膜症との関係を調査した研究では,イタリアのセベソにおけるダイオキシン類汚染地区の研究があり,この研究では血中2,3,7,8-TCDD 濃度と子宮内膜症発症の関連性を検討している.血中2,3,7,8-TCDD 濃度が20 ppt 以下の低曝露群と比較して,100 ppt を超える高曝露群で子宮内膜症の発症率が2.1倍と増加傾向を示したが,統計学的な有意差は認められなかった.

3. 現在の研究

子宮内膜症の発生には家族性があり,疾患感受性(いわゆる個人差)や環境要因との関連性を解明するため遺伝子多型の解析が行われている.遺伝子多型のうち,1つの塩基が他の塩基と置き換わっているものは1塩基多型(Single Nucleotide Polymorphism, SNP)と呼ばれ,約1000塩基に1つの頻度で存在している.SNP は薬物代謝酵素やレセプターをコードしている遺伝子内にも存在しており,その発現量や活性に影響を及ぼすことがある.

子宮内膜症はエストロゲン依存性疾患であり,エストロゲン代謝における薬物代謝酵素

Table 4. 2,3,7,8-TCDD 投与による子宮内膜症の発症率・重症化

2,3,7,8-TCDD 投与量 (ppt)	子宮内膜症 なし	I	II	III	IV	発生率 (%)	P 値
0(対照群)	4	2	0	0	0	33	
5	2	2	0	2	1	71	0.034
25	1	0	1	1	4	86	0.005

進行期は r-AFS (revised American Fertility Society) 分類

やレセプターなどで子宮内膜症の発症と遺伝子多型との関連性をみる研究が行われている(Fig. 3).

これまでにCYP1A1, CYP19, グルタチオンS-トランスフェラーゼ(GSTM), N-アセチルトランスフェラーゼ(NAT), エストロゲンレセプターαの遺伝子多型と子宮内膜症発症リスクとの関連性が報告されている [35]. CYP1A1はエストロゲンの中で最も活性の強い17β-Estradiol(E2)の代謝に関与する酵素であり, 多環芳香族炭化水素類によって誘導されることから内分泌撹乱物質のバイオマーカーとしての意義をもつと考えられる. また多くの化学物質は第1相薬物代謝酵素(CYP1A1など)により活性化され, 第2相薬物代謝酵素(GST, NATなど)によって抱合・解毒される. CYP1A1やGST, NATのバリアントタイプではワイルドタイプと比べて酵素活性が低下していることが報告されており, 子宮内膜症を発症するリスクが高くなっている可能性がある.

CYP19はアンドロゲンをエストロゲンに変換するアロマターゼであり, エストロゲン合成の律速段階となる重要な酵素である. これまでにCYP19のバリアントタイプで子宮内膜症発症のリスクが高くなるという報告がある. これはバリアントタイプでアロマターゼ活性が上昇している可能性を示唆する.

ダイオキシン類以外の多くの内分泌撹乱物質は, 直接エストロゲンレセプターと結合してその作用をもたらす. エストロゲンレセプターには2つのサブタイプ, エストロゲンレセプターαとエストロゲンレセプターβが存在することが報告されている. エストロゲンレセプターαのイントロンには制限酵素Pvu Ⅱで認識される遺伝子多型が知られており, この遺伝子多型はエストロゲンレセプターの発現やエストロゲンとの結合に何らかの影響を及ぼしている可能性が考えられる.

それぞれの遺伝子多型における分子生物学的な機能の変化はほとんど解明されておらず, 子宮内膜症の発症に遺伝子多型がどれだけ関与しているのか, 未だ一致した見解は得られていない.

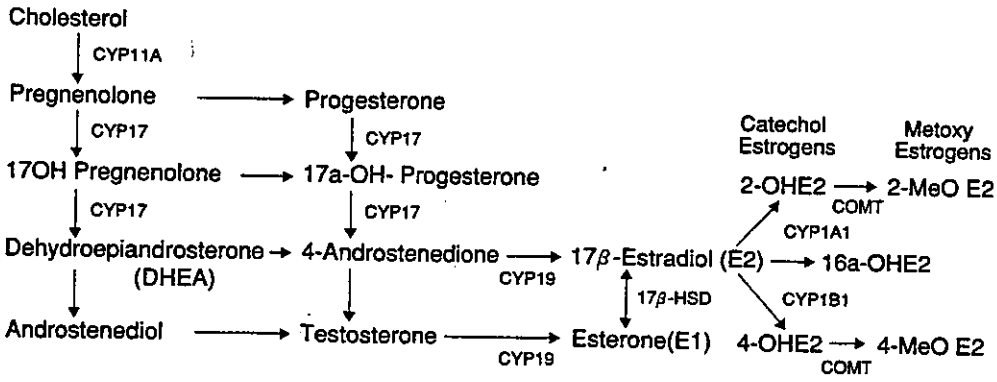

Fig. 3. エストロゲンの代謝機構.