each plate, transferred into a Brucella broth liquid culture medium containing 10% fetal calf serum, and cultured for 24 h with agitation under the same conditions as described above. Some of the bacterial suspension was stored at −80 °C in 0.01 M PBS containing 20% glycerol. DNA from each *H. pylori* isolate was extracted from the pellet of the bacterial suspension using the protease/phenol–chloroform method, suspended in a TE buffer (10 mM Tris–HCl, pH 8.0 and 1 mM EDTA), and stored at 4 °C until amplification was performed.

2.4. Real-time PCR

All PCRs were performed by real-time methods using the "ABI PRISM 7700 Sequence Detection System" (Applied Biosystems). PCR was performed in a 50-μl reaction mixture containing 25 μl of "TaqMan Universal PCR Master Mix" (Applied Biosystems), 200–800 nM of each primer, 400–600 nM probe, 5 μl of each DNA sample or an approximately 10-fold serial diluted standard *H. pylori* DNA (0.1–10^6 fg), and double distilled H_2O in 0.2 ml MicroAmp optical tubes (Applied Biosystems). Thermal cycling conditions comprised an initial denaturation step at 95 °C for 10 min and 50 cycles at 95 °C for 15 s and 60 °C for 1 min. Several PCR products were also examined by 3% agarose gel electrophoresis. The gels were stained with ethidium bromide.

In addition, a few PCR products were then purified with Centricon-100 Concentrator columns (Amicon, Beverly, MA, USA). DNA direct sequencing was performed using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI PRISM 3100-*Avant* Genetic Analyzer (Applied Biosystems) according to the manufactures' recommendations. Nucleotide sequences were aligned and analyzed by GENETYX-Mac software (version 11.2.3, Software Development, Tokyo, Japan).

3. Results

3.1. Verification of the system

We could detect the *H. pylori* 16S rRNA gene, Western and East Asian-*cagA* genes from the DNA of gastric biopsies. Fig. 2 shows results of two typical Western-*cagA* samples (Thailand-080, 104) and two typical East Asian-*cagA* samples (Thailand-100, 116). In 16S rRNA, all *H. pylori*-positive samples were successfully amplified, but then, typical Western or East Asian-*cagA* samples were only successfully amplified by each specific PCR set (Fig. 2, upper panels). We could also show these results by agarose gel electrophoresis for the PCR products (Fig. 2, lower panels), but the sensitivity of the real-time PCR system used was higher than that using agarose gel electrophoresis.

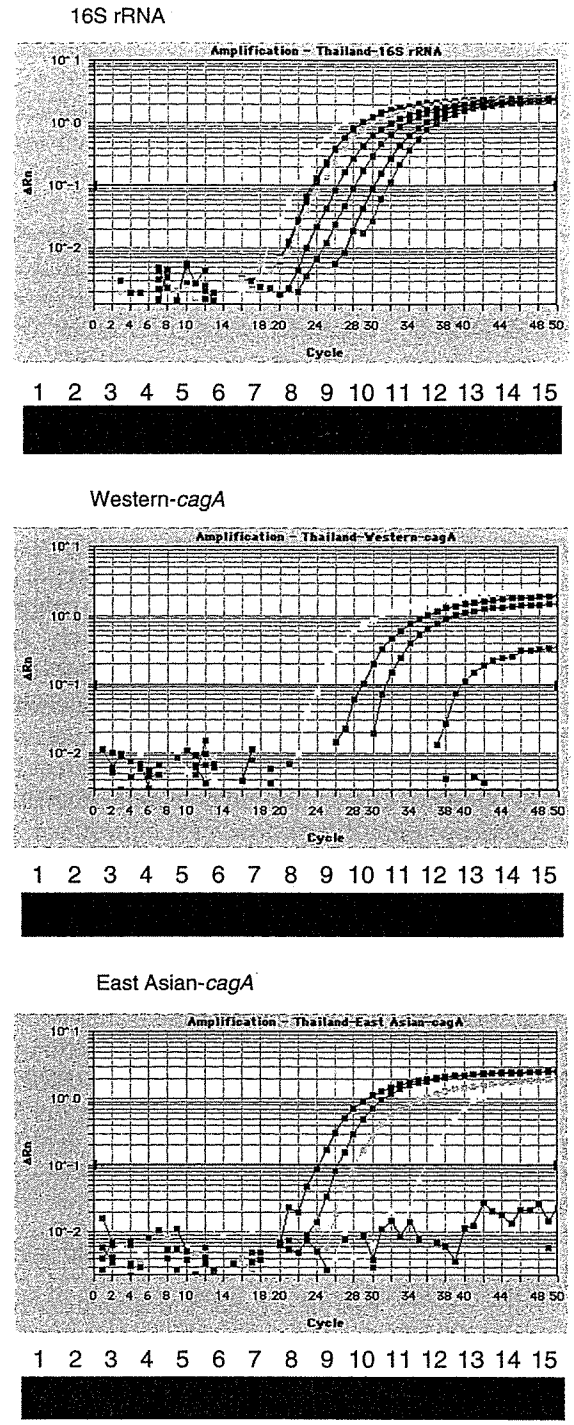

Fig. 2. Representative 16S rRNA, Western-*cagA*, and East Asian-*cagA* amplification plots and agarose gel electrophoresis. Thailand-080 (red, lane 8) and 104 (pink, lane 9) were typical Western-*cagA* samples. Thailand-100 (green, lane 10) and 116 (blue, lane 11) were typical East Asian-*cagA* samples. Then, Thailand-019 (dark green, lane 12) and 112 (yellow, lane 13) were considered to be multiple infections samples, detected by both Western and East Asian type PCR sets. Thailand-070 (light blue, lane 14) and 075 (dark blue, lane 15) were considered to be *cagA*-negative or AB-type CagA samples, detected by neither Western nor East Asian type PCR sets. Lanes 1–7, standard dilution series (10^6–1 fg).

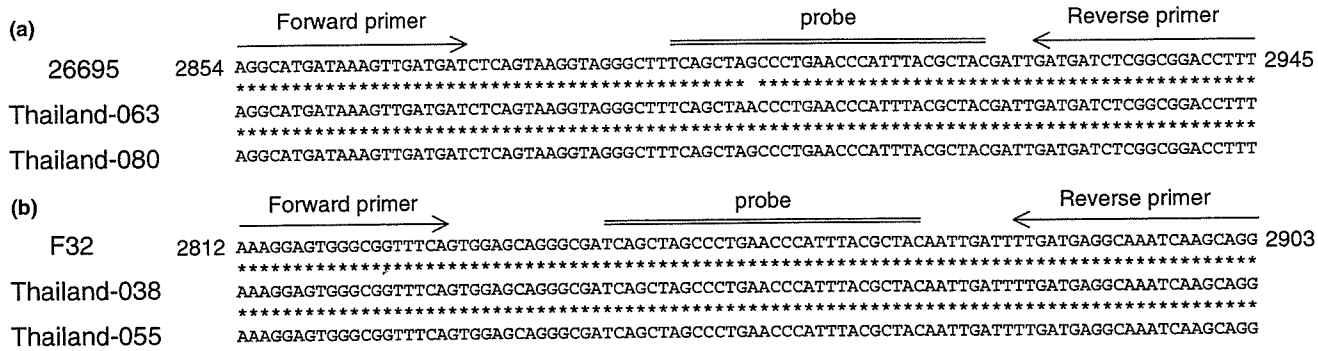

Fig. 3. Alignment of the nucleotide sequence in the 3′ region of the *cagA* gene. (a) Western-*cagA* sequence among strains 26695, Thailand-063, and Thailand-080. Each 92-bp sequence presented here starts at position 2824 of strain 26695 *cagA* open reading frame (GenBank accession number: AE000569). (b) East Asian-*cagA* sequence among strains F32, Thailand-038, and Thailand-055. Each 92-bp sequence presented here starts at position 2812 of strain F32 *cagA* open reading frame (GenBank accession number: AF202972).

Furthermore, we checked nucleotide sequences of the Western and East Asian-*cagA* PCR products (Fig. 3). In the East Asian-*cagA* sequence among strains F32, Thailand-038, and 055, each 92-bp sequence was completely identified. In the Western-*cagA* sequence among strains 26695, Thailand-063, and 080, the 92-bp sequences were highly homologous, except for only a 1-bp replacement from G to A in Thailand-063.

3.2. Distribution of CagA diversity in Thai patients

All *H. pylori*-positive patients examined were *H. pylori*-positive, since *H. pylori* 16S rRNA was detected (Table 2), and all four *H. pylori*-negative patients were negative. Therefore, comparing with histology the sensitivity and specificity of this real-time PCR system were 100%. Concerning CagA diversity, 53.7% (22/41) were East Asian CagA-positive, and 26.8% (11/41) were Western CagA-positive. Three cases (7.3%) (Thailand-019, 053, 112) were not classed as both types because they were detected by both PCR sets, whereas either East Asian or Western CagA had higher intensity. These cases were considered to be multiple infections (Fig. 2). Five cases (Thailand-070, 075, 102, 122, 125) (12.2%) had undetectable CagA in this study (Fig. 2). *H. pylori* 16S rRNA was detected in these cases, so they were *cagA*-negative or AB-type CagA, which did not contain either the ESS or WSS sequence (Table 2).

4. Discussion

In East Asia, the prevalence of gastric cancer is much higher than in Western countries. It is known that there are significant geographic differences among *H. pylori* [12,25], and large sequence differences distinguish the *cagA* gene fragments from East Asian and Western strains [26,27]. We previously reported that the grades of inflammation, activity of gastritis, and atrophy are significantly higher in patients with gastritis infected by the East Asian CagA-positive *H. pylori* strains than in patients with gastritis infected by the *cagA*-negative or Western CagA-positive strains [33]. Furthermore, it was demonstrated by use of in vitro transfection experiments using a human gastric cancer cell line (AGS), that the East Asian-specific sequence of CagA protein confers stronger SHP-2 binding and transforming activities than does the Western-specific sequence [27], meaning that the potential of East Asian CagA to disturb host cell functions as a virulence factor may be higher than that of Western CagA. Based on these data, it is thought that endemic circulation of *H. pylori* organisms with more virulent CagA proteins may affect the prevalence of gastric cancer in East Asian countries, and that the East Asian CagA-positive strains may be more virulent than the Western CagA-positive strains. Thus, it may be important to distinguish between East Asian and Western type CagA to expedite clinical procedures in areas where two types of *H. pylori* are mixed-present. Therefore, in the present study, we developed a highly sensitive real-time PCR system to identify *H. pylori* and the *cagA* gene type in DNA samples from gastric biopsies.

A real-time PCR method based on TaqMan fluorescence methodology requires fewer steps after PCR than conventional PCR methods, simplifying the procedures. Using these primers sets, it was also possible to show these results by agarose gel electrophoresis for the PCR products, but the sensitivity of our real-time PCR system may be higher than using agarose gel electrophoresis. We were also able to detect the *H. pylori* 16S rRNA and Western or East Asian-*cagA* gene from several DNA samples of paraffin-blocked tissues, gastric juices, and feces (data not shown). Although these samples contain almost all host DNA, there are very small concentrations of *H. pylori* DNA if these are present. Since extracted DNA from host samples is required in this system, we can examine the *cagA*-typing if there are no facilities for *H. pylori* culturing. Furthermore, it is known that host immunogenic factors, such as polymorphisms in

Table 2
Discrimination between Western and East Asian-*cagA* in gastric biopsies from Thai patients

Sample	Age	Sex	Diagnosis	16s rRNA	*cagA*		Decision HP(+)
					Western	East Asian	
006	77	M	CG	+++	–	+++	East Asian
011	38	M	CG	+++	–	+	East Asian
013	45	F	CG	+++	–	++	East Asian
019	28	F	CG	+++	++	+++	East Asian/Western
026	44	F	CG	+++	–	+++	East Asian
029	57	M	CG	+++	–	+++	East Asian
033	25	M	CG	+	–	+	East Asian
034	78	M	CG	+	–	++	East Asian
037	27	F	CG	+++	+++	–	Western
038	63	M	GU	+++	–	+++	East Asian
040	41	M	CG	++	–	++	East Asian
045	80	M	GU	+++	–	+++	East Asian
051	58	M	GU	+++	–	+++	East Asian
053	65	M	GU	+++	+++	+	Western/East Asian
055	45	F	GCA	+++	–	+++	East Asian
057	45	M	GCA	+++	–	+++	East Asian
059	70	M	GCA	+++	+++	–	Western
061	55	M	GCA	+++	–	++	East Asian
063	40	M	GCA	+++	+++	–	Western
067	60	M	GCA	++	–	+	East Asian
068	78	F	GCA	+++	–	+	East Asian
070	66	M	GCA	+++	–	–	*cagA*(–)/AB type
075	70	M	CG	+++	–	–	*cagA*(–)/AB type
076	50	F	CG	+++	++	–	Western
078	37	M	CG	++	–	+	East Asian
080	44	F	CG	+++	+++	–	Western
082	64	F	CG	+	++	–	Western
084	29	M	CG	+++	–	+	East Asian
088	73	F	CG	+++	+++	–	Western
092	59	M	CG	+++	–	++	East Asian
094	41	M	CG	+++	+++	–	Western
096	33	F	CG	+++	+	–	Western
098	43	F	CG	+++	++	–	Western
100	63	F	CG	+++	–	+++	East Asian
102	22	M	CG	+	–	–	*cagA*(–)/AB type
104	34	M	CG	+++	+++	–	Western
112	59	M	DU	+++	+++	++	Western/East Asian
116	35	F	DU	+++	–	+++	East Asian
120	76	M	DU	+++	–	+++	East Asian
122	32	M	DU	+	–	–	*cagA*(–)/AB type
125	61	M	DU	++	–	–	*cagA*(–)/AB type

CG, chronic gastritis; GU, gastric ulcer; GCA, gastric cancer; DU, duodenal ulcer.
+++ ≧ 100 fg; 100 fg > ++ ≧ 10 fg; 10 fg > + ≧ 1 fg; –<1 fg.

the interleukin-1 gene cluster and HLA genes have been proposed to modulate the risk of gastric cancer [34–36]; we may also be able to investigate host immunogenic factors at once since we used almost all host DNA samples in this system.

In the present study, we examined the distribution of CagA diversity in gastric biopsies from Thai patients. The prevalence of East Asian CagA and Western CagA was 53.7% and 26.8%, respectively, which was quite different from our previous data from East Asia. We previously reported that over 90% of samples isolated from Fukui, Japan (100%, 65/65) and Hangzhou, China (94.4%, 17/18) had East Asian type CagA [33,37]. We confirmed these previous data using a real-time PCR method in this study. The CagA types by the conventional PCR methods were consistent with those by the real-time PCR method (data not shown). In contrast, there were mixed-present *H. pylori* that had the East Asian or Western type CagA in Thailand. Three cases (7.3%) could not be classed as both types because these were detected by both PCR sets, whereas either East Asian or Western CagA had a higher intensity. These cases were considered to have multiple infections. Five cases (12.2%) had undetectable CagA in this study. *H. pylori* 16S rRNA was detected in these cases, and they were *cagA*-negative or AB-type CagA, which did not contain either the ESS or WSS sequence. Ultimately, in this case, we could distinguish *cagA*-typing com-

pletely at about 80% using this system. The prevalence of East Asian CagA-positive *H. pylori* infection varied according to clinical outcome (54.2% in chronic gastritis, 62.5% in gastric cancer, 100% in gastric ulcer, and 60.0% in duodenal ulcer patients), although the differences were not significant.

The present study suggests that real-time PCR can provide a highly sensitive assessment of CagA type of *H. pylori* in gastric biopsies, and it may be a useful and simple new diagnostic tool. We plan to investigate the geographical and clinical significance of the diversity of CagA using this system in a large number of cases in a further study.

Acknowledgement

This work was supported by a grant-in-aid for Scientific Research (B) and (C) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

[1] Parsonnet, J., Friedman, G.P., Vandersteen, D.P., Chang, Y., Vogelman, J.H., Orentriech, N. and Sibley, R.K. (1991) *Helicobacter pylori* infection and the risk of gastric carcinoma. N. Engl. J. Med. 325, 1127–1131.

[2] The Eurogast Study Group. (1993) An association between *Helicobacter pylori* infection and gastric cancer. Lancet 341, 1359–1362.

[3] Uemura, N., Okamoto, S., Yamamoto, S., Matsumura, N., Yamaguchi, S., Yamakido, M., Taniyama, K., Sasaki, N. and Schlemper, R.J. (2001) *Helicobacter pylori* infection and the development of gastric cancer. N. Engl. J. Med. 345, 784–789.

[4] International Agency for Research on Cancer Working Group. (1994) Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the evaluation of carcinogenic risks to humans. IARC Monogr. Eval. Carcinog. Risks Hum. 61, 1–124.

[5] Blaser, M.J., Perez-Prez, G.I., Kleanthous, H., Cover, T.L., Peek, R.M., Chyou, P.H., Stemmermann, G.N. and Nomura, A. (1995) Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res. 55, 2111–2115.

[6] Kuipers, E.J., Perez-Perez, G.I., Meuwissen, S.G. and Blaser, M.J. (1995) *Helicobacter pylori* and atrophic gastritis: importance of the *cagA* status. J. Natl. Cancer Inst. 87, 1777–1780.

[7] Peek, R.M., Moss, S.F., Tham, K.T., Perez-Perez, G.I., Wang, S., Miller, G.G., Atherton, J.C., Holt, P.R. and Blaser, M.J. (1997) *Helicobacter pylori* cagA+ strains and dissociation of gastric epithelial cell proliferation from apoptosis. J. Natl. Cancer Inst. 89, 863–868.

[8] Rokkas, T., Ladas, S., Liatsos, C., Petridou, E., Papatheodorou, G., Theocharis, S., Karameris, A. and Raptis, S. (1999) Relationship of *Helicobacter pylori* CagA status to gastric cell proliferation and apoptosis. Digest. Dis. Sci. 44, 487–493.

[9] Huang, J.Q., Zheng, G.F., Sumanac, K., Irvine, E.J. and Hunt, R.H. (2003) Meta-analysis of relationship between *cagA* seropositivity and gastric cancer. Gastroenterology 125, 1636–1644.

[10] Censini, S., Lange, C., Xiang, Z., Crabtree, J.E., Ghiara, P., Borodovsky, M., Rappuori, R. and Covacci, A. (1996) cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA 93, 14648–14653.

[11] Akopyants, N.S., Clifton, S.W., Kersulyte, D., Crabtree, J.E., Youree, B.E., Reece, C.A., Bukanov, N.O., Drazek, E.S., Roe, B.A. and Berg, D.E. (1998) Analyses of the cag pathogenicity island of *Helicobacter pylori*. Mol. Microbiol. 28, 37–53.

[12] Covacci, A., Telford, J.L., DelGiudice, G., Parsonnet, J. and Rappuori, R. (1999) *Helicobacter pylori* virulence and genetic geography. Science 284, 1328–1333.

[13] Segal, E.D., Cha, J., Lo, J., Falkow, S. and Tompkins, L.S. (1999) Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA 96, 14559–14564.

[14] Asahi, M., Azuma, T., Ito, S., Ito, Y., Suto, H., Nagai, Y., Tsubokawa, M., Tohyama, Y., Maeda, S., Omata, M., Suzuki, T. and Sasakawa, C. (2000) *Helicobacter pylori* CagA protein can be tyrosine phosphorylated in gastric epithelial cells. J. Exp. Med. 191, 593–602.

[15] Backert, S., Ziska, E., Brinkmann, V., Zimny-Arndt, U., Fauconnier, A., Jungblut, P.R., Naumann, M. and Meyer, T.F. (2000) Translocation of the *Helicobacter pylori* CagA protein in gastric epithelial cells by a type IV secretion apparatus. Cell. Microbiol. 2, 155–164.

[16] Odenbreit, S., Puls, J., Sedlmaier, B., Gerland, E., Fischer, W. and Haas, R. (2000) Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science 287, 1497–1500.

[17] Stein, M., Rappuoli, R. and Covacci, A. (2000) Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after *cag*-driven host cell translocation. Proc. Natl. Acad. Sci. USA 97, 1263–1268.

[18] Backert, S., Moese, S., Selbach, M., Brinkmann, V. and Meyer, T.F. (2001) Phosphorylation of tyrosine 972 of the *Helicobacter pylori* CagA protein is essential for induction of a scattering phenotype in gastric epithelial cells. Mol. Microbiol. 42, 631–644.

[19] Stein, M., Bagnoli, F., Halenback, R., Rappuoli, R., Fantl, W.J. and Covacci, A. (2002) c-Src/Lyn kinases active *Helicobacter pylori* CagA through tyrosine phosphorylation of the EPIYA motifs. Mol. Microbiol. 43, 971–980.

[20] Higashi, H., Tsutsumi, R., Muto, S., Sugiyama, T., Azuma, T., Asaka, M. and Hatakeyama, M. (2002) SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 295, 683–686.

[21] Feng, G., Hui, C. and Pawson, T. (1993) SH-2 containing phosphotyrosine phosphatase as a target of protein-tyrosine kinases. Science 259, 1607–1611.

[22] Freeman, R., Plutzky, J. and Neel, B.G. (1992) Identification of a human src homology 2-containing protein-tyrosine-phophatase: a putative homolog of *Drosophila* corkscrew. Proc. Natl. Acad. Sci. USA 89, 11239–11243.

[23] Yu, D., Qu, C., Henegariu, O., Lu, X. and Feng, G. (1998) Protein-tyrosine phosphatase Shp-2 regulates cell spreading, migration, and focal adhesion. J. Biol. Chem. 273, 21125–21131.

[24] Yamazaki, S., Yamakawa, A., Ito, Y., Ohtani, M., Higashi, H., Hatakeyama, M. and Azuma, T. (2003) The CagA protein of *Helicobacter pylori* is translocated into epithelial cells and binds to SHP-2 in human gastric mucosa. J. Infect. Dis. 187, 334–337.

[25] Falush, D., Wirth, T., Linz, B., Pritchard, J.K., Stephens, M., Kidd, M., Blaser, M.J., Graham, D.Y., Vacher, S., Perez-Perez, G.I., Yamaoka, Y., Megraud, F., Otto, K., Reichard, U., Katzowitsch, E., Wang, X., Achtman, M. and Suerbaum, S. (2003) Traces of human migrations in *Helicobacter pylori* populations. Science 299, 1582–1585.

[26] Achtman, M., Azuma, T., Berg, D.E., Ito, Y., Morelli, G., Pan, Z., Suerbaum, S., Thomson, S.A., van der Endo, A. and van Doorn, L.J. (1999) Recombination and clonal groupings within

Helicobacter pylori from different geographical regions. Mol. Microbiol. 32, 459–470.

[27] Higashi, H., Tsutsumi, R., Fujita, A., Yamazaki, S., Asaka, M., Azuma, T. and Hatakeyama, M. (2002) Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc. Natl. Acad. Sci. USA 99, 14428–14433.

[28] Covacci, A., Censini, S., Bugnoli, M., Petracca, R., Burroni, D., Macchia, G., Massone, A., Papini, E., Xiang, Z., Figura, N. and Rappuoli, R. (1993) Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. USA 90, 5791–5795.

[29] Yamaoka, Y., Kodama, T., Kashima, K., Graham, D.Y. and Sepulvena, A.R. (1998) Variants of the 3′ region of the *cagA* gene in *Helicobacter pylori* isolates from patients with different *H. pylori*-associated diseases. J. Clin. Microbiol. 36, 2258–2263.

[30] Yamaoka, Y., El-Zimaity, H.M., Gutierrez, O., Figura, N., Kim, J.G., Kodama, T., Kashima, K., Graham, D.Y. and Kim, J.K. (1999) Relationship between the *cagA* 3′ repeat region of *Helicobacter pylori*, gastric histology, and susceptibility to low pH. Gastroenterology 117, 342–349.

[31] Yamaoka, Y., Osato, M.S., Sepulveda, A.R., Gutierrez, O., Figura, N., Kim, J.G., Kodama, T., Kashima, K. and Graham, D.Y. (2000) Molecular epidemiology of *Helicobacter pylori*: separation of *H. pylori* from East Asian and non-Asian countries. Epidemiol. Infect. 124, 91–96.

[32] Matsuhisa, T., Matsukura, N. and Yamada, N. (2004) Topography of chronic active gastritis in *Helicobacter pylori*-positive Asian populations: age-, gender-, and endoscopic diagnosis-matched study. J. Gastroenterol. 39, 324–328.

[33] Azuma, T., Yamazaki, S., Yamakawa, A., Ohtani, M., Muramatsu, A., Suto, H., Ito, Y., Dojo, M., Yamazaki, Y., Kuriyama, M., Keida, Y., Higashi, H. and Hatakeyama, M. (2004) Association between diversity in the Src homology 2 domain-containing tyrosine phosphatase binding site of *Helicobacter pylori* CagA protein and gastric atropy and cancer. J. Infect. Dis. 189, 820–827.

[34] Azuma, T., Ito, S., Sato, F., Yamazaki, Y., Miyaji, H., Ito, Y., Suto, H., Kuriyama, M., Kato, T. and Kohli, Y. (1998) The role of the HLA-DQA1 gene in resistance to atrophic gastritis and gastric adenocarcinoma induced by *Helicobacter pylori* infection. Cancer 82, 1013–1018.

[35] Ohtani, M., Azuma, T., Yamazaki, S., Yamakawa, A., Ito, Y., Muramatsu, A., Dojo, M., Yamazaki, Y. and Kuriyama, M. (2003) Association of the HLA-DRB1 gene locus with gastric adenocarcinoma in Japan. Digest. Liver Dis. 35, 468–472.

[36] El-Omar, E.M., Carrington, M., Chow, W.H., McColl, K.E., Bream, J.H., Young, H.A., Herrera, J., Lissowska, J., Yuan, C.C., Rothman, N., Lanyon, G., Martin, M., Fraumeni, J.F. and Rabkin, C.S. (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404, 398–402.

[37] Zhou, W., Yamazaki, S., Yamakawa, A., Ohtani, M., Ito, Y., Keida, Y., Higashi, H., Hatakeyama, M., Si, J. and Azuma, T. (2004) The diversity of *vacA* and *cagA* genes of *Helicobacter pylori* in East Asia. FEMS Immunol. Med. Microbiol. 40, 81–87.

NUTRITION AND CANCER, 51(2), 170–177

Increased Risk of Colorectal Cancer Due to Interactions Between Meat Consumption and the *CD36* Gene *A52C* Polymorphism Among Japanese

Kiyonori Kuriki, Nobuyuki Hamajima, Hitoshi Chiba, Yukihide Kanemitsu, Takashi Hirai, Tomoyuki Kato, Toshiko Saito, Keitaro Matsuo, Koichi Koike, Shinkan Tokudome, and Kazuo Tajima

Abstract: *A previous study showed expression of CD36, recently reported to play important roles in metabolism of oxidized low-density lipoprotein and long-chain fatty acids and to be positively correlated with colon cancer prognosis. To examine relationships between colorectal cancer and the* CD36 *gene* A52C *polymorphism according to meat consumption as a surrogate for saturated fatty acid intake, we conducted the present hospital-based, case-control study of 128 cases and 238 non-cancer controls. Consumption of meat and vegetables/fruit was divided into three (low, moderate, and high) and two (low and high) groups, respectively. Regarding the risk of colorectal cancer on cross-classifying subjects for the* CD36 *genotype and meat consumption, the odds ratio (OR) for the* C/C *genotype with moderate meat consumption relative to the* A/A *genotype with low meat consumption was 8.30 (95% confidence interval, CI = 2.15–32.00). None of individuals with the* C/C *genotype was in the high meat consumption group. In the low vegetables/fruit consumption group, the OR for the* C/C *genotype relative to the* A/A *genotype was 3.03 (95% CI = 1.12–7.90). Our findings suggest that interactions between moderate-high meat consumption and the* CD36 *gene* A52C *polymorphism may increase the risk of colorectal cancer.*

Introduction

High consumption of red meat has been reported as a probable risk factor for colorectal cancer, with diets high in total fat, animal fat/saturated fatty acid (SFA), and processed meat possibly increasing the likelihood of cancer development (1,2). On the other hand, high consumption of vegetables is suggested as a convincing protective factor for colorectal cancer. A large-scale cohort study in Japan demonstrated a markedly increased risk of colon cancer for men consuming meat daily but green or yellow vegetables rarely, relative to individuals eating both foods daily (3). Doll and Peto and Kye et al. have advocated that the majority of colorectal cancer in industrial countries may be preventable through modification of dietary habits (4,5).

Data from the National Nutritional Survey during the last 50 yr illustrated that Japanese had a 20-fold increase in intake of milk, followed by meat (9-fold), eggs (7-fold), animal fat and vegetable oil (6-fold), and fruit (3-fold), whereas the intakes of rice and potatoes gradually decreased (6,7). The intakes of fish, beans, green or yellow vegetables, and other vegetables remained relatively constant. Dietary energy and fat intake derived from meat were 1.3 and 2.2 times higher than those of sea foods, respectively. Both dietary energy and fat intake derived from pork, chicken, and processed meats as reference to beef (100%) were 150–160%, 70–80%, and 60%, in that order. The westernization of dietary habits has been considered one of the most important risk factors for increasing incidence and mortality rates of colorectal cancer among Japanese (8–12). Cancer registry information shows that the incidence rate of colon cancer among Japanese immigrants in the United States has rapidly increased and is the same or rather higher than that among native Caucasians (13).

Meat and meat products are suggested to be major contributors of animal fat/SFA in Japan as well as in the United States and European countries (14,15). For the risk of colorectal cancer, however, there have been few reports of meat consumption focusing on interactions with gene polymorphisms related to

K. Kuriki, T. Saito, K. Matsuo, and K. Tajima are affiliated with the Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464–8681, Japan. N. Hamajima is affiliated with the Department of Preventive Medicine/Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya 466–8550, Japan. H. Chiba is affiliated with the Department of Laboratory Medicine, Hokkaido University School of Medicine, Sapporo 060–8648, Japan. Y. Kanemitsu, Takashi Hirai, and T. Kato are affiliated with the Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Chikusa-ku, Nagoya 464–8681, Japan. K. Koike is affiliated with the Division of Clinical Laboratories, Aichi Cancer Center Hospital, Chikusa-ku, Nagoya 464–8681, Japan. S. Tokudome is affiliated with the Department of Health Promotion and Preventive Medicine, Nagoya City University Graduate School of Medical Sciences, Mizuho-cho, Mizuho-ku, Nagoya 467–8601, Japan.

fat metabolism. CD36 has been demonstrated to play important roles not only in monocyte adhesion to thrombospondins or collagen type I and IV but also in metabolism of dietary fat and serum lipids as a fatty acid translocase (FAT) and as a scavenger receptor of oxidized low-density lipoprotein (LDL) (16–18). A previous study showed that the expression of CD36 regulated stromal antiangiogenetic activity of thrombospondin 1 and was correlated with colon cancer prognosis and the decreased stromal vascularization (19). The frequency of CD36 deficiency is higher among Japanese than among American-European people (3–11% and 0.3%, respectively) (18,20). The regular translation initiation codon and another ATG codon are located at exon 3 position +290 and at exon 1 position +62 within its promoter region, respectively, and such upstream codons have been reported to be present in many protooncogenes (21). Recently, the *CD36* gene *A52C* polymorphism has been detected, featuring *A* to *C* substitution at exon 1 position +52 within its promoter region, which is predicted to modulate CD36 expression and the abundance of transcription-binding sites (21,22).

CD36 has not hitherto been linked to carcinogenesis according to intake of meat/SFA. Regarding the increment of colorectal cancer along with the westernization of dietary habits among Japanese, our hypothesis is that the *CD36* gene *A52C* polymorphism might have a positive association with colorectal cancer according to tumor promotion by intake of meat/SFA. To explore possible gene–environmental interactions between meat consumption and the *CD36* gene *A52C* polymorphism for the risk of colorectal cancer, we therefore conducted the present hospital-based case-control study.

Subjects and Methods

Study Subjects

This case-control study was conducted as part of a series in a major project on genetic polymorphisms and cancer risk in Aichi Cancer Center Research Institute (23). We recruited the subjects from patients aged 40–79 yr who visited the outpatient services at Aichi Cancer Center Hospital between March 1999 and July 2000 (24). The cases comprised incident (within 1 yr before the study entry) and prevalent cases that had been histopathologically diagnosed as having colon or rectal cancer at the Department of Gastroenterological Surgery. Cases who were under 40 yr at the time of cancer diagnosis and had hereditary ailments such as familial adenomatous polyposis were excluded because those cases were more likely to be affected by host-related than environmental factors. The rate of incident patients was 49.6%, and all prevalent cases were diagnosed within 5 yr, except for one patient. Controls were first- and re-visit outpatients who visited the Department of Gastroenterology during the same term and were confirmed to be cancer-free; these non-cancer outpatients had gastric/duodenal ulcers (9.5%), gastritis (9.2%), hypertension (6.3%), pain including arthritis and lumbago (3.3%), diabetes mellitus (2.9%), hyperlipidemia (2.9%), and miscellaneous other diseases (9.5%) (not confirmed in their medical records). Our previous study demonstrated that it is feasible to use general lifestyle of non-cancer outpatients at Aichi Cancer Center Hospital as controls in epidemiological studies (25).

All subjects were Japanese and lived in the Tokai area, including Aichi Prefecture, central Japan. They were provided with an explanatory document and gave their written informed consent for participation in this study. One trained interviewer collected and checked information on food consumption, lifestyle (including habitual exercise, drinking, and smoking habits), and medical histories from self-administrated questionnaires, and we performed 7-ml blood sampling from a peripheral vein. We excluded two cases with missing information from the questionnaires. Eligible subjects were 128 colon and rectal cancer cases and 238 controls.

This study was approved by the Ethics Committee of the Aichi Cancer Center.

Lifestyle Assessment

Regarding dietary habits, the frequency of intake for whole meat (including beef, pork, and chicken), fish, raw vegetables (over one small bowl), fruit and tofu, and three beverages (Japanese tea, coffee, and milk) and preference for salt were assessed. Level of consumption was divided into three groups, that is, less than once a week ("low"), one to three times per week ("moderate"), and four or more times per week ("high"). "Vegetables/fruit" was defined as a variable that included raw vegetables and fruit and divided into two groups, that is, "high" for four and more times per week of both food items and "low" for other category combinations of these. The consumption of the three beverages was categorized as rarely, sometimes, or daily.

Lifestyle factors were also classified into three groups: 1) habitual exercise, which was other than work for more than 15 min, less than once a week, one to two times per week, and three or more times per week; 2) drinking habit, less than once a week, one to four times per week, and five or more times per week; and 3) smoking status, current, former, and never-smokers. We defined former smokers as those who quit smoking more than 2 yr before the questionnaire study. We asked the cases to provide information about their lifestyle before the onset of disease and the controls at the study enrollment.

Laboratory Methods

DNA was extracted from buffy coat fractions with a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). Genotyping of *A* to *C* the substitution at position +52 (*A52C*) from the first nucleotide on the 5′-proximal promoter region of the *CD36* gene exon 1 (accession no. L06849, *A1160C*) was conducted by the polymerase chain reaction (PCR) with confronting two-pair primers (PCR-CTPP) (26): F1, 5′-AGT CTA TCC AAA GTC GTC AAT-3′ (sense) and R1, 5′-AGA

CAT GAA TTT TAC AGG AAA G-3′ (antisense) for the *C* allele and F2, 5′-TTT CTG TGA CTC ATC AGT TCA-3′ (sense) and R2, 5′-TTA GTT GCA TCC TCA CTA TCT-3′ (antisense) for the *A* allele. Genomic DNA (30–100 ng) was assessed in 25 μl of reaction mixture, with 0.15 mM dTNPs, 25 pmol of each primer, 0.5 units of Takara Taq (Takara Shuzo, Otsu, Japan), 2.5 μl 10 × PCR buffer, including 15 ml $MgCl_2$ (Takara Shuzo), and 1 μl glycerol. Amplification conditions were 5 min of initial denaturation at 94°C followed by 30 cycles of 60 s at 94°C, 60 s at 46°C, and 60 s at 72°C and 5 min final extension at 72°C. PCR products were visualized on a 2% agarose gel with ethidium bromide staining. Genotypes were defined with reference to allele-specific bands of 300 and 212 bp for the *C* and *A* alleles, respectively, as well as the common 470 bp band.

Statistical Analysis

The χ^2 test was performed for nonparametric comparisons of data. Accordance with the Hardy-Weinberg equilibrium, which indicates an absence of discrepancies between genotype and allele frequencies, was also checked for controls with the χ^2 test. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using the unconditional logistic regression model. To control for the effects of potential environmental confounding factors, ORs were calculated after adjustment for age (continuous), sex, habitual exercise, drinking and smoking habits, and others. Furthermore, dietary variables such as meat were entered in the model stated previously when testing for trend. Due to the small number of cases and controls with the *C/C* genotype, no formal statistical test of the gene by environmental interaction was performed. Significance was considered at $P < 0.05$. All statistical analyses were performed with the STATA statistical package (STATA, College Station, TX).

Results

The mean age of colorectal cancer cases in women was older than that of controls (Table 1). Sex ratios between cases and controls did not differ. The rate for ≥3 times per week of habitual exercisers was higher in controls than in cases ($P < 0.05$). In men, the rate for ≥5 times per week of alcohol consumption was higher in cases than in controls ($P < 0.05$), but in women the rate for <1 time per week was over 80% in both groups. Proportions of smokers in men and women did not differ between cases and controls.

Meat consumption increased the risk of colorectal cancer ($P = 0.05$ for trend), and the ORs for moderate and high meat consumption relative to low meat consumption were 1.84 (1.01–3.35) and 1.99 (0.93–4.27), respectively (Table 2). In contrast, the risk of colorectal cancer demonstrated a tendency for decrease with consumption of milk ($P = 0.06$ for trend), but there were no obvious associations with consumption of raw vegetables, fruit, and vegetables/fruit.

Figure 1 shows a representative result of the *CD36* genotyping by the PCR-CTPP method. No differences in genotype and allele frequencies of the *A52C* polymorphism of the *CD36* gene were evident between cases and controls, and the allelic distribution for controls was in the Hardy-Weinberg equilibrium ($P = 0.16$) (Table 3). There were no gender differences in those frequencies, and the allelic distributions for men and women in controls were also in the Hardy-Weinberg equilibrium ($P = 0.32$ for men and $P = 0.31$ for women, respectively). The OR for the *C/C* genotype relative to the *A/A*

Table 1. Background Characteristics of Colorectal Cancer Cases and Controls[a]

	Controls	Cases	*P* Value for χ^2 or *t*-Test
Number of subjects			
Men	116 (48.7)	74 (57.8)	
Women	122 (51.3)	54 (42.2)	NS
Age (yr)[b]			
Men	57.9 ± 8.1	59.6 ± 8.8	NS
Women	56.0 ± 7.4	60.1 ± 9.2	<0.01
Habitual exercise			
<1 time/wk	124 (52.1)	84 (65.6)	
1–2 times/wk	46 (19.3)	19 (14.8)	
≥3 times/wk	68 (28.6)	25 (19.5)	<0.05
Drinking habit			
<1 time/wk	135 (56.7)	57 (44.5)	
1–4 times/wk	45 (18.9)	17 (13.3)	
≥5 times/wk	58 (24.4)	54 (42.2)	<0.05
Smoking habit			
Non-smokers	139 (58.4)	59 (46.1)	
Ex-smokers	43 (18.1)	34 (26.6)	
Smokers	56 (23.5)	35 (27.3)	NS

a: Values in parentheses are percentages. NS, not significant.
b: Mean ± SD.

Table 2. ORs and 95% CIs for Colorectal Cancer According to Food Consumption[a]

	Controls	Cases	OR[b] (95% CI)	OR[c] (95% CI)
Meat[d]				
Low (<1 time/wk)	59 (24.8)	21 (16.4)	1.00	1.00
Moderate (1–3 times/wk)	142 (59.7)	83 (64.8)	1.72 (0.97–3.06)	1.84 (1.01–3.35)
High (≥4 times/wk)	37 (15.5)	24 (18.8)	2.02 (0.95–4.26)	1.99 (0.93–4.27)
P value for trend			NS	0.05
Milk[e]				
Rarely	42 (17.7)	37 (28.9)	1.00	1.00
Sometimes	77 (32.5)	39 (30.5)	0.60 (0.33–1.10)	0.62 (0.33–1.16)
Daily	118 (49.8)	52 (40.6)	0.46 (0.25–0.82)	0.53 (0.29–0.97)
P value for trend			<0.05	NS
Raw vegetables				
Low (<1 time/wk)	45 (18.9)	19 (14.8)	1.00	1.00
Moderate (1–3 times/wk)	114 (47.9)	69 (53.9)	1.81 (0.95–3.45)	2.26 (1.12–4.53)
High (≥4 times/wk)	79 (33.2)	40 (31.3)	1.37 (0.69–2.70)	1.66 (0.80–3.46)
P value for trend			NS	NS
Fruit				
Low (<1 time/wk)	32 (13.4)	26 (20.3)	1.00	1.00
Moderate (1–3 times/wk)	79 (33.2)	41 (32.0)	0.61 (0.31–1.20)	0.80 (0.39–1.66)
High (≥4 times/wk)	127 (53.4)	61 (47.7)	0.65 (0.33–1.26)	0.88 (0.42–1.83)
P value for trend			NS	NS
Vegetables/fruit[f]				
Low (<4 times/wk)	183 (76.9)	102 (79.7)	1.00	1.00
High (≥4 times/wk)	55 (23.1)	26 (20.3)	0.86 (0.50–1.48)	0.99 (0.57–1.74)

a: ORs, odds ratios; CIs, confidence intervals; NS, not significant. Values in parentheses are percentages.
b: ORs were adjusted for age and sex.
c: ORs were adjusted for age, sex, and habitual exercise, drinking, and smoking habits.
d: Meat included beef, pork, and chicken.
e: One male control missing milk consumption was excluded from analyses.
f: High consumption of "vegetables/fruit" was defined as a variable of ≥4 times/wk of both raw vegetables and fruit consumption, and other combinations of those were categorized into the low consumption group.

genotype was 2.23 (95% confidence interval, CI = 0.97–5.15).

With regard to the risk of colorectal cancer adjusted for age and sex on cross-classifying subjects for the *CD36* genotype and meat consumption, the OR for the *C/C* genotype with moderate meat consumption relative to the *A/A* genotype with low meat consumption was 8.30 (95% CI = 2.15–32.00) (Table 4). In the *A/A* genotype group, the OR for high meat consumption relative to low meat consumption was 3.29 (95% CI = 1.10–9.84). None of individuals with the *C/C* genotype was in the high meat consumption group. High level of milk consumption was not associated with the risk of colorectal cancer. For consumption of raw vegetables and fruit, there were inconsistent interactions with the *CD36* gene *A52C* polymorphism for the risk of colorectal cancer. In the low vegetables/fruit consumption group, however, the OR for the *C/C* genotype relative to the *A/A* genotype was 3.03 (95% CI = 1.16–7.90).

Discussion

The present study demonstrated that the risk of colorectal cancer for meat consumption increased from 1.99 to 8.30 according to an interaction between moderate meat consumption and the *C/C* genotype of the *CD36* gene *A52C* polymorphism relative to high meat consumption ignoring this gene polymorphism. Consequently, we found that individuals with the *C/C* genotype in particular might be advised to reduce meat intake for the prevention of colorectal cancer.

CD36 is a highly glycosylated 88-kDa protein and is expressed in a broad variety of human cells, such as platelets, monocytes, capillary endothelial cells, adipocytes, and mammary epithelial cells (22). The *CD36* gene is located on chromosome 7q11.12 and encodes 15 exons that extend over more than 32 kilobases (27). The gene sequence of CD36 in humans is 85% homologous with that of FAT in rat (17). The

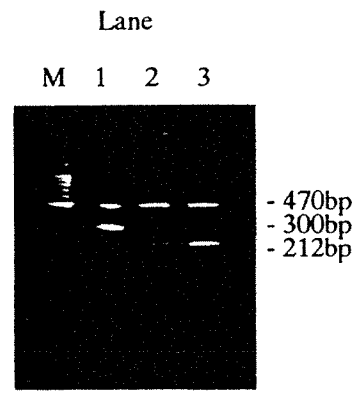

Figure 1. Representative results for the *CD36* gene *A52C* polymorphism by the PCR-CTPP method. DNA fragments stained with ethidium bromide are shown. Lane M, markers; lane 1, *C/C*; lane 2, *A/C*; lane 3, *A/A*.

Table 3. ORs and 95% CIs for Colorectal Cancer According to *CD36* Gene *A52C* Polymorphisms in Colorectal Cancer Cases and Controls[a]

	Controls	Case	Crude OR (95% CI)	OR[b] (95% CI)
Genotype				
A/A	122 (51.5)	62 (49.6)	1.00	1.00
A/C	102 (43.0)	49 (39.2)	0.95 (0.60–1.50)	0.93 (0.58–1.49)
C/C	13 (5.5)	14 (11.2)	2.12 (0.94–4.79)	2.23 (0.97–5.15)
Allele frequency				
A	346 (73.0)	173 (69.2)		
C	128 (27.0)	77 (30.8)		

a: The *CD36* genotypes and the allele frequencies in three colorectal cancer cases (one colon and two rectal cancer cases) and one control were unknown because DNA was not amplified by PCR-CTPP. Values in parentheses are percentages.

b: Adjusted for age and sex.

Table 4. ORs and 95% CIs for Colorectal Cancer According to the *CD36* Genotype With Reference to Food Consumption

	Number of Cases/Controls[a]			OR[b] (95% CI)		
	Low[c,d] (<1 time/wk)	Moderate[c] (1–3 times/wk)	High[c] (≥4 times/wk)	Low[c,d] (<1 time/wk)	Moderate[c] (1–3 times/wk)	High[c] (≥4 times/wk)
Meat[e]						
A/A	8/32	40/69	14/21	1.00	2.74 (1.10–6.82)	3.29 (1.10–9.84)
A/C	9/20	32/66	8/16	1.80 (0.59–5.52)	2.04 (0.83–5.04)	3.15 (0.85–11.67)
C/C	3/7	11/6	0/0	2.17 (0.42–11.21)	8.30 (2.15–32.00)	NE[f]
Milk[c]						
A/A	14/23	20/38	28/61	1.00	0.95 (0.38–2.36)	0.72 (0.31–1.63)
A/C	13/15	15/35	21/52	1.54 (0.52–4.55)	0.66 (0.26–1.67)	0.57 (0.24–1.36)
C/C	7/4	4/4	3/5	2.80 (0.60–13.16)	1.62 (0.35–7.61)	1.06 (0.21–5.29)
Raw vegetables						
A/A	8/26	34/57	20/39	1.00	2.33 (0.91–5.97)	1.75 (0.63–4.83)
A/C	7/15	26/52	16/35	1.35 (0.38–4.80)	1.73 (0.68–4.42)	1.52 (0.56–4.12)
C/C	2/4	8/5	4/4	1.60 (0.24–10.72)	6.88 (1.55–30.55)	3.53 (0.67–18.64)
Fruit						
A/A	13/15	17/43	32/64	1.00	0.42 (0.15–1.17)	0.77 (0.27–1.70)
A/C	9/13	20/33	20/56	1.07 (0.31–3.70)	0.76 (0.28–2.07)	0.29 (0.10–0.82)
C/C	2/4	4/3	8/6	0.58 (0.08–4.27)	2.78 (0.41–18.82)	1.78 (0.41–7.69)
Vegetables/fruit[d]						
A/A	47/98		15/24	1.00		1.35 (0.62–2.92)
A/C	40/76		9/26	1.10 (0.65–1.87)		0.70 (0.29–1.66)
C/C	12/9		2/4	3.03 (1.16–7.90)		0.97 (0.17–5.72)

a: Three colorectal cancer cases and one control with unknown *CD36* genotype were excluded from analyses.

b: ORs were adjusted for age and sex.

c: Milk intake was rarely, sometimes, and daily and was represented as low, moderate, and high, respectively.

d: High consumption of "vegetables/fruit" was defined as a variable for ≥4 times/wk of both raw vegetables and fruit consumption, and other combinations of those were categorized into the low consumption group.

e: Meat included beef, pork, and chicken.

f: NE, not estimated because case and control were absent in this category.

variant allele frequency of the *A52C* polymorphism was found to be 26.7% among 750 Japanese (21). Expression of the *CD36* gene is regulated in a tissue-specific manner by peroxisome proliferator-activator receptor ligand-responsive promotion (28,29).

The major cause of CD36 deficiency is found with the *Pro90Ser* polymorphism, but the *Ser* allele frequency was only 4.2% among our study subjects (30,31). CD36 deficiency is reported to be linked with a high serum level of LDL cholesterol, but this biological marker has been controversial with regard to the risk of colorectal cancer (32–41). Oxidized LDL has been suggested as a potent independent mitogenic and/or cytotoxic factor under some conditions (42). Therefore, we considered that the variant allele of the *A52C* polymorphism might have the lower ability for FAT and oxidized LDL scavenger receptor and effect colorectal tumor promotion through the increased secretion of bile acid as a tumor promoter, with modulation of cell membrane fluidity, and increased responsiveness to insulin and insulin-like growth factor-I as growth hormonal factors for colonic mucosal cells (43–46).

Clear results from epidemiological studies demonstrating intake of meat, meat products, and animal fat/SFA to increase the risk of colorectal cancer have been limited, and many in-

vestigations failed to confirm this hypothesis after adjusting for total energy (1,47–51). Meat and its products (8 foods) contribute 18.0% of the 65.8% cumulative contribution (20 foods) of SFA, followed by 12.2% for chicken egg, 10.2% for milk, 2.9% for salad oil, and 2.6% for butter (14). Intake of meat, therefore, can be thought of as a good surrogate for SFA. In contrast to meat, milk is rich in short- and middle-chain SFAs (about 25%), which are absorbed at the portal vein, but not the lymphatic system for long-chain fatty acids, and metabolized to energy immediately. Furthermore, milk is also rich in calcium and has been reported to reduce the risk of colorectal cancer (52).

Incidentally, high consumption of dietary fiber (53) supports a protective role in the dilution of bile acids related to high intake of animal fat. From our finding that the combination of low vegetables/fruit consumption and the *C/C* genotype confers a threefold elevated risk for colorectal cancer, we feel that the *A52C* polymorphism might be related to the function of FAT.

Regarding risks for colorectal cancer and adenomas, gene–environmental interactions for subgroups of cytochrome P450 CYP1A2 and *N*-acetyltransferases-2 for heterocyclic amines (HCAs), glutathione *S*-transferases for polycyclic aromatic hydrocarbons, and cytochrome P450 CYP2A6 and CYP2E1 for *N*-nitroso compounds have been reported (54–62). Cigarette smoking and alcohol consumption as inducers of those metabolic enzymes and activators of organic xenobiotics, however, critically affected the magnitude of those risks, (58–60). Intake of HCAs generated during high-temperature cooking has been summarized as follows: 1) fish, but not meat, was the highest source of HCAs in the Japanese diet and 2) daily intakes of HCAs among Japanese, Swedish, and American populations were 1.1, 2.3, and 9.0 ng/kg, respectively (63). Thus, at least among Japanese the impact of HCAs for colorectal cancer might be limited.

Further study on larger populations is needed to confirm and extend our observations because our study subjects were relatively small, especially in the *C/C* genotype group. The function of the *CD36* gene *A52C* polymorphism has also yet to be clarified. The type of meat, such as beef, pork, chicken, and processed meat, was not distinguished in our questionnaire, and the accuracy of food consumption data was not examined. However, trends in colorectal cancer risk showed that the food-frequency questionnaire might be a sufficiently reliable instrument, with classification into three consumption groups (low, moderate, and high) for meat, milk, raw vegetables, and fruit. Risks of colorectal cancer may be underestimated with data from prevalent cases because these might have already modified their lifestyle with reference to the recommended primary cancer prevention. Our findings, therefore, are not conclusive.

In conclusion, the present study is the first to our knowledge to examine the association between the *CD36* gene *A52C* polymorphism and consumption of meat as a good surrogate for SFA regarding the risk of colorectal cancer. Positive interactions between the *CD36* gene *A52C* polymorphism and meat consumption of more than one time per week were found after adjusting for confounding effects of lifestyle factors and vegetables/fruit consumption. We need to stress that lifestyle modification for primary cancer prevention may be more effective when taking into account the individual genetic background.

Acknowledgments and Notes

The authors are grateful to Ms. M. Kato, Ms. N. Takeuchi, and Ms. M. Yagyu for genotyping; Ms. H. Fujikura for the data collection; and Dr. M. A. Moore for checking the English language. The work was supported by a Grant-in-Aid for Scientific Research on Special Priority Areas of Cancer from the Japanese Ministry of Education, Culture, Sports, Science and Technology, and a Grant-in-Aid for a Research Fellow of the Japan Society for the Promotion of Science (JSPS). K. K. was supported by a Research Fellowship of the JSPS for Young Scientists during much of the performance of this study. Address correspondence to K. Kuriki, Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1–1 Kanokoden, Chikusa-ku, Nagoya 464–8681, Japan. Phone: +81–52–762–6111. FAX: +81–52–763–5233. E-mail: kkuriki@aichi-cc.jp.

Submitted 7 October 2003; accepted in final form 3 January 2005.

References

1. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition and the Prevention of Cancer: A Global Perspective. Washington, DC: American Institute for Cancer Research, 1997.
2. Ogimoto I, Shibata A, and Fukuda K: World Cancer Research Fund/American Institute of Cancer Research 1997 recommendations: applicability to digestive tract cancer in Japan. *Cancer Causes Control* **11,** 9–23, 2000.
3. Hirayama T: A large scale cohort study on cancer risks by diet—with special reference to the risk reducing effects of green-yellow vegetable consumption. *Princess Takamatsu Symp* **16,** 41–53, 1985.
4. Doll R and Peto R: The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *JNCI* **66,** 1191–1308, 1981.
5. Key TJ, Allen NE, Spencer EA, and Travis RC: The effect of diet on risk of cancer. *Lancet* **360,** 861–868, 2002.
6. Ministry of Health, Labor and Welfare, Division of Health and Nutrition, Kokumin-Eiyo-no-Genjo (Current Status of National Nutrition) 2000 (in Japanese). Tokyo: Dai-ichi Shuppann, 2002.
7. Kuriki K, Tokudome S, and Tajima K: Association between type II diabetes and colon cancer among Japanese with reference to changes in food intake. *Asian Pac J Cancer Prev* **5,** 28–35, 2004.
8. Tominaga S and Kuroishi T: An ecological study on diet/nutrition and cancer in Japan. *Int J Cancer* **10,** S2–S6, 1997.
9. Tajima K and Tominaga S: Dietary habits and gastro-intestinal cancers: a comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. *Jpn J Cancer Res* **76,** 705–716, 1985.
10. Broder S: Perspectives on cancer in Japan and the United States. *Jpn J Cancer Res* **84,** 821–830, 1993.
11. Flood DM, Weiss NS, Cook LS, Emerson JC, Schwartz SM, et al.: Colorectal cancer incidence in Asian migrants to the United States and their descendants. *Cancer Causes Control* **11,** 403–411, 2000.
12. Le Marchand L: Combined influence of genetic and dietary factors on colorectal cancer incidence in Japanese Americans. *Monogr Natl Cancer Inst* **26,** 101–105, 1999.
13. Parkin DM, Whelan SL, Ferlay J, Teppo L, and Thomas DB: *Cancer Incidence in Five Continents VIII*, IARC Scientific Publications No. 155, Lyon, France: IARC, 2002.

14. Tokudome Y, Imaeda N, Ikeda M, Kitagawa I, Fujiwara N, et al.: Foods contributing to absolute intake and variance in intake of fat, fatty acids and cholesterol in middle-aged Japanese. *J Epidemiol* **9**, 78–90, 1999.
15. Willett W and Lenart E: Reproducibility and validity of food frequency questionnaires. In *Nutritional Epidemiology*, Willett W (ed). New York: Oxford University Press, 1990, pp 101–147.
16. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, et al.: A CD36 is a receptor for oxidized low density lipoprotein. *J Biol Chem* **268**, 11811–11816, 1993.
17. Abumrad NA, El-Maghrabi MR, Amri EZ, Lopez E, and Grimaldi PA: Cloning a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. *J Biol Chem* **268**, 17665–17668, 1993.
18. Greenwalt DE, Lipsky RH, Ockenhouse CF, Ikeda H, Tandon NN, et al.: Membrane glycoprotein CD36: a review of its roles in adherence, signal transduction, and transfusion medicine. *Blood* **80**, 1105–1115, 1992.
19. Tsuchida T, Kijima H, Tokunaga T, Oshika Y, Hatanaka H, et al.: Expression of the thrombospondin 1 receptor CD36 is correlated with decreased stromal vascularisation in colon cancer. *Int J Oncol* **14**, 47–51, 1999.
20. Yamamoto N, Greco NJ, Barnard MR, Tanoue K, Yamazaki H, et al.: GPIb-dependent and GPIb-independent pathways of thrombin-induced platelet activation. *Blood* **77**, 1740–1747, 1991.
21. Armesilla AL and Vega MA: Structural organization of the gene for human CD36 glycoprotein. *J Biol Chem* **269**, 18985–18991, 1994.
22. Database of Japanese Single Nucleotide Polymorphisms. http://snp.ims.u-tokyo.ac.jp/.
23. Takezaki T, Hamajima N, Matsuo K, Tanaka R, Hirai T, et al.: Association of polymorphism in the beta-2 and beta-3 adrenoceptor genes with risk of colorectal cancer in Japan. *Int J Clin Oncol* **6**, 117–122, 2001.
24. Hamajima N, Matsuo K, Suzuki T, Nakamura T, Matsuura A, et al.: Low expression myeloperoxidase genotype negatively associated with Helicobacter pylori infection. *Jpn J Cancer Res* **92**, 488–493, 2001.
25. Inoue M, Tajima K, Hirose K, Hamajima N, Takezaki T, et al. Epidemiological features of first-visit outpatients in Japan: comparison with general population and variation by sex, age, and season. *J Clin Epidemiol* **50**, 69–77, 1997.
26. Hamajima N, Saito T, Matsuo K, Kozaki K, Takahashi T, et al.: Polymerase chain reaction with confronting two-pair primers for polymorphism genotyping. *Jpn J Cancer Res* **91**, 865–868, 2000.
27. Fernandez-Ruiz E, Armesilla AL, Sanchez-Madrid F, and Vega MA: The gene encoding type IV and thrombospondin receptor CD36 is located on chromosome 7q11.12. *Genomics* **17**, 759–761, 1993.
28. Sato O, Kuriki C, Fukui Y, and Motojima K: Dual promoter structure of mouse and human fatty acid translocase/CD36 genes and unique transcriptional activation by peroxisome proliferator-activated receptor alpha and gamma ligands. *J Biol Chem* **277**, 15703–15711, 2002.
29. Kuriki C, Tanaka T, Fukui Y, Sato O, and Motojima K: Structural and functional analysis of a new upstream promoter of the human FAT/CD36 gene. *Biol Pharm Bull* **25**, 1476–1478, 2002.
30. Kashiwagi H, Tomiyama Y, Honda S, Kosugi S, Shiraga M, et al.: Molecular basis of CD36 deficiency. *J Clin Invest* **95**, 1040–1046, 1995.
31. Hamajima N, Saito T, Matsuo K, Suzuki T, Nakamura T, et al.: Genotype frequency of 50 polymorphisms for 241 Japanese non-cancer patients. *J Epidemiol* **12**, 229–236, 2002.
32. Yanai H, Chiba H, Morimoto M, Abe K, Fujiwara H, et al.: Human CD36 deficiency is associated with elevation in low-density lipoprotein-cholesterol. *Am J Med Genet* **93**, 299–304, 2000.
33. Nozaki S, Kashiwagi H, Yamashita S, Nakagawa T, Kostner B, et al.: Reduced uptake of oxidized low density lipoproteins in monocyte-derived macrophages from CD36-deficient subjects. *J Clin Invest* **96**, 1859–1865, 1995.
34. Kreger BE, Anderson KM, Schatzkin A, and Splansky GL: Serum cholesterol level, body mass index, and the risk of colon cancer: the Framingham Study. *Cancer* **70**, 1038–1043, 1992.
35. Cowan LD, O'Connell DL, Criqui MH, Barrett-Connor E, Bush TL, et al.: Cancer mortality and lipid and lipoprotein levels. *Am J Epidemiol* **131**, 468–482, 1990.
36. Gaard M, Tretli S, and Urdal P: Blood lipid and lipoprotein levels and the risk of cancer of the colon and rectum: a prospective study of 62,173 Norwegian men and women. *Scand J Gastroenterol* **32**, 162–168, 1997.
37. Bird CL, Ingles SA, Frankl HD, Lee ER, Longnecker MP, et al.: Serum lipids and adenomas of the left colon and rectum. *Cancer Epidemiol Biomarkers Prev* **5**, 607–612, 1996.
38. Schoen RE, Tangen CM, Kuller LH, Burke GL, Cushman M, et al.: Increased blood glucose and insulin, body size, and incident colorectal cancer. *JNCI* **91**, 1147–1154, 1999.
39. Kono S, Imanishi K, Shinchi K, and Yanai F: Serum lipids and left-sided adenomas of the large bowel: an extended study of self-defense officials in Japan. *Cancer Causes Control* **4**, 117–121, 1993.
40. Tornberg SA, Holm LE, Carstensen JM, and Eklund GA: Risks of cancer of the colon and rectum in relation to serum cholesterol and beta-lipoprotein. *N Engl J Med* **315**, 1629–1633, 1986.
41. Bayerdorffer E, Mannes GA, Richter WO, Ochsenkuhn T, Sceholzer G, et al.: Decreased high-density lipoprotein cholesterol and increased low-density cholesterol levels in patients with colorectal adenomas. *Ann Intern Med* **118**, 481–487, 1993.
42. Zettler ME, Prociuk MA, Austria JA, Massaeli H, Zhong G, et al.: OxLDL stimulates cell proliferation through a general induction of cell cycle proteins. *Am J Physiol Heart Circ Physiol* **284**, H644–H653, 2003.
43. Weisburger JH: Mechanisms of macronutrient carcinogenesis. In *Macronutrients: Investigating Their Role in Cancer*, Micozzi MC and Moon TE (eds). New York: Marcel Dekker, 1992, pp 3–31.
44. Field CJ, Ryan EA, Thomson AB, and Clandinin MT: Dietary fat and the diabetic state alter insulin binding and the fatty acyl composition of the adipocyte plasma membrane. *Biochem J* **253**, 417–424, 1988.
45. Hagve TA: Effects of unsaturated fatty acids on cell membrane functions. *Scand J Clin Lab Invest* **48**, 381–388, 1988.
46. Guo YS, Narayan S, Yallampalli C, and Singh P: Characterization of insulin-like growth factor-I and II receptors in human colon cancer. *Gastroenterology* **102**, 1101–1108, 1992.
47. Willett WC, Stampfer MJ, Colditz GA, Rosner BA, and Speizer FE: Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. *N Engl J Med* **323**, 1664–1672, 1990.
48. Goldbohm RA, van den Brandt PA, van 't Veer P, Brants HA, Dorant E, et al.: A prospective cohort study on the relation between meat consumption and the risk of colon cancer. *Cancer Res* **54**, 718–723, 1994.
49. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, et al.: Intake of fat, meat, and fiber in relation to risk of colon cancer in men. *Cancer Res* **54**, 2390–2397, 1994.
50. Sandhu MS, White IR, and McPherson K: Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. *Cancer Epidemiol Biomarkers Prev* **10**, 439–446, 2001.
51. Willett WC: Diet and cancer: one view at the start of the millennium. *Cancer Epidemiol Biomarkers Prev* **10**, 3–8, 2001.
52. Cho E, Smith-Warner SA, Spiegelman D, Beeson WL, van den Brandt PA, et al.: Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. *JNCI* **96**, 1015–1022, 2004.
53. Cummings JH, Branch W, Jenkins DJA, Southgate DAT, Houston H, et al.: Colonic response to dietary fiber from carrot, cabbage, apple bran and guar gum. *Lancet* **1**, 5–9, 1978.
54. Le Marchand L: Meat intake, metabolic genes and colorectal cancer. In *Nutrition and Lifestyle: Opportunities for Cancer Prevention*, Riboli E and Lambert R (eds). IARC Scientific Publications No. 156, Lyon, France: IARC, 2002; pp 481–485.
55. Le Marchand L, Hankin JH, Pierce LM, Sinha R, Nerurkar PV, et al.: Well-done red meat, metabolic phenotypes and colorectal cancer in Hawaii. *Mutat Res* **506–507**, 205–214, 2002.

56. Chen J, Stampfer MJ, Hough HL, Garcia-Closas M, Willett WC, et al.: A prospective study of *N*-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. *Cancer Res* **58,** 3307–3311, 1998.
57. Sweeney C, Coles BF, Nowell S, Lang NP, and Kadlubar FF: Novel markers of susceptibility to carcinogens in diet: associations with colorectal cancer. *Toxicology* **181–182,** 83–87, 2002.
58. Barrett JH, Smith G, Waxman R, Gooderham N, Lightfoot T, et al.: Investigation of interaction between *N*-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer. *Carcinogenesis* **24,** 275–282, 2003.
59. Tiemersma EW, Kampman E, Bueno de Mesquita HB, Bunschoten A, van Schothorst EM, et al.: Meat consumption, cigarette smoking, and genetic susceptibility in the etiology of colorectal cancer: results from a Dutch prospective study. *Cancer Causes Control* **13,** 383–393, 2002.
60. Cortessis V, Siegmund K, Chen Q, Zhou N, Diep A, et al.: A case-control study of microsomal epoxide hydrolase, smoking, meat consumption, glutathione *S*-transferase M3, and risk of colorectal adenomas. *Cancer Res* **61,** 2381–2385, 2001.
61. Le Marchand L, Donlon T, Seifried A, and Wilkens LR: Red meat intake, *CYP2E1* genetic polymorphisms, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* **11,** 1019–1024, 2002.
62. Kampman E, Slattery ML, Bigler J, Leppert M, Samowitz W, et al.: Meat consumption, genetic susceptibility, and colon cancer risk: a United States multicenter case-control study. *Cancer Epidemiol Biomarkers Prev* **8,** 15–24, 1999.
63. Kobayashi M, Hanaoka T, Nishioka S, Kataoka H, and Tsugane S: Estimation of dietary HCA intakes in a large-scale population-based prospective study in Japan. *Mutat Res* **506–507,** 233–241, 2002.

J. Obstet. Gynaecol. Res. Vol. 31, No. 2: 144–151, April 2005

Cigarette smoking and the risk of ovarian cancer in the Japanese population: Findings from the Japanese Collaborate Cohort study

Yoshimitsu Niwa[1], Kenji Wakai[2], Sadao Suzuki[3], Koji Tamakoshi[4], Yingsong Lin[5], Hiroshi Yatsuya[4], Takaaki Kondo[4], Kazuko Nishio[1], Akio Yamamoto[6], Shinkan Tokudome[3], Nobuyuki Hamajima[1], Hideaki Toyoshima[4] and Akiko Tamakoshi[1] for the JACC Study Group

Departments of [1]Preventive Medicine/Biostatistics and Medical Decision Making and [4]Public Health/Health Information Dynamics, Nagoya University Graduate School of Medicine, [2]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, [3]Department of Health Promotion and Preventive Medicine, Nagoya City University Graduate School of Medical Sciences, Nagoya, [5]Department of Public Health, Aichi Medical University, Nagakute and [6]Department of Epidemiology, Hyogo Prefectural Institute of Public Health and Environmental Science, Kobe, Japan

Abstract

Aim: The many studies into the relation between cigarette smoking and the risk of ovarian cancer have produced inconsistent results. Here we investigated this relation using data from the Japan Collaborative Cohort Study for Evaluation of Cancer Risk, initiated in 1988.

Methods: A self-administered questionnaire on smoking habits and other risk factors for cancer was completed by 34 639 Japanese women. After 7.6 years of follow up, 39 cases of ovarian cancer were available for analyses. Cox proportional-hazards models were used to compute relative risks (RR) and to adjust for confounders.

Results: Relative to those who had never smoked, the RR of ovarian cancer were 1.63 (95% confidence interval [CI] = 0.21–12.50) for former smokers and 2.27 (95% CI = 0.85–6.08) for current smokers. Among current smokers, the RR were 1.48 (95% CI = 0.20–10.92), 5.56 (95% CI = 1.68–19.06), and 1.86 (95% CI = 0.25–14.30) among women who smoked <10, 10–19, and at least 20 pack-years ([number of cigarettes smoked per day/20] × number of years subject has smoked), respectively, relative to those who had never smoked. A test for trend was statistically significant ($P = 0.044$).

Conclusions: These data indicate that cigarette smoking increases the risk of developing ovarian cancer in the Japanese population.

Key words: cohort study, Japanese; ovarian cancer, proportional-hazards model, smoking.

Introduction

Ovarian cancer is one of the most common cancers among women worldwide.[1] Although the frequency of ovarian cancer in Japan is low relative to the rate in many other industrialized countries, the age-adjusted incidence rate increased approximately 1.5-fold (from 3.6 to 5.7) from 1975 to 1993.[2,3] Previous reports have attributed this increase to declining parity[3] and increasing obesity,[4] but possible contributions from

Received: July 30 2004.
Accepted: December 8 2004.
Reprint request to: Dr Yoshimitsu Niwa, Department of Preventive Medicine/Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Email: ytt-niwa@xg7.so-net.ne.jp

environmental factors, such as diet, coffee, alcohol, and cigarette smoking, remain unclear. Moreover, the prevalence of smoking in young Japanese women is steadily increasing.[5]

Benzo[α]-pyrene is one of the mutagens and carcinogens present in cigarettes.[6] Zenzes *et al.* reported on the immunodetection and quantification of benzo[α]-pyrene DNA adducts in ovarian granulosa-lutein cells of patients undergoing *in vitro* fertilization and embryo transfer who were exposed to cigarette smoking.[7] The presence of these adducts in ovarian cells from women exposed to cigarette smoke indicates the possibility of an increased risk for DNA damage caused by smoking.

Many epidemiological studies have investigated associations between cigarette smoking and ovarian cancer risk (mainly using case–control study designs), but the findings have been equivocal.[8–26] Several of these studies have indicated a positive association between smoking and ovarian cancer,[24–27] but only one prospective cohort study identified a significant relation.[28]

We therefore examined the association between cigarette smoking and the risk of ovarian cancer in a prospective cohort study involving Japanese subjects.

Materials and Methods

Study population

The investigation was conducted using data from the Japan Collaborative Cohort Study for Evaluation of Cancer Risk sponsored by the Ministry of Education, Culture, Sports, Science and Technology in Japan (JACC study), which was a multicenter collaborative study in which 24 institutions participated. The design and conduct of the JACC study has been described elsewhere in detail.[29] Between 1988 and 1990, 64 327 women from 45 areas of Japan and aged 40–79 years registered based mainly on a basic health examination that was conducted under the Health and Medical Service Law for the Aged.

On enrollment in the JACC study, all participants completed a self-administered questionnaire that comprised personal identifiers, demographic characteristics, personal and family medical history, reproductive history, smoking and alcohol consumption status, dietary exposures, education, and various behavioral, environmental and occupational exposures. For cigarette smoking, subjects were asked to describe their smoking status (never/former/current), age at starting smoking, average number of cigarettes smoked per day, years of smoking, depth of smoke inhalation, and years of smoking cessation. In this study, we did not assess passive smoking.

The data and cause of death were annually or biannually confirmed, with the permission of the director-general of the Prime Minister's Office. Population registries in the municipalities were used to determine the vital and residential status of subjects. Registration of death is required under the Family Registration Law in Japan, which applies throughout the country. Incidences of cancer were confirmed using records from the population-based cancer registries, which were supplemented by a systematic review of death certificates;[29] in some areas, medical records were also reviewed in major local hospitals. Analyses were restricted to data from the participants who lived in the 24 study areas in which cancer registries were available. The data of subjects who had moved from the study area were also verified by the investigator in each area by reviewing population-register sheets of the cohort members. The mortality/incidence ratio for ovarian cancer was 0.62 in the cohort covered by cancer registries, which is comparable with data in population-based cancer registries in Japan that are considered acceptably accurate (0.50–0.71);[30] this correspondence indicates that a reasonably high proportion of ovarian cancer cases was identified in the present study.

The follow-up periods ran from the time of the baseline survey through to the end of 1999 in all areas. The endpoint of the study was defined as the incidence of ovarian cancer (10th Revision of the International Classification of Disease; ICD-10: C56). For each participant, the person-years of follow up were calculated from the enrollment to development of ovarian cancer, death from any other causes, moving from the study area, or the end of the follow-up period, whichever occurred first. However, for cases in which the time of development could not be ascertained, we defined the person-years measure as the time period from the enrollment to death from ovarian cancer. Those who moved from the study areas or died from causes other than ovarian cancer were treated as censored cases. The Ethical Board at Nagoya University School of Medicine approved this investigation.

The following exclusion criteria were applied to our analytic cohort: women with a history of ovarian cancer at the time of enrollment, women registered from areas with a zero incidence of ovarian cancer, and women with an unknown smoking status. After these exclusions, a total of 34 639 women and 39 ovarian cancer cases were eligible for analysis.

Statistical analysis

The strength of the association between cigarette smoking and risk of ovarian cancer incidence was examined. We used Cox proportional-hazards models to estimate relative risks (RR) and 95% confidence intervals (CI), adjusted for age at enrollment and study area.[31] In another multivariate analysis, all RR were adjusted for the following potential confounders: body mass index (<18.5, 18.5–24.9, ≥25 kg/m^2), height (<150, 150–155, ≥156 cm), hormone replacement therapy (HRT; yes, no), family history of breast cancer and/or ovarian cancer (yes, no), age at menarche (≤12, 13–15, ≥16 years), age at menopause (≤43, 44–47, 48–51, ≥52 years), parity (0, 1–2, ≥3 children), alcohol consumption (non-drinker, former drinker, current drinker), and education (less than high school, high school, college or higher). In the analyses, all variables were entered as dummy variables. These variables were assessed using the baseline questionnaire and were selected as confounders because they are either suspected to be or are modifiers of ovarian cancer risk.[32–39] However, we did not adjust for HRT in the final models because there was no ovarian cancer case among individuals with HRT. In this study, oral contraceptives were not measured because the study subjects in the cohort were aged 40–79 years at baseline. Missing values for each covariate were treated as an additional category in the variable and were included in the model. Trends in risk with pack-years (i.e. [number of cigarettes smoked per day/20] × number of years the subject has smoked) were evaluated among all smokers by treating ordinal-score variables as continuous variables in the proportional-hazards model.

All statistical analyses in this study were carried out with SAS software (SAS Institute, Cary, NC). All reported *P*-values are two-sided, and considered statistically significant when <0.05.

Results

A total of 34 639 women (mean age = 58.3 years) were followed for an average of 7.6 years (range = 0–10.0 years) (266 366 person-years). At the initiation of the JACC study the proportions of those who had never smoked and former and current smokers were 93.1%, 1.6%, and 5.3%, respectively. A total of 39 women were diagnosed with ovarian cancer, although the time of development could not be ascertained in four of them. Of these 39 cases, 35.9% (14 cases) had histologic diagnosis of serous carcinoma, 10.3% (four cases) had endometrioid carcinoma, and 5% (two cases) had mucinous carcinoma, whereas 48.7% (19 cases) had no detailed histologic diagnosis. The baseline characteristics of the study cohort according to smoking status are given in Table 1. Former smokers were older and heavier than current smokers and those who had never smoked. Current smokers were taller than former smokers and those who had never smoked. Both current smokers and former smokers were more likely to be nullipara and current drinkers than those who had never smoked.

Table 2 lists the age- and study-area-adjusted and multivariate-adjusted RR of ovarian cancer for smoking status. After adjustment for study area and age at registration, current smokers showed a significantly increased risk of ovarian cancer compared with those who had never smoked (RR = 2.63, 95% CI = 1.02–6.78). However, multivariate analysis with the other confounders showed that current smokers were associated with an increased (although non-significant) risk of ovarian cancer (RR = 2.27, 95% CI = 0.85–6.08). Former smokers were also positively associated with the risk of ovarian cancer, but this was also not significant.

Table 3 lists the age- and study-area-adjusted and multivariate-adjusted RR of ovarian cancer for cigarettes per day, years smoked, and consumption in pack-years among current smokers. Women who currently smoked 10–19 cigarettes per day had a significantly higher risk of developing ovarian cancer (RR = 3.50, 95% CI = 1.05–11.68) compared with those who had never smoked. The risk was also increased in women who were long-term smokers at enrollment. Women who smoked 10–19 years had a risk that was more than four-fold that of never smokers (RR = 4.58, 95% CI = 1.07–19.59). Women who currently consumed 10–19 pack-years showed the highest RR compared with those who had never smoked after adjustment by multiple possible confounders (RR = 5.56, 95% CI = 1.68–19.06). Smokers with a consumption of at least 20 pack-years had an increased risk of ovarian cancer, but this was also not significant (RR = 1.86, 95% CI = 0.25–14.30). A test for trend was statistically significant ($P = 0.044$). The depth of smoke inhalation was not associated with risk of ovarian cancer.

Discussion

The results from this prospective study support the existing evidence that long-term exposure to tobacco carcinogens is associated with a higher incidence of ovarian cancer.[28] There is evidence that

Table 1 Baseline characteristics of the study cohort by smoking status

Covariates	Baseline smoking status					
	Current (n = 1825)		Former (n = 571)		Never (n = 32 243)	
	No.	%	No.	%	No.	%
Age at baseline (years)						
40–49	578	31.7	98	17.2	7 700	23.9
50–59	535	29.3	134	23.5	10 166	31.5
60–69	463	25.4	199	34.9	9 851	30.6
70–79	249	13.6	140	24.5	4 526	14.0
Body mass index (kg/m^2)						
<18.5	163	8.9	38	6.7	1 933	6.0
18.5–24.9	1155	63.3	335	58.7	21 980	68.2
≥25	387	21.2	161	28.2	6 796	21.1
Height (cm)						
<150	708	38.8	268	46.9	14 662	45.5
150–155	603	33.0	168	29.4	11 025	34.2
≥156	408	22.4	102	17.9	5 190	16.1
Hormone replacement therapy						
No	1389	76.1	428	75.0	25 907	80.3
Yes	113	6.2	47	8.2	1 359	4.2
Family history of ovarian cancer or breast cancer						
No	1794	98.3	563	98.6	31 730	98.4
Yes	31	1.7	8	1.4	513	1.6
Age at menarche (years)						
≤12	148	8.1	43	7.5	2 047	6.3
13–15	911	49.9	286	50.1	17 980	55.8
≥16	586	32.1	190	33.3	10 162	31.5
Age at menopause (years)						
≤43	179	9.8	68	11.9	2 562	7.9
44–47	209	11.5	72	12.6	3 590	11.1
48–51	432	23.7	138	24.2	9 544	29.6
≥52	246	13.5	129	22.6	5 833	18.1
Parity (number of children)						
0	152	8.3	54	9.5	1 426	4.4
1–2	788	43.2	232	40.6	13 088	40.6
≥3	658	36.1	212	37.1	15 558	48.3
Alcohol consumption						
Non-drinker	835	45.8	241	42.2	24 288	75.3
Former drinker	123	6.7	63	11.0	384	1.2
Current drinker	748	41.0	235	41.2	6 770	21.0
Education						
Less than high school	148	8.1	43	7.5	2 047	6.3
High school	911	49.9	286	50.1	17 980	55.8
College or higher	586	32.1	190	33.3	10 162	31.5

Table 2 Relative risks of ovarian cancer with 95% confidence intervals according to smoking status

Smoking status	Cases/person-years[†]	RR (95% CI)[‡]	RR (95% CI)[§]
Never smoked	33/248 929	1 (reference)	1 (reference)
Former smoker	1/3900	2.06 (0.28–15.16)	1.63 (0.21–12.50)
Current smoker	5/13 536	2.63 (1.02–6.78)	2.27 (0.85–6.08)

[†]Number of ovarian cancer cases per 100 000 person-years. [‡]Multivariate models included age and study areas. [§]Multivariate models included age, study areas, body mass index, height, family history of breast cancer and/or ovarian cancer, age at menarche, age at menopause, parity, alcohol consumption, and education. CI, confidence interval; RR, relative risk.

Table 3 Relative risk of ovarian cancer with 95% confidence interval according to cigarettes per day, years smoked, and consumption in pack-years among current smokers

Characteristics	Cases/person-years†	RR (95% CI)‡	RR (95% CI)§
Cigarettes/day			
Never smokers	33/248 929	1 (reference)	1 (reference)
<10	1/3320	2.09 (0.29–15.31)	2.08 (0.28–15.43)
10–19	3/5766	3.78 (1.16–12.33)	3.50 (1.05–11.68)
≥20	1/3795	1.93 (0.26–14.14)	1.81 (0.24–13.77)
		P for trend = 0.049	*P* for trend = 0.08
Years smoked			
Never smoked	33/248 929	1 (reference)	1 (reference)
<10	1/1741	4.10 (0.56–30.10)	3.95 (0.53–29.49)
10–19	2/3127	4.60 (1.10–19.22)	4.58 (1.07–19.59)
≥20	2/7482	1.95 (0.47–8.14)	1.82 (0.42–7.85)
		P for trend = 0.035	*P* for trend = 0.11
Pack-years¶			
Never smoked	33/248 929	1 (reference)	1 (reference)
<10	1/4627	1.13 (0.21–11.15)	1.48 (0.20–10.92)
10–19	3/3816	5.74 (1.76–18.73)	5.56 (1.68–19.06)
≥20	1/3738	1.98 (0.27–14.50)	1.86 (0.25–14.30)
		P for trend = 0.027	*P* for trend = 0.044

†Number of ovarian cancer cases per 100 000 person-years. ‡Multivariate models included age and study area. §Multivariate models included age, study area, body mass index, height, family history of breast cancer and/or ovarian cancer, age at menarche, age at menopause, parity, alcohol consumption, and education. ¶Pack-years = (cigarettes smoked per day/20) multiplied by years smoked. relative risk.

benzo[α]-pyrene DNA adduct levels in ovarian cells are related to the dose and duration of smoke exposure, both current and long-term.[7] In this study, we used cumulative cigarette smoking as a surrogate measure of tobacco carcinogen exposure. Even though our study included only a small number of cases, it revealed a positive association between cigarette smoking and ovarian cancer incidence in Japanese women. In terms of smoking status at the time of study entry, a statistically significant association with ovarian cancer was found for current smokers, adjusted by age and study area. After adjustment for the other confounders, however, the association was not statistically significant. In terms of dose and duration, although not significant for individuals with a consumption of at least 20 pack-years, a significant association was found between ovarian cancer and individuals with 10–19 pack-years of consumption. Compared with the Canadian Cohort Study, which showed that women who had consumed at least 40 pack-years of cigarettes had a statistically non-significant 29% increased risk,[28] a relatively small consumption in terms of pack-years seemed to affect the ovarian cancer pathogenesis in our Japanese cohort. However, our subjects were on average approximately 10 years older at baseline than those included in the Canadian study, and it is possible that there was a longer time between initial exposure to cigarettes in the Japanese cohort, and therefore more time for ovarian cancer to develop in that cohort. Alternatively, there might be genetic race-related factors that influence the association between environmental factors and susceptibility to ovarian cancer.

The strengths of the present study include its prospective design and the large size of its cohort. Data on exposure were collected before the diagnosis of ovarian cancer, which should preclude recall bias. Moreover, to our knowledge this is the first prospective cohort study that has examined the association between cigarette smoking and the risk of ovarian cancer in an Asian population.

The main limitation of this study is the small numbers of cohort members who developed ovarian cancer. This limits the statistical power of the study to detect associations. The incidence of ovarian cancer is lower in Japanese than in Caucasians women, which contributed to our results being based on relatively few cases. This difference might be associated with a lower rate of cigarette smoking among Japanese women compared with Caucasians (14.7% and 21–30%, respectively).[40]

Second, our study had insufficient information on the histologic type of ovarian cancer, which prevented us from examining histologic specificity. Many previous studies have reported that the influence of smoking habits differed between histologic types of epithelial ovarian cancer. The results from some case–

control studies have suggested that smoking, even over relatively short periods, is particularly related to mucinous adenocarcinoma of the ovary.[24–27] A cohort study by Terry *et al.* found that the risk of mucinous tumors was twofold higher in current smokers (but not former smokers) than in those who had never smoked, with a relatively short period between the cigarette smoking and the disease diagnosis.[28] In contrast, the risk of non-mucinous tumors in their data was highest among women who were long-term smokers. The incidence of ovarian clear-cell carcinoma among Japanese women is relatively high. Such differences in the distribution of histologic subtypes between races, as well as genetic factors, might influence the association between smoking habits and ovarian cancer risk.

Third, information on cigarette smoking was not updated after the enrollment. It is likely that some current smokers ceased smoking and others commenced smoking during follow up, which would have attenuated the association between smoking and ovarian cancer.

Future large prospective studies with improved histologic information and follow-up data should attempt to reveal the pathogenesis of ovarian cancer in Japan.

In summary, despite some limitations in our prospective study, our results suggest that cigarette smoking is associated with an increased risk of ovarian-cancer incidence in Japanese subjects.

Acknowledgments

The authors sincerely express their appreciation to Dr Kunio Aoki, Professor Emeritus, Nagoya University School of Medicine and the former chairman of the Monbusho ECC, and Dr Haruo Sugano, the former Director, Cancer Institute, Tokyo, who greatly contributed to the initiation of the JACC Study.

The present investigators involved, with the coauthorship of this paper, in the JACC Study and their affiliations are as follows: Dr Akiko Tamakoshi (present chairman of the study group), Nagoya University Graduate School of Medicine; Dr Mitsuru Mori, Sapporo Medical University School of Medicine; Dr Yutaka Motohashi, Akita University School of Medicine; Dr Ichiro Tsuji, Tohoku University Graduate School of Medicine; Dr Yosikazu Nakamura, Jichi Medical School; Dr Hiroyasu Iso, Institute of Community Medicine, University of Tsukuba; Dr Haruo Mikami, Chiba Cancer Center; Dr Yutaka Inaba, Juntendo University School of Medicine; Dr Yoshiharu Hoshiyama, University of Human Arts and Sciences; Dr Hiroshi Suzuki, Niigata University School of Medicine; Dr Hiroyuki Shimizu, Gifu University School of Medicine; Dr Hideaki Toyoshima, Nagoya University Graduate School of Medicine; Dr Kenji Wakai, Aichi Cancer Center Research Institute; Dr Shinkan Tokudome, Nagoya City University Graduate School of Medical Sciences; Dr Yoshinori Ito, Fujita Health University School of Health Sciences; Dr Shuji Hashimoto, Fujita Health University School of Medicine; Dr Shogo Kikuchi, Aichi Medical University School of Medicine; Dr Akio Koizumi, Graduate School of Medicine and Faculty of Medicine, Kyoto University; Dr Takashi Kawamura, Kyoto University Center for Student Health; Dr Yoshiyuki Watanabe, Kyoto Prefectural University of Medicine Graduate School of Medical Science; Dr Tsuneharu Miki, Graduate School of Medical Science, Kyoto Prefectural University of Medicine; Dr Chigusa Date, Faculty of Human Environmental Sciences, Mukogawa Women's University; Dr Kiyomi Sakata, Wakayama Medical University; Dr Takayuki Nose, Tottori University Faculty of Medicine; Dr Norihiko Hayakawa, Research Institute for Radiation Biology and Medicine, Hiroshima University; Dr Takesumi Yoshimura, Fukuoka Institute of Health and Environmental Sciences; Dr Akira Shibata, Kurume University School of Medicine; Dr Naoyuki Okamoto, Kanagawa Cancer Center; Dr Hideo Shio, Moriyama Municipal Hospital; Dr Yoshiyuki Ohno, Asahi Rosai Hospital; Dr Tomoyuki Kitagawa, Cancer Institute of the Japanese Foundation for Cancer Research; Dr Toshio Kuroki, Gifu University; and Dr Kazuo Tajima, Aichi Cancer Center Research Institute.

The past investigators of the study group were listed in reference number 29, except for the following eight members (affiliations are those where they participated in the study): Dr Takashi Shimamoto, Institute of Community Medicine, University of Tsukuba; Dr Heizo Tanaka, Medical Research Institute, Tokyo Medical and Dental University; Dr Shigeru Hisamichi, Tohoku University Graduate School of Medicine; Dr Masahiro Nakao, Kyoto Prefectural University of Medicine; Dr Takaichiro Suzuki, Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases; Dr Tsutomu Hashimoto, Wakayama Medical University; Dr Teruo Ishibashi, Asama General Hospital; and Dr Katsuhiro Fukuda, Kurume University School of Medicine.

The JACC Study has been supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Monbusho) (No. 61010076, 62010074, 63010074,

1010068, 2151065, 3151064, 4151063, 5151069, 6279102 and 11181101).

References

1. Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. *Int J Cancer* 1999; **83**: 18–29.
2. Ioka A, Tsukuma H, Ajiki W, Oshima A. Ovarian cancer incidence and survival by histologic type in Osaka, Japan. *Cancer Sci* 2003; **94**: 292–296.
3. Tamakoshi K, Kondo T, Yatsuya H, Hori Y, Kikkawa F, Toyoshima H. Trends in the mortality (1950–1970) and incidence (1975–1993) of malignant ovarian neoplasm among Japanese women: analyses by age, time, and birth cohort. *Gynecol Oncol* 2001; **83**: 64–71.
4. Mori M, Nishida T, Sugiyama T *et al.* Anthropometric and other risk factors for ovarian cancer in a case–control study. *Jpn J Cancer Res* 1998; **89**: 246–253.
5. Ohida T, Kamal AMM, Takemura S, Sone T, Mochizuki Y, Kawaminami K. Relation between smoking prevalence and various social factors in Japan. *Keio J Med* 2001; **50**: 263–268.
6. Jeffrey AM, Weinstein IB, Jennette KW *et al.* Structures of benzo(α)pyrene nuclei acid adducts formed in human and bovine bronchial explants. *Nature* 1977; **269**: 348–350.
7. Zenzes MT, Puy LA, Bielecki R. Immunodetection of benzo[α]pyrene adducts in ovarian cells of women exposed to cigarette smoke. *Mol Hum Reprod* 1998; **4**: 159–165.
8. Doll R, Gray R, Hafner B, Peto R. Mortality in relation to smoking. 22 year's observations on female British doctors. *Br Med J* 1980; **280**: 967–971.
9. Byers T, Marshall J, Graham S, Mettlin C, Swanson M. A case–control study of dietary and nondietary factors in ovarian cancer. *J Natl Cancer Inst* 1983; **71**: 681–686.
10. Tzonou A, Day NE, Trichopoulos D *et al.* The epidemiology of ovarian cancer in Greece: A case–control study. *Eur J Cancer Clin Oncol* 1984; **20**: 1045–1052.
11. Smith EM, Sowers MF, Burns TL. Effects of smoking on the development of female reproductive cancers. *J Natl Cancer Inst* 1984; **73**: 371–376.
12. Baron JA, Byers T, Greenberg ER, Cummings KM, Swanson M. Cigarette smoking in women with cancers of the breast and reproductive organs. *J Natl Cancer Inst* 1986; 77: 677–680.
13. Franks AL, Lee NC, Kendrick JS, Rubin GL, Layde PM. Cigarette smoking and the risk of epithelial ovarian cancer. *Am J Epidemiol* 1987; **126**: 112–117.
14. Stockwell HG, Lyman GH. Cigarette smoking and the risk of female reproductive cancer. *Am J Obstet Gynecol* 1987; **157**: 35–40.
15. Mori M, Harabuchi I, Miyake H, Casagrande JT, Henderson BE, Ross RK. Reproductive, genetic, and dietary risk factors for ovarian cancer. *Am J Epidemiol* 1988; **128**: 771–777.
16. Whittemore AS, Wu ML, Paffenbarger RS Jr *et al.* Personal and environmental characteristics related to epithelial ovarian cancer: II. Exposures to talcum powder, tobacco, alcohol, and coffee. *Am J Epidemiol* 1988; **128**: 1228–1240.
17. Hartge P, Schiffman MH, Hoover R, McGowan L, Lesher L, Norris HJ. A case–control study of epithelial ovarian cancer. *c* 1989; **161**: 10–16.
18. Tverdal A, Thelle D, Stensvold I, Leren P, Bjartveit K. Mortality in relation to smoking history: 13 years' follow-up of 68 000 Norwegian men and women 35–49 years. *J Clin Epidemiol* 1993; **46**: 475–487.
19. Engeland A, Andersen A, Haldrosen T, Tretli S. Smoking habits and risk of cancers other than lung cancer: 28 years' follow-up of 26 000 Norwegian men and women. *Cancer Causes Control* 1996; **7**: 497–506.
20. Polychronopoulou A, Tzonou A, Hsieh CC *et al.* Reproductive variables, tobacco, ethanol, coffee, and somatometry as risk factors for ovarian cancer. *Int J Cancer* 1993; **55**: 402–407.
21. Nandakumar A, Anantha N, Dhar M *et al.* A case–control investigation on cancer of the ovary in Bangalore, India. *Int J Cancer* 1995; **63**: 361–365.
22. Kuper H, Tistu-Ernstoff L, Harlow BL, Cramer DW. Population based study of coffee, alcohol and tobacco use and risk of ovarian cancer. *Int J Cancer* 2000; **88**: 313–318.
23. Goodman MT, Tung KH. Active and passive tobacco smoking and the risk of borderline and invasive ovarian cancer (United States). *Cancer Causes Control* 2003; **14**: 569–577.
24. Marchbancks PA, Wilson H, Bastos E, Cramer DW, Schildkraut JM, Peterson HB. Cigarette smoking and epithelial ovarian cancer by histologic type. *Obstet Gynecol* 2000; **13**: 467–471.
25. Green A, Purdie D, Bain C, Siskind V, Webb PM. Cigarette smoking and risk of epithelial ovarian cancer (Australia). *Cancer Causes Control* 2000; **12**: 713–719.
26. Modugno F, Ness RB, Cottreau CM. Cigarette smoking and the risk of mucinous and nonmucinous epithelial ovarian cancer. *Epidemiology* 2002; **13**: 467–471.
27. Zhang Y, Coogan PF, Palmer JR, Strom BL, Rosenberg L. Cigarette smoking and increased risk of mucinous epithelial ovarian cancer. *Am J Epidemiol* 2004; **159**: 133–139.
28. Terry PD, Miller AB, Jones JG, Rohan TE. Cigarette smoking and the risk of invasive epithelial ovarian cancer in a prospective cohort study. *Eur J Cancer* 2003; **39**: 1157–1164.
29. Ohno Y, Tamakoshi A, JACC Study Group. Japan Collaborative Cohort Study for evaluation of cancer risk sponsored by Monbusho (JACC Study). *J Epidemiol* 2001; **11**: 144–150.
30. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J. *Cancer Incidence in Five Continents*, Vol. VII. Lyon: International Agency for Research on Cancer, 1997.
31. Cox DR. Regression models and life tables. *J R Stat Soc* 1972; **34**: 187–220.
32. Parazzini F, Franceschi S, La Vecchia C, Fasoli M. The epidemiology of ovarian cancer. *Gynecol Oncol* 1991; **43**: 9–23.
33. Negri E, Franceschi S, Tzonou A *et al.* Pooled analysis of 3 European case–control studies: I. Reproductive factors and risk of epithelial ovarian cancer. *Int J Cancer* 1991; **49**: 50–56.
34. Franceschi S, La Vecchia C, Booth M *et al.* Pooled analysis of 3 European case–control studies: II. Age at menarche and at menopause. *Int J Cancer* 1991; **49**: 57–60.
35. Franceschi S, Parazzini F, Negri E *et al.* Pooled analysis of 3 European case–control studies: III. Oral contraceptive use. *Int J Cancer* 1999; **49**: 61–65.
36. Frank TS, Manley SA, Olopade OI *et al.* Sequence analysis of *BRCA1* and *BRCA2*: Correlation of mutations with family history and ovarian cancer risk. *J Clin Oncol* 1998; **16**: 2417–2425.
37. Rodriguez C, Calle EE, Fakharabadi-Shokoohi D, Jacobs EJ, Thun MJ. Body mass index, height, and the risk of ovarian