Genetic polymorphism of cholesterol 7α-hydroxylase (CYP7A1) and colorectal adenomas: Self Defense Forces Health Study

Shinji Tabata,[1,2] Guang Yin,[1] Shinsaku Ogawa,[2] Keizo Yamaguchi,[1,3] Masamichi Mineshita[3] and Suminori Kono[1,4]

[1]Department of Preventive Medicine, Faculty of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582;
[2]Self Defense Forces Fukuoka Hospital, 1-61 Kokurahigashi, Kasuga-shi 816-0826; and
[3]Self Defense Forces Kumamoto Hospital, 15-1 Higashihon-machi, Kumamoto 862-0902, Japan

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Bile acids have long been implicated in colorectal carcinogenesis, but epidemiological evidence is limited. Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme producing bile acids from cholesterol. A recent case-control study showed a decreased risk of proximal colon cancer associated with the *CC* genotype of the *CYP7A1 A-203C* polymorphism. The present study examined the relationship between the *CYP7A1 A-203C* polymorphism and colorectal adenoma, which is a well-established precursor lesion of colorectal cancer. The study subjects comprised 446 cases of colorectal adenomas and 914 controls of normal total colonoscopy among men receiving a preretirement health examination at two hospitals of the Self Defense Forces (SDF). The *CYP7A1* genotype was determined by the polymerase chain reaction–restriction fragment length polymorphism method. Statistical adjustment was made for age, hospital, rank in the SDF, smoking, alcohol use, body mass index, physical activity and parental history of colorectal cancer. The *CYP7A1* polymorphism was not measurably related to the overall risk of colorectal adenomas. However, the *CC* genotype was associated with a decreased risk of proximal colon adenomas, but not of distal colon and rectal adenomas. Adjusted odds ratios of proximal colon adenomas (95% confidence intervals) for the *AC* and *CC* genotype versus *AA* genotype were 0.82 (0.54–1.24) and 0.56 (0.34–0.95), respectively. The findings add to evidence for the role of bile acids in colorectal carcinogenesis. The *CC* genotype of the *CYP7A1 A-203C* polymorphism probably renders lower activity of the enzyme synthesizing bile acids. (*Cancer Sci* 2006; 97: 406–410)

Bile acids have long been implicated in colorectal carcinogenesis.[1] Primary bile acids, such as cholic and chenodeoxycholic acids, are produced from cholesterol in the liver, and more than 95% of those passing through the ileum are reabsorbed and return to the liver. Secondary bile acids, mainly deoxycholic and lithocholic acids, are formed by the anaerobic bacterial flora in the large bowel from primary bile acids that escape absorption in the ileum.[1] Secondary bile acids are known to promote colorectal carcinogenesis in animals,[2,3] and molecular mechanisms have been found regarding the effect of bile acids promoting colorectal carcinogenesis.[4,5] However, epidemiological evidence remains elusive regarding the relationship between bile acids and colorectal cancer. Fecal concentrations of secondary bile acids are higher in populations at high risk of colorectal cancer.[6,7] Several case-control studies have shown that fecal or serum levels of secondary bile acids are higher in patients with colorectal cancer or adenoma than in those without these lesions.[8–11] A high ratio of serum deoxycholic acid to cholic acid tended to be associated with an increased risk of colorectal cancer in a prospective study.[12]

Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme that converts cholesterol into cholesterol 7α-hydroxycholesterol in the first step of the classical pathway of bile acid synthesis.[13] Overexpression of cholesterol 7α-hydroxylase activity in hamsters results in a dose-dependent decrease in plasma cholesterol concentrations.[14] In mice deficient in cholesterol 7α-hydroxylase, fecal excretion of bile acids as well as the bile acid pool is decreased.[15] In humans, a polymorphism in the promoter region of the *CYP7A1* gene (*CYP7A1 A-203C*) is associated with plasma concentrations of total or low density lipoprotein (LDL) cholesterol, suggesting lower enzyme activities in those with the *-203CC* genotype,[16,17] although this finding was not replicated in another study.[18] The *CYP7A1 A-203C* polymorphism may modulate transcription of the *CYP7A1* gene and consequently the rate of bile acid synthesis.

In a case-control study of colorectal cancer,[19] individuals with the *CYP7A1-203CC* genotype were associated with a lower risk of proximal colon cancer, but not of distal colon and rectal cancer. In the present study, we examined the relationship between the *CYP7A1 A-203C* polymorphism and colorectal adenomas, which is a well-established precursor lesion of colorectal cancer.[20,21]

Materials and Methods

Subjects

Study subjects were male self-defense officials who received a preretirement health examination at the Self Defense

[4]To whom correspondence should be addressed.
E-mail: skono@phealth.med.kyushu-u.ac.jp

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Forces (SDF) Fukuoka or Kumamoto Hospital from January 1997 to March 2001. The preretirement health examination is a program offering comprehensive medical examination for those retiring from the SDF. Details of the health examination and a lifestyle survey have been described elsewhere.[22,23] In addition to blood samples for routine use in the health examination, a sample of 7 mL fasting venous blood was obtained for the purpose of medical research with written informed consent. The study was approved by the ethical committee of Kyushu University.

The study subjects comprised 446 cases of colorectal adenomas and 914 controls of normal total colonoscopy. In the consecutive series of 2454 men, except for eight who refused to participate in the survey, 2377 (97%) underwent total or partial colonoscopy. We excluded 242 men with a prior history of colectomy ($n = 17$), colorectal polypectomy ($n = 212$), malignant neoplasm ($n = 27$) or inflammatory bowel disease ($n = 1$). Numbers of men according to the macroscopic findings of colonoscopy were: normal 1073, colorectal polyps 938, carcinoma one and other non-polyp benign lesions 123. Of the 938 with colorectal polyps, 461 were found to have at least one histologically confirmed adenoma without *in situ* or invasive carcinoma. Of the 1073 men with a normal result, 949 underwent total colonoscopy and were used as controls subjects. Of these cases and controls, DNA was not available for six cases and 13 controls, and genotyping was unsuccessful with nine cases and 22 controls, thus the remaining 446 cases and 914 controls were used in the analysis.

Numbers of adenoma cases by subsite were: proximal colon only 146, distal colon only 180, rectum only 42, and multiple sites 78. Proximal colon included cecum, ascending colon and transverse colon. Cases of adenoma with sizes of <5 mm, 5–10 mm and ≥10 mm (the largest size for multiple adenomas) numbered 260, 149 and 37, respectively.

Genotyping

DNA was extracted from buffy coat stored at −80°C by using a commercial kit (QIAGEN, Hilden, German). The *CYP7A1* genotype was determined by the polymerase chain reaction (PCR)–restriction fragment length polymorphism method, as described previously by Han *et al.*,[18] using primers 5′-AATGT TTTTC CCAGT TCTCT TTC-3′ (sense) and 5′-AATTA GCCAT TTGTT CATTC TATTA G-3′ (antisense). The PCR was carried out in a reaction mixture of 10 μL containing 0.5 IU of *Taq* and 1 μL of template DNA with a concentration of approximately 50–150 ng/μL. After the initial denaturation at 94°C for 4 min, 30 cycles of PCR were carried out for 30 s at 94°C, 30 s at 53°C and 30 s at 72°C, with a final extension at 72°C for 7 min. The PCR product of 393 bp was digested with 10 IU of *Bsa*I in a reaction mixture of 20 μL for 3 h at 50°C. The digestion resulted in fragments of 300 and 93 bp for the *A* allele, and fragments of 261, 93 and 39 bp for the *C* allele. The digested fragments were electrophoresed on a 3% agarose gel (NuSieve GTG), and visualized using ethidium bromide. The polymorphism was described as A-204C by Couture *et al.*,[17] but we confirmed, by sequencing, the actual site of the polymorphism at 203 bp upstream of the transcription start site. Genotyping was carried out with case and control status unknown.

Lifestyle factors

Height and bodyweight were recorded, and body mass index (kg/m^2) was calculated. Body mass index was categorized by using the 30th, 60th and 90th percentiles in the distribution of the controls. A self-administered questionnaire ascertained smoking habit, alcohol use, leisure-time physical activity and other lifestyle factors. Lifetime exposure to cigarette smoking was expressed as cigarette-years (the average number of cigarettes smoked per day multiplied by years of smoking), and classified into four levels of 0, 1–399, 400–799 and ≥800 cigarette-years. Alcohol drinking was defined as having drunk alcoholic beverages at least once per week for 1 year or longer, and former alcohol use was separated from lifetime non-use of alcohol. The amount of alcohol consumed per day was calculated for current alcohol drinkers on the basis of consumption frequencies and amounts per occasion of five types of alcoholic beverages on average in the past year. Alcohol use was categorized into never, former and current use with consumption of <30, 30–59, or ≥60 mL of ethanol per day.

Questions on leisure-time physical activity were slightly changed in April 1999. In the earlier version, subjects were first asked about the frequency of regular participation in exercise and sport during leisure time on average in the past year using a closed-ended question (none, 1–2, 3–4, 5–6 times per week and daily). If the subjects participated in recreational physical activity at least once per week, they reported type of activity and time spent per occasion regarding at most three types of regular exercise. In the revised questionnaire, the subjects were first asked whether they participated in recreational activity regularly (one or more times per week) in the past year. Those with a regular participation reported at most three types of physical activities together with frequency per week and time spent per occasion for each activity. Type of physical activity was classified into light, moderate, heavy or very heavy activity in terms of metabolic equivalent (MET).[24] The time spent in recreational exercise was multiplied by the corresponding MET value (light 2, moderate 4, heavy 6 and very heavy 8) to yield a MET-hour score per week. Individuals were classified into four groups with the quartiles in the control group as cut-off points. Parental history of colorectal cancer was also elicited.

Statistical analysis

The association between the *CYP7A1* polymorphism and colorectal adenomas was assessed by means of adjusted odds ratio (OR) and 95% confidence interval (CI), which were derived from multiple logistic regression analysis. Statistical adjustment was made for age (continuous variable), hospital, rank of the SDF (low, intermediate and high), cigarette smoking, alcohol use, body mass index, physical activity and parental colorectal cancer. Interactions of the *CYP7A1* polymorphism with selected lifestyle factors were evaluated by the likelihood ratio test. Statistical significance was declared if a two-sided *P*-value was less than 0.05 or if the 95% CI did not include unity. All statistical analyses were carried out using SAS version 8.2 (SAS Institute, Cary, NC, USA).

Results

The characteristics of colorectal adenoma cases and controls are summarized in Table 1. The age ranges were 50–57 years in the cases and 47–59 years in the controls, but the mean ages were identical in the two groups. Cigarette and alcohol consumption were greater in the cases than in the controls, and body mass index was also greater in the former group. Men with low physical activity and those with parental history of colorectal cancer were slightly more frequent in the cases.

In the cases, proportions of the *AA*, *AC* and *CC* genotypes were 26, 50 and 25%, respectively (Table 2). The corresponding proportions in the control group were 24, 49, and 27%, respectively. These frequencies were in agreement with the Hardy–Weinberg equilibrium ($P = 0.98$ for cases and $P = 0.95$ for controls). Overall, the *CYP7A1* polymorphism was not measurably associated with colorectal adenomas, although the OR for the *CC* genotype versus *AA* genotype was slightly lower than unity.

When the association with the *CYP7A1* polymorphism was examined for adenomas of the proximal colon, distal colon and rectum separately, the OR of proximal colon adenomas showed a statistically significant decrease among individuals with the *CC* genotype compared with those with the *AA* genotype. The *CYP7A1* polymorphism was unrelated to adenomas at the distal colon and rectum (Table 3).

Adjusted OR of small colorectal adenomas (<5 mm) for the *AC* and *CC* genotypes versus *AA* genotype were 1.11 (95% CI 0.78–1.58) and 0.87 (95% CI 0.58–1.32), respectively. The corresponding OR of large colorectal adenomas (≥5 mm) were 0.74 (95% CI 0.50–1.09) and 0.86 (95% CI 0.55–1.33), respectively. Adjusted OR of small proximal colon adenomas ($n = 86$) were 1.09 (95% CI 0.64–1.86) for the *AC* genotype and 0.41 (95% CI 0.20–0.87) for the *CC* genotype compared with the *AA* genotype, and adjusted OR of large proximal colon adenomas ($n = 60$) for the *AC* and *CC* genotypes were 0.52 (95% CI 0.28–0.98) and 0.71 (95% CI 0.36–1.41), respectively.

Finally, we explored interactions between the *CYP7A1* polymorphism (three genotypes) and lifestyle factors for the risk of proximal colon adenomas with two categories used for smoking (<400 cigarette-years vs ≥400 cigarette-years), alcohol use (<30 mL/day including past alcohol use versus ≥30 mL/day), body mass index (<25.0 kg/m^2 vs ≥25.0 kg/m^2), and physical activity (<15 MET-hours/week vs ≥15 MET-hours/week). There was no measurable interaction for any of the lifestyle factors under study: smoking ($P = 0.38$), alcohol use ($P = 0.93$), body mass index ($P = 0.62$) and physical activity ($P = 0.39$).

Table 1. Characteristics of colorectal adenoma cases and controls

Variable	Cases (n = 446)	Controls (n = 914)	P-value†
Age (years), mean (SD)	52.4 (0.83)	52.4 (0.92)	0.82
Hospital (%)			
Fukuoka	71.1	68.4	0.31
Kumamoto	28.9	31.6	
Rank (%)			
Low	60.3	62.3	0.68
Intermediate	25.3	23.2	
High	14.3	14.6	
Cigarette-years (%)			
0	21.3	34.1	<0.0001
1–399	14.3	19.1	
400–799	44.8	33.8	
≥800	19.5	12.9	
Alcohol use (%)			
None	11.2	14.6	0.0002
Past	2.9	3.1	
<30 (mL/day)	21.5	31.0	
30–59	34.1	28.8	
≥60	30.3	22.6	
BMI (kg/m^2), mean (SD)	24.1 (2.79)	23.7 (2.46)	0.007
MET-hours/week (%)			
<5	28.5	23.9	0.32
5–14	23.8	25.9	
15–24	24.2	24.9	
≥25	23.5	25.3	
Parental CRC (%)			
Negative‡	95.3	96.6	0.23
Positive	4.7	3.4	

†Based on t-test or χ^2- test. ‡Including two cases and two controls with parental colorectal cancer (CRC) unknown. BMI, body mass index; MET, metabolic equivalent; SD, standard deviation.

Table 2. *CYP7A1 A-203C* polymorphism and risk of colorectal adenoma

Genotype	Cases		Controls		Crude OR	Adjusted OR (95% CI)†
	n	%	n	%		
AA	115	25.8	219	24.0	1.00	1.00 (referent)
AC	221	49.6	452	49.5	0.93	0.93 (0.70–1.24)
CC	110	24.7	243	26.6	0.86	0.87 (0.62–1.20)

†Adjusted for age, hospital, rank, body mass index, cigarette smoking, alcohol use, physical activity and parental history of colorectal cancer. CI, confidence interval; OR, odds ratio.

Table 3. *CYP7A1 A-203C* polymorphism and risk of colorectal adenoma by location

Genotype	Proximal colon		Distal colon		Rectum	
	n†	OR (95% CI)‡	n†	OR (95% CI)‡	n†	OR (95% CI)‡
AA	44/219	1.00 (referent)	42/219	1.00 (referent)	10/219	1.00 (referent)
AC	73/452	0.82 (0.54–1.24)	86/452	1.01 (0.67–1.52)	23/452	1.07 (0.49–2.35)
CC	29/243	0.56 (0.34–0.95)	52/243	1.14 (0.72–1.80)	9/243	0.89 (0.35–2.31)

†Numbers of cases/controls. ‡Adjusted for age, hospital, rank, body mass index, cigarette smoking, alcohol use, physical activity and parental history of colorectal cancer. CI, confidence interval; OR, odds ratio.

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Discussion

The present study was the first that examined the relationship between a functional *CYP7A1* polymorphism and colorectal adenomas, and showed a statistically significant decrease in the risk of proximal colon adenomas, but not of distal colon and rectal adenomas, associated with the *CC* genotype of the *CYP7A1 A-203C* polymorphism. The findings are consistent with the recent observation in Japan that the *CC* genotype of this polymorphism is related to a decreased risk of proximal colon cancer exclusively,[19] and provide further evidence for the role of bile acids in colorectal carcinogenesis. Although the relationship between the *CYP7A1 A-203C* polymorphism and fecal or serum bile acids has not been investigated directly, individuals with the *CC* genotype probably have lower exposure to bile acids due to decreased activity of 7α-hydroxylase.[25] However, further studies are required to assess functionality of the *CYP7A1 A-203C* polymorphism. It is possible that this polymorphism does not directly modulate the gene expression. Another linked polymorphism in the *CYP7A1* gene may be of functional relevance to bile acid production.

The *CYP7A1 A-203C* polymorphism was first identified as a genetic determinant of plasma total and LDL cholesterol levels through sequential steps of sibling-pair linkage analysis, DNA sequencing and association studies within families and in unrelated individuals in the USA.[16] In that study, the *CC* homozygotes had higher levels of total and LDL cholesterol in plasma than the *AA* homozygotes. A supportive finding was reported for men but not for women in the Framingham Offspring Study.[17] In contrast, a study of Micronesian islanders showed no association between the *CYP7A1 A-203C* polymorphism and serum apolipoprotein B, a surrogate of LDL cholesterol.[18] In this regard, it is of interest whether serum cholesterol levels differ by the polymorphism in the present study subjects. A preliminary analysis showed no measurable variation in serum total cholesterol levels according to the *CYP7A1 A-203C* polymorphism; in the whole subjects excluding men under lipid-lowering medication (20 cases and 41 controls), adjusted means (and standard errors) of fasting total cholesterol concentrations for the *AA*, *AC* and *CC* genotypes were 203.1 (1.9), 202.5 (1.3) and 203.2 (1.8) mg/dL, respectively, after controlling for age, hospital, rank, smoking, alcohol use, body mass index and physical activity. This lack of association is not necessarily surprising because many other factors, including dietary fat, influence the between-individual variation in serum total and LDL cholesterol. Different dietary habits may have masked the genotype–lipid association in individuals.

Several lines of epidemiological evidence have suggested that bile acids may increase the risk of proximal colon cancer selectively. Patients with the gallbladder removed have an increased risk of proximal colon cancer.[26–28] Cholecystectomy results in increased fecal excretion of secondary bile acids due to an increase in the bile acid pool in the enterohepatic circulation and increased degradation of primary bile acids.[29,30]

Low concentrations of serum total or LDL cholesterol have been related to increased risk of colon cancer in many prospective studies.[31] Whereas this inverse association has generally been ascribed to the effect of preclinical cancer existing at the baseline,[31] an increased risk of proximal colon cancer associated with low cholesterol levels persisted 10–20 years later in a prospective study of Japanese people in Hawaii.[32] In a case-control study, lower levels of serum total and LDL cholesterol were observed in cases of proximal colon cancer alone than in controls.[33] In that study, individuals with the *E4* allele for apolipoprotein E, in whom bile acid synthesis is decreased,[34] had a lower risk of proximal colon cancer and adenomas.

The present study had methodological advantages in that colonoscopy was carried out non-selectively in a defined population and in that the absence of polyp lesions was confirmed in the control subjects by total colonoscopy. The study subjects were not representative of Japanese men in the general population, but selection was unlikely to exist with regard to the genetic polymorphism under study. The allele frequency of *CYP7A1-203C* (51%) in the control subjects in the present study was quite similar to that observed in a random sample of adult residents in an area in Japan (50%),[19] whereas these values are slightly greater than those reported in Caucasians (approximately 40%).[16,17] Lack of dietary information was a weakness in the present study. High-fat diet increases bile acid excretion,[35,36] and thus the interaction between the *CYP7A1 A-203C* polymorphism and fat intake deserves further studies. Another weakness was the small number in the subgroup analysis, especially regarding size-specific risks of proximal colon adenomas. Decreased risk of proximal colon adenomas associated with the *CC* genotype did not seem to differ by size, but the observed OR were variable. Moreover, it was uncertain whether the risk of proximal colon adenomas was decreased in individuals with the heterozygous *AC* genotype. Larger studies are required to address these questions conclusively.

In conclusion, a case-control study demonstrated a decreased risk of proximal colon adenoma in individuals with the *CC* genotype of the *CYP7A1 A-203C* polymorphism, which probably renders lower activity of the enzyme synthesizing bile acids. The findings add to evidence for the role of bile acids in colorectal carcinogenesis.

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References

1 Nagengast FM, Grubben MJ, van Munster IP. Role of bile acids in colorectal carcinogenesis. *Eur J Cancer* 1995; **31A**: 1067–70.

2 Narisawa T, Magadia NE, Weisburger JH, Wynder EL. Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in rats. *J Natl Cancer Inst* 1974; **53**: 1093–7.

3 Reddy BS, Narasawa T, Weisburger JH, Wynder EL. Promoting effect of sodium deoxycholate on colon adenocarcinomas in germfree rats. *J Natl Cancer Inst* 1976; **56**: 441–2.
4 Qiao D, Chen W, Stratagoules ED, Martinez JD. Bile acid-induced activation of activator protein-1 requires both extracellular signal-regulated kinase and protein kinase C signaling. *J Biol Chem* 2000; **275**: 15 090–8.
5 Milovic V, Teller IC, Faust D, Caspary WF, Stein J. Effects of deoxycholate on human colon cancer cells: apoptosis or proliferation. *Eur J Clin Invest* 2002; **32**: 29–34.
6 Reddy BS, Wynder EL. Large-bowel carcinogenesis: fecal constituents of populations with diverse incidence rates of colon cancer. *J Natl Cancer Inst* 1973; **50**: 1437–42.
7 Wynder EL, Reddy BS. Metabolic epidemiology of colorectal cancer. *Cancer* 1974; **34**: S801–6.
8 Hill MJ, Drasar BS, Williams RE *et al*. Faecal bile-acids and clostridia in patients with cancer of the large bowel. *Lancet* 1975; **i**: 535–9.
9 Reddy BS, Wynder EL. Metabolic epidemiology of colon cancer. Fecal bile acids and neutral sterols in colon cancer patients and patients with adenomatous polyps. *Cancer* 1977; **39**: 2533–9.
10 Bayerdörffer E, Mannes GA, Richter WO *et al*. Increased serum deoxycholic acid levels in men with colorectal adenomas. *Gastroenterology* 1993; **104**: 145–51.
11 Bayerdörffer E, Mannes GA, Ochsenkühn T, Dirschedl P, Wiebecke B, Paumgartner G. Unconjugated secondary bile acids in the serum of patients with colorectal adenomas. *Gut* 1995; **36**: 268–73.
12 Costarelli V, Key TJ, Appleby PN, Allen DS, Fentiman IS, Sanders TA. A prospective study of serum bile acid concentrations and colorectal cancer risk in post-menopausal women on the island of Guernsey. *Br J Cancer* 2002; **86**: 174–4.
13 Schwaltz M, Lund EG, Russell DW. Two 7 alpha-hydroxylase enzymes in bile acid biosynthesis. *Curr Opin Lipidol* 1998; **9**: 113–18.
14 Spady DK, Cuthbert JA, Willard MN, Meidell RS. Adenovirus-mediated transfer of a gene encoding cholesterol 7α-hydroxylase into hamsters increases hepatic enzyme activity and reduces plasma total and low density lipoprotein cholesterol. *J Clin Invest* 1995; **96**: 700–9.
15 Schwarz M, Russell DW, Dietschy JM, Turley SD. Marked reduction in bile acid synthesis in cholesterol 7α-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. *J Lipid Res* 1998; **39**: 1833–43.
16 Wang J, Freeman DJ, Grundy SM, Levine DM, Guerra R, Cohen JC. Linkage between cholesterol 7α-hydroxylase and high plasma low-density lipoprotein cholesterol concentrations. *J Clin Invest* 1998; **101**: 1283–91.
17 Couture P, Otvos JD, Cupples LA, Wilson PW, Schaefer EJ, Ordovas JM. Association of the A-204C polymorphism in the cholesterol 7α-hydroxylase gene with variations in plasma low density lipoprotein cholesterol levels in the Framingham Offspring Study. *J Lipid Res* 1999; **40**: 1883–9.
18 Han Z, Heath SC, Shmulewitz D *et al*. Candidate genes involved in cardiovascular risk factors by a family-based association study on the island of Kosrae, Federated States of Micronesia. *Am J Med Genet* 2002; **110**: 234–42.
19 Hagiwara T, Kono S, Yin G *et al*. Genetic polymorphism in cytochrome P450 (CYP) 7A1 and risk of colorectal cancer: The Fukuoka Colorectal Cancer Study. *Cancer Res* 2005; **65**: 2979–82.
20 Hill MJ, Morson BC, Bussey HJ. Aetiology of adenoma–carcinoma sequence in large bowel. *Lancet* 1978; **i**: 245–7.
21 Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759–67.
22 Kono S, Handa K, Hayabuchi H *et al*. Obesity, weight gain and risk of colon adenomas in Japanese men. *Jpn J Cancer Res* 1999; **90**: 805–11.
23 Toyomura K, Yamaguchi K, Kawamoto H *et al*. Relation of cigarette smoking and alcohol use to colorectal adenomas by subsite: the self-defense forces health study. *Cancer Sci* 2004; **95**: 72–6.
24 Ainsworth BE, Haskell WL, Leon AS *et al*. Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* 1993; **25**: 71–80.
25 Cohen JC. Contribution of cholesterol 7α-hydroxylase to the regulation of lipoprotein metabolism. *Curr Opin Lipidol* 1999; **10**: 303–7.
26 Giovannucci E, Colditz GA, Stampfer MJ. A meta-analysis of cholecystectomy and risk of colorectal cancer. *Gastroenterology* 1993; **105**: 130–41.
27 Reid FD, Mercer PM, Harrison M, Bates T. Cholecystectomy as a risk factor for colorectal cancer: a meta-analysis. *Scand J Gastroenterol* 1996; **31**: 160–9.
28 Lagergren J, Ye W, Ekbom A. Intestinal cancer after cholecystectomy: is bile involved in carcinogenesis? *Gastroenterology* 2001; **121**: 542–7.
29 Pomare EW, Heaton KW. The effect of cholecystectomy on bile salt metabolism. *Gut* 1973; **14**: 753–62.
30 Hepner GW, Hofmann AF, Malagelada JR, Szczepanik PA, Klein PD. Increased bacterial degradation of bile acids in cholecystectomized patients. *Gastroenterology* 1974; **66**: 556–64.
31 Law MR, Thompson SG. Low serum cholesterol and the risk of cancer: an analysis of the published prospective studies. *Cancer Causes Control* 1991; **2**: 253–61.
32 Nomura AM, Stemmermann GN, Chyou PH. Prospective study of serum cholesterol levels and large-bowel cancer. *J Natl Cancer Inst* 1991; **83**: 1403–7.
33 Kervinen K, Södervik H, Mäkelä J *et al*. Is the development of adenoma and carcinoma in proximal colon related to apolipoprotein E phenotype? *Gastroenterology* 1996; **110**: 1785–90.
34 Miettinen TA. Impact of ApoE phenotype on the regulation of cholesterol metabolism. *Ann Med* 1991; **23**: 181–6.
35 Reddy BS. Diet and excretion of bile acids. *Cancer Res* 1981; **41**: 3766–8.
36 Reddy BS. Dietary fat and colon cancer: animal model studies. *Lipids* 1992; **27**: 807–13.

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