III. 考　察

本邦で認められているPPI＋アモキシシリン＋クラリスロマイシンによる *H. pylori* 除菌治療の成功率は約80%であり，残りの症例は感染が持続する。2次除菌を行っても10%程度は感染が持続する[5]。治療効果を上げるには薬物の投与量，種類を増やすことが必要であるが，逆に副作用も増加することになる。そこで副作用を増加させずに治療効果を上げることを目的として，probioticsなどの併用が報告されている[6,7]。

牛乳の成分ではラクトフェリンが *H. pylori* の発育を阻害すること[8,9]，κカゼインが *H. pylori* の胃粘膜への接着を阻害すること[10,11]が報告されている。しかし，今回の検討では牛乳摂取群と非摂取群で，*H. pylori* 感染率に差はなかった。喫煙および飲酒期間が長い高齢者において，牛乳摂取による萎縮性胃炎の進展防止効果が期待されたが，萎縮性胃炎の程度を反映するとされる[12]血清PG 1/2比に有意差はみられなかった。1日200 cc程度の牛乳摂取では，*H. pylori* 感染および萎縮性胃炎の進展に及ぼす影響は少なかった。

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消化器科，39(2)：154-158，2004

特集I 呼気テストによる消化器疾患の病態診断

呼気試験による慢性胃炎の解析*

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Key Words：atrophic gastritis, breath test, bacterial overgrowth, fermentation

はじめに

消化管内腔には多数の気体が存在し，その一部は吸収されて血液循環を介し，肺へ到達して呼気中へ排出される．呼気中に出現するガス成分は200種類以上にのぼる[1]が，消化管内腔で同定されている気体は少なく[2]，ごく一部であると言わざるをえない．

消化管内腔のガスの起源をまとめると，図1のように大きく分類することができる．嚥下とともに内腔へ流入するとされる窒素(N_2)および酸素(O_2)，腸内細菌や口腔内細菌による発酵反応により生成される水素(H_2)，メタン(CH_4)，二酸化炭素(CO_2)，酸化還元反応によるCO_2，一酸化窒素(NO)，酵素反応によるCO_2，アンモニア(NH_3)，一酸化炭素(CO)などがあげられる．これらの気体が消化器疾患あるいは腹部症状の発症に関与しているかは明らかではない．慢性胃炎はdyspepsia症状を有する場合があり，今回，慢性胃炎症例ついて，呼気および消化管内腔の気体を解析し，慢性胃炎をとりまく消化管環境の変化について述べる．

##萎縮性胃炎と消化管bacterial overgrowth

Helicobacter pylori(*H. pylori*)が発見される前から，酸分泌の低下した症例では多くの細菌が胃内に存在することが報告されている[3][4]．胃酸は胃および小腸での細菌増殖に防御的に作用しているため，萎縮性胃炎では酸分泌が低下し，上部消化管においてbacterial overgrowthが起こっている可能性が高い[5][6]．増殖した細菌は嫌気的に発酵反応により生命維持に必要なエネルギーを獲得する場合が多い．いかなる発酵反応においても水素，二酸化炭素が生成されるため，呼気中水素ガスは全消化管の発酵反応の指標と考えられ，空腹時の呼気中水素ガス濃度からもある程度，腸管でのbacterial overgrowthの有無を推定できる[7]．しかし，呼気中水素ガスが消化管のどの部位から発生したのか同定することはできない．

ラクチュロース負荷による発酵部位の同定(図2)

大腸内視鏡検査を行った連続50例(平均年齢63.6±10.1歳，男女比26/24)を対象とし，腸管洗浄液ニフレック®2,000mlにラクチュロース12gを混入して2時間で飲用させ，15分間隔で4時間後まで呼気を採取し，呼気中水素濃度を呼気分析装

* Breath tests for the study on chronic gastritis.

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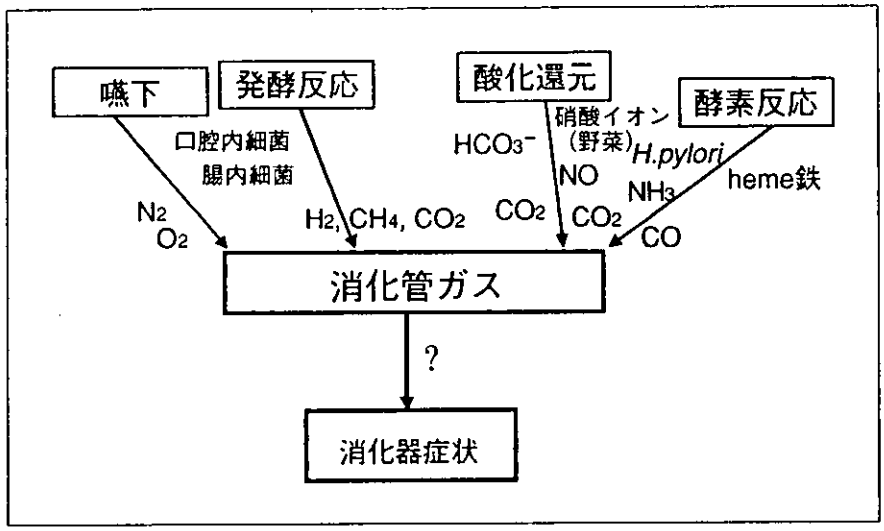

図1 消化管ガスの起源

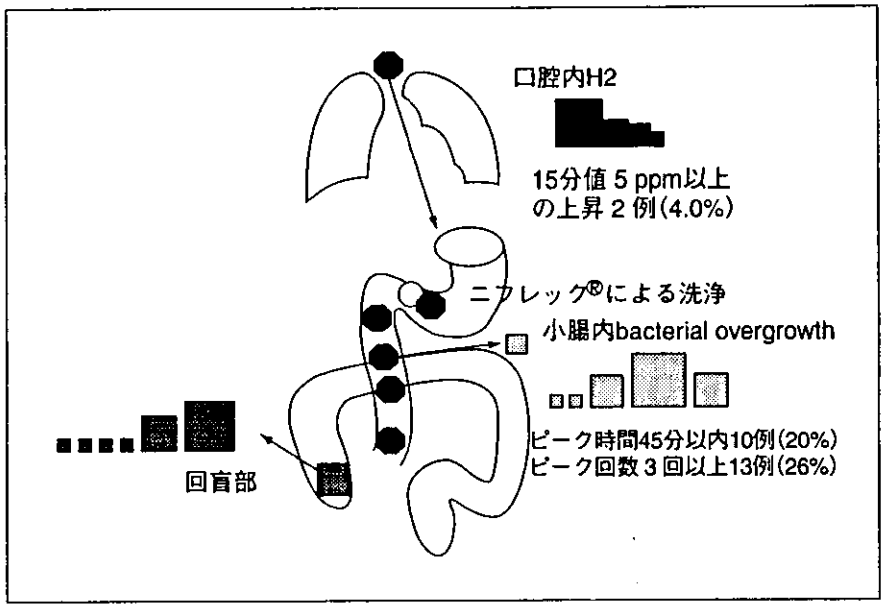

図2 ラクチュロース飲用後の呼気中水素ガス上昇

置TGA-2000(テラメックス社)で測定した[8]．

呼気中水素ガス濃度がPEG飲用後5 ppm以上上昇した時間は，45分以内が8例(16%)あり，これらの症例では回盲部にPEGが到達する前に水素ガスが発生したものと考えられ，小腸でのbacterial overgrowthが示唆された．飲用後15分以内に呼気中水素ガスが5 ppm以上上昇した症例が2例(4 %)存在し，口腔内細菌による水素産生が疑われた．また，呼気中水素ガス濃度のピークが3回以上出現した症例が13例(26%)あり，小腸でのbacterial overgrowthと考えられた．さらに発酵部位同定が困難な症例には，^{13}C-acetate呼気試験を同時に施行すると，胃排出時間との関連から同定が容易となる[9]．

内視鏡を用いた発酵部位の同定

生体での水素ガスは発酵反応が唯一の起源であると考えられている[10]．すなわち，胃内腔に水素発酵菌が存在する場合，食餌中の炭水化物を気質として発酵が生じ，水素ガスが生成されることになる．同様に上部小腸でbacterial overgrowthが起こった場合，十二指腸内腔の水素ガスが上昇することが推測される．そこで，内視鏡を用いて消化管内腔の気体を採取し，水素ガス濃度を測定し，発酵部位の同定を試みた．

萎縮性胃炎の指標を内視鏡的萎縮境界で示す

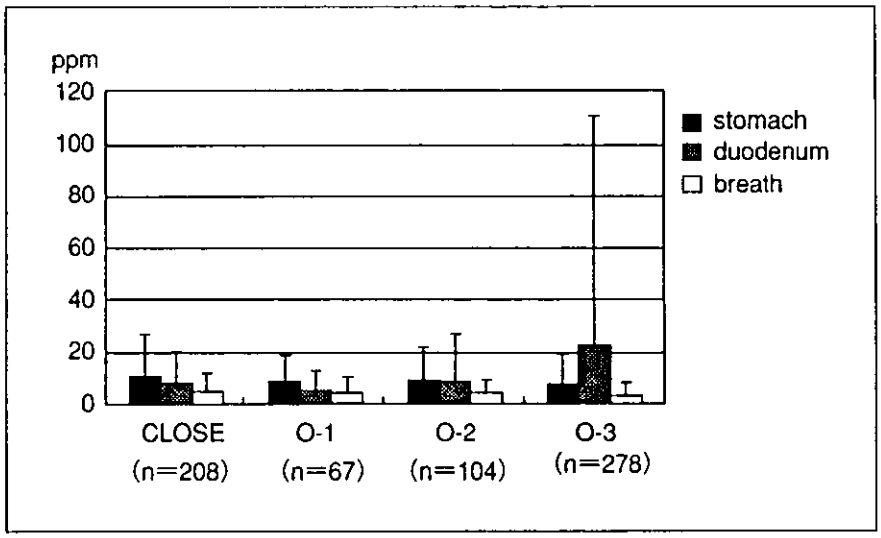

図3　萎縮性胃炎の進展と消化管内腔・呼気中水素ガスの変化

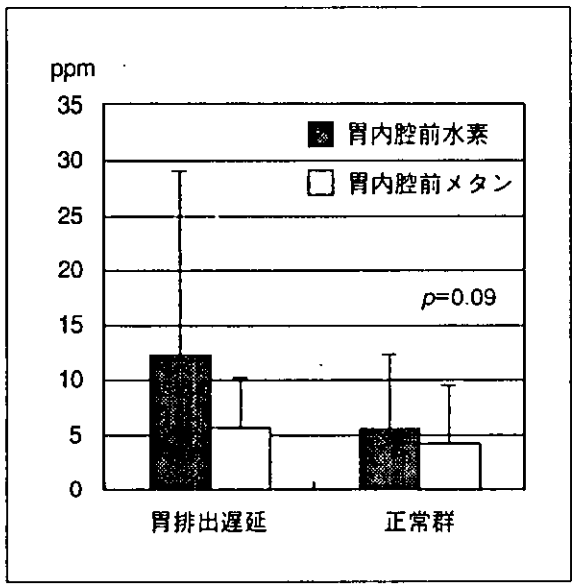

図4　胃排出速度と胃内腔水素・メタンガス濃度

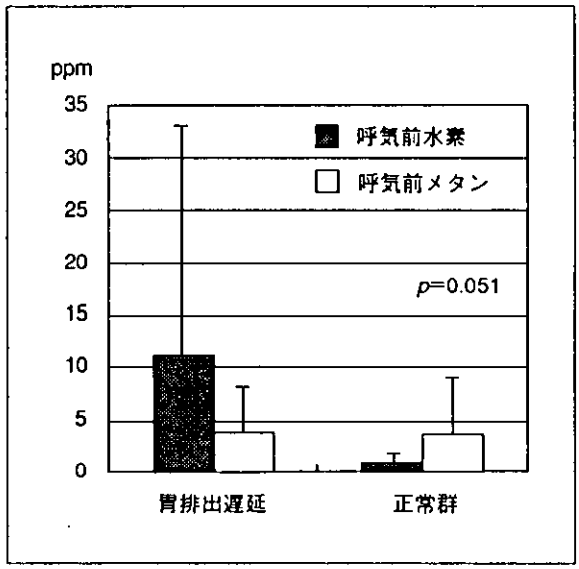

図5　胃排出速度と呼気中水素・メタンガス濃度

と，図3のように萎縮の進展したO-3型の症例で十二指腸内腔の水素ガスが高値であった．胃内腔および呼気中水素ガス濃度は萎縮の程度と差はなかった．以上から，萎縮性胃炎においては，胃内腔よりも小腸でのbacterial overgrowthが高率に起こっていると考えられる．

消化管運動とbacterial overgrowth

胃粘膜萎縮性変化のほかに，細菌と発酵気質(食餌)との接触時間が延長した場合，水素ガス発生が亢進すると予想される．^{13}C-acetate呼気試験にて胃排出遅延群と正常群に分類し，胃内腔の水素・メタンガス濃度を比較すると，図4，図5のように胃内腔および呼気中水素ガス濃度が胃排出遅延群で高値であった．すなわち，胃排出が遅延した場合，胃内腔での発酵反応が亢進するのみならず，全消化管において亢進していると考えられた．

食後の消化管ガス発生

消化管内腔におけるガス発生は，細菌が食餌中の発酵基質を発酵に利用することによる．そこで空腹時に消化管ガスが少ない症例でも，食後に大量のガスが発生して腹部膨満感を生じる場合が予想される．そこで，胃内腔に基質を供給し，水素ガスがどの程度発生するかを検討した[11]．

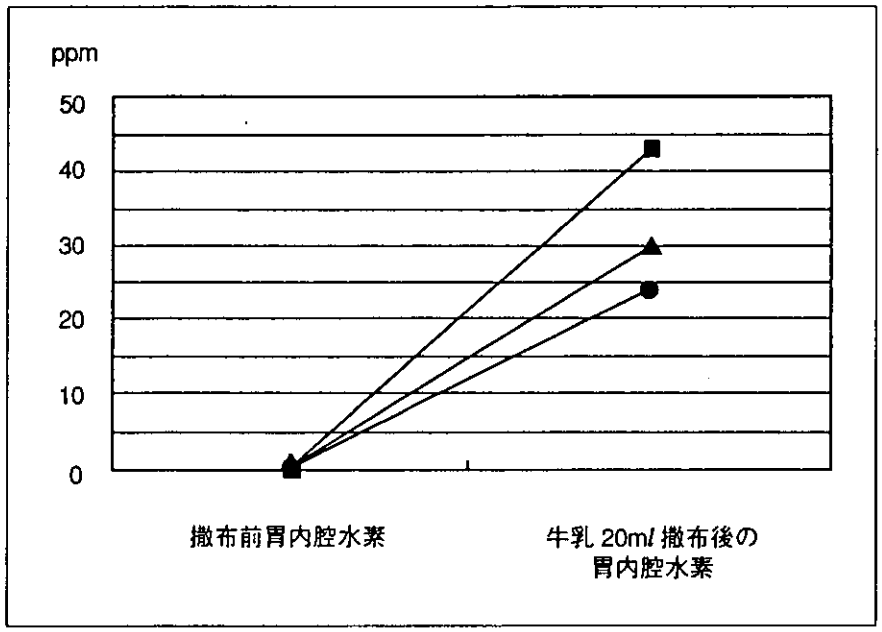

図6　普通牛乳散布による胃内腔水素ガス濃度上昇例

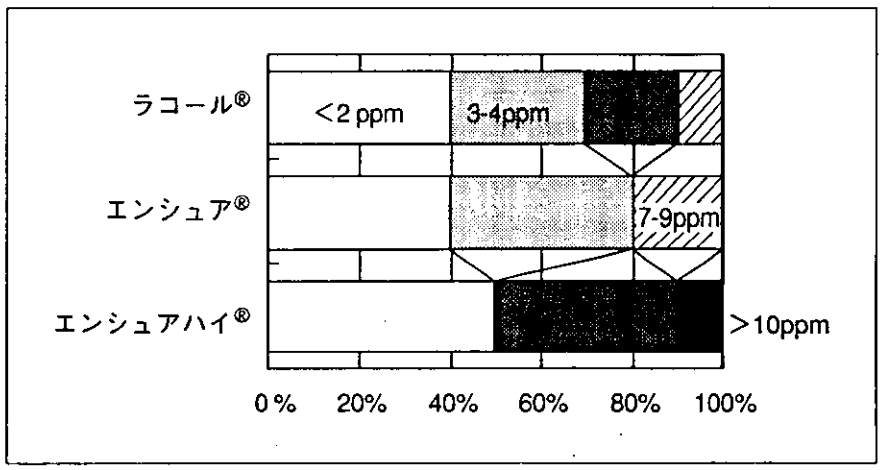

図7　液状食飲用後の呼気中水素ガス濃度の変化

内視鏡下にラクチュロース6gを散布し，10～15秒後に胃内腔の気体を採取し，その中に含まれる水素ガス濃度の変化を検討した．256例中10例(3.9%)で3 ppm以上の上昇がみられた．また，カロリーメイト20ml(20kcal，糖質3.3g，脂質0.44g，蛋白質0.67g，線維0.2g)を散布した場合，91例中9例(9.9%)，普通牛乳20ml(13kcal，糖質0.9g，脂質0.67g，蛋白質0.6g，線維 0)では55例中3例(5.5%)で水素ガスの上昇がみられた(図6)．

食餌摂取による腸管内でのガス発生

胃内腔では通常の食餌をわずか20ml投与しただけでも，10%前後の症例で水素ガスが発生した．細菌の多い腸管内ではさらに高率にガスが発生する可能性が高い．そこで，200ccの液状食を飲用後，経時的に240分まで呼気中水素ガス濃度を測定したところ，半数以上の症例で3 ppm以上の上昇がみられた(図7)．

消化管発酵生成物の消化管機能への影響

発酵反応では水素，二酸化炭素，短鎖脂肪酸が生成される．この短鎖脂肪酸には食後の一過性LES弛緩の増加，胃排出抑制などの作用があり，逆流性食道炎を惹起する可能性が報告されている[12]．われわれの検討でも，逆流性食道炎群で呼気中水素あるいはメタンガスが10ppm以上の症例が，非食道炎群よりも有意に多かった(図8)．逆流性食道炎の発症には酸分泌，胃食道協調運動

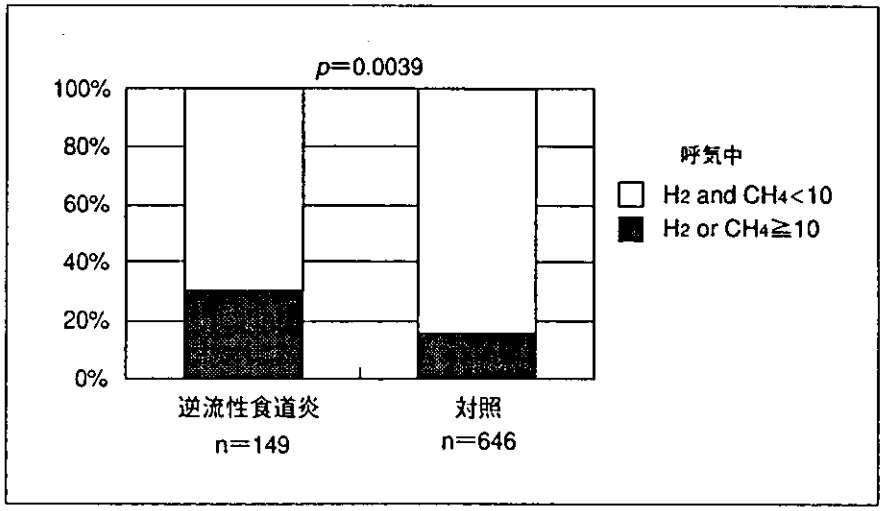

図8　逆流性食道炎と呼気中水素・メタンガス濃度

障害が重要であるが，一部の逆流性食道炎症例では消化管発酵が関与している可能性もある．

おわりに

慢性胃炎を取り巻く消化管環境の変化について，消化管発酵反応を中心に述べた．消化管内腔の細菌は*H. pylori*のように粘膜に接着して増殖しなければ病原性はほとんどないと考えられてきた．しかし，発酵生成物が消化管機能に影響を及ぼす可能性があり，胃内腔に持続的に流入する口腔内細菌など，いわゆる胃内通過菌においても消化器症状を惹起する可能性がある．萎縮性胃炎と消化器症状との関連は明らかではないが，発酵亢進例については，抗菌剤投与や食事指導など発酵反応を抑える治療も選択肢のひとつと考えられる．

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Individual and joint impact of family history and *Helicobacter pylori* infection on the risk of stomach cancer: a nested case–control study

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We used 202 cases of stomach cancer and 394 controls nested within the Japan Collaborative Cohort Study For Evaluation of Cancer Risk (JACC study) to investigate whether family history has an independent effect on the risk of stomach cancer after controlling for the *Helicobacter pylori* infection. A positive history of stomach cancer in one or more first-degree relatives was associated with an increased risk of the disease in women, but not in men after controlling for *H. pylori* infection and other confounding variables. Women with both a family history and *H. pylori* infection were associated with more than five-fold increased risk of the disease (OR 5.10, 95% CI 1.58–16.5) compared to those without these factors. These results suggest the existence of inherited susceptibility to the disease in women, and that measurements of *H. pylori* infection together with the family history allow meaningful evaluation of risk beyond that provided by either factor alone.
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Keywords: family history; *Helicobacter pylori*; stomach cancer; sex difference; nested case–control study; JACC study

Familial aggregation of stomach cancer has long been noted (Macklin, 1960; Toyoshima *et al*, 1997; Yatsuya *et al*, 2002; Kondo *et al*, 2003). Certain evidence, however, suggests that both genetic and environmental factors are responsible for familial clustering (Palli *et al*, 2001). One environmental risk factor is infection with *Helicobacter pylori* (*H. pylori*) (Talley *et al*, 1991), and previous studies have revealed that this also aggregates within families (Dominici *et al*, 1999).

A case–control study found that a family history of stomach cancer significantly increased the risk of the disease independent of *H. pylori* infection (Brenner *et al*, 2000). In addition, positive family history in individuals with *H. pylori* infection appeared to be a stronger risk factor for the disease compared to those without such an infection. There are, however, no prospective studies of this subject. We, therefore, conducted a nested case–control investigation within a cohort study to examine the independent effect of family history on the risk of stomach cancer after controlling first for the *H. pylori* infection, and, second, to evaluate any joint contribution of these two factors to the disease risk.

MATERIALS AND METHODS

JACC study

The study was part of the Japan Collaborative Cohort Study For Evaluation of Cancer Risk Sponsored by the Ministry of Education, Science, Sports and Culture of Japan (JACC Study), a nation-wide multicenter collaborative study to evaluate prospectively various risks or protective factors on cancer mortality and incidence. Details of the study design were reported previously (Ohno and Tamakoshi, 2001; Hoshiyama *et al*, 2002). Briefly, the cohort included 110 792 men and women (46 465 and 64 327, respectively), 40–79 years old at recruitment, enrolled in 1988–1990. Enrollment was based on the participants of a general health checkup that is periodically provided by the 45 municipalities involved. Informed consent procedures were approved by the Ethics Committee of Medical Care and Research, University of Occupational and Environmental Health, Kitakyushu, where the chief investigator of stomach cancer group is affiliated, and the Ethical Board of the

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[10] See Appendix A for the investigators (name and affiliation) involved in the JACC Study
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Nagoya University School of Medicine, Japan, where the present chairman of the JACC study is affiliated.

At the time of recruitment, baseline characteristics were gathered by self-administered questionnaires, which covered the medical history and included lifestyle-related items such as drinking and smoking, level of education, and family history of several medical conditions including cancer. About one-third of the cohort members ($n = 39\,293$) also donated a residual serum sample (about 2 ml) used for the general health checkup. It was partitioned into 0.3–0.5 ml aliquots and stored at −80°C until laboratory analyses.

Follow-up and identification of stomach cancer cases, and selection of control subjects

Vital statuses of the participants were checked annually by each regional research centre, with permission to review their population-register sheets from the Ministry of Public Management, Home Affairs, Post and Telecommunications. Incidence of cancer was ascertained in 24 study areas ($n = 65\,184$) and coded according to the tenth revision of International Classification of Diseases and the second edition of International Classification of Diseases for Oncology. These data were collected at the central office of the Research Committee.

We first restricted the subjects to those who lived in the study areas where cancer incidence was ascertained. We then excluded 857 participants with a self-reported history of cancer at any site. Among the remaining 64 327 subjects, diagnosis of stomach cancer 12 or more months after cohort recruitment was documented in 804 cases until the end of 1997. Serum had been obtained from 218 out of the initial 804 cases. However, seven cases without enough serum for laboratory analysis, and one case without an eligible control subject were excluded. In addition, one of the 24 study areas where family history was not assessed was excluded from the analysis. Thus, the study reported here included 202 cases (105 men and 97 women) in total. Lag time between blood sampling and stomach cancer diagnosis varied between 12 and 113 months (median 50 months). Each of these subjects was matched with two control subjects for gender, age at recruitment (as near as possible), and study area, who had also provided an adequate baseline blood sample and who were alive and remained free of confirmed cancer as of the end of 1997. Owing to a lack of eligible subjects, a few sets ($n = 10$) contained only one control; a total of 394 controls was available for the present analysis. As information on the location of cancer within the stomach or the histological type was not available in all cases, we did not use it to classify cases.

Laboratory assays

Serum samples from each case and matched controls were retrieved from storage and shipped on dry ice to a single laboratory for the assay. None of the samples had been previously defrosted. *H. pylori* infection was investigated serologically using HM-CAPTM (Enteric Products, Westbury, NY, USA) with antigen from Japanese (J-HM-CAP), and the serum titer of immunoglobulin G antibodies 2.3 or greater was defined as positive infection.

Definition of family history and covariates

Family history of stomach cancer was defined as when the subject had at least one first-degree relative with a history of stomach cancer. Risk factors that could potentially confound the relation between family history and stomach cancer other than *H. pylori* infection were collected at the baseline, using a self-administered questionnaire. A drinking habit was first categorised into three statuses (none, past, present). If present, it was further categorised into two levels by weekly consumption (light, heavy), that is, daily alcohol consumption times days of drinking per week. Smoking status was classified into three levels (never, past, current). Consumption frequency of vegetables, citrus fruits, and green tea was initially assessed in five levels (everyday, 3–4 times a week, 1–2 times a week, 1–2 times a month and seldom). For the present analysis, the former two and the latter three categories were combined. Salty-food preference was categorised into three levels (dislike, neutral, like). Information on educational level was measured as the age of formal schooling completed and was classified into two categories: $\leqslant 15$ years old (corresponds to $\leqslant 9$ years of schooling) and $\geqslant 16$ years old (corresponds to $\geqslant 10$ years of schooling). Missing values in each variable were treated as an additional category in the variable, and were included in the analyses.

Statistical analysis

We compared the baseline characteristics of case subjects and control subjects by one-way analysis of variance for continuous variables and χ^2 tests for categorical variables. We then performed logistic regression analysis, conditioned on the matching variables of gender, age, and study area, and presented the odds ratios (ORs) that represent the risk associated with a positive family history of stomach cancer. Adjusted estimates of risk were obtained with multivariate models that controlled for *H. pylori* infection, and other covariates listed above. To assess the joint effect of family history and *H. pylori* infection on the risk of stomach cancer, four categories were created by the combination of these two factors. Another logistic regression analysis was performed taking a category with no family history and no *H. pylori* infection as a reference. The 95% confidence intervals (95% CIs) are presented for all ORs. All reported *P*-values are two-sided. All analyses were performed separately for men and women with SPSS statistical package for windows version 11.5.

RESULTS

Table 1 shows the baseline characteristics of the 202 cases and the 394 matched controls. In this sample, the women diagnosed with stomach cancer were more likely to have a family history of stomach cancer, whereas men were not. The proportion of case subjects who reported a history of stomach cancer in a first-degree relative was 15.2 and 24.7% in men and women, respectively, against 16.3% in men and 15.1% in women in control subjects (*P*-values for the χ^2 test were 0.87 in men, 0.054 in women; case *vs* control). The proportion of individuals infected with *H. pylori* was high even in control subjects (79.7 and 78.6% for men and women, respectively). However, it was higher in cases with stomach cancer in men and especially in women. Cases and controls did not differ significantly in terms of smoking status, alcohol intake, or other diet-related items for both sexes. The proportion of women with a higher educational level seemed to be higher in controls compared to that in cases.

Table 2 shows the relation of family history of stomach cancer to the risk of the disease incidence. Family history of stomach cancer was significantly related to the risk of the disease incidence only in women. This association became attenuated after adjustment for *H. pylori* infection or other potentially confounding variables. A family history of stomach cancer did not seem to be related to the risk of the disease incidence in men in the present dataset.

The prevalence of *H. pylori* infection in men with a family history was 81.6%, against 82.2% in men without such a history (Table 3). In women, the prevalence was 88.7 and 81.4% in those with and without a family history, respectively. The difference in the proportion was not significant, showing that a positive family history of stomach cancer and *H. pylori* infection were not related

Table 1 Baseline characteristics of the study participants

Characteristic	Men ($n=307$)			Women ($n=289$)		
	Cases ($n=105$)	Controls ($n=202$)	*P*-value	Cases ($n=97$)	Controls ($n=192$)	*P*-value
Age category: no. (%)						
40–49	6 (5.7)	12 (5.9)	Matching factor	9 (9.3)	18 (9.4)	Matching factor
50–59	22 (21.0)	42 (20.8)		32 (33.0)	64 (33.3)	
60–69	52 (49.5)	104 (51.5)		39 (40.2)	77 (40.1)	
70–79	25 (23.8)	44 (21.8)		17 (17.5)	33 (17.2)	
Age (years): mean ± s.d.	63.6 ± 7.9	63.3 ± 7.8		61.6 ± 8.3	61.4 ± 8.3	
H. pylori infection: no. (%)						
Present	91 (86.7)	161 (79.7)	0.16	88 (90.7)	151 (78.6)	0.013
Absent	14 (13.3)	41 (20.3)		9 (9.3)	41 (21.4)	
Family history of stomach cancer: no. (%)						
Present	16 (15.2)	33 (16.3)	0.87	24 (24.7)	29 (15.1)	0.054
Absent	89 (84.8)	169 (83.7)		73 (75.3)	163 (84.9)	
Number of siblings						
0–2	11 (10.5)	28 (13.9)	0.73	13 (13.4)	25 (13.0)	0.97
3–5	46 (43.8)	79 (39.1)		42 (43.3)	88 (45.8)	
6–	31 (29.5)	57 (28.2)		28 (28.9)	51 (26.6)	
Missing	17 (16.2)	38 (18.8)		14 (14.4)	28 (14.6)	
Smoking status: no. (%)						
Never	17 (16.2)	35 (17.3)	0.35	86 (88.7)	168 (87.5)	0.52
Past	31 (29.5)	57 (28.2)		1 (1.0)	1 (0.5)	
Current	55 (52.4)	97 (48.0)		4 (4.1)	4 (2.1)	
Missing	2 (1.9)	13 (6.4)		6 (6.2)	19 (9.9)	
Alcohol intake: no. (%)						
None	21 (20.0)	42 (20.8)	0.41	69 (71.1)	146(76.0)	0.82
Past	7 (6.7)	6 (3.0)		3 (3.1)	4 (2.1)	
Light drinker	37 (35.2)	83 (41.1)		13 (13.4)	21 (10.9)	
Heavy drinker	23 (21.9)	34 (16.8)		0 (0.0)	1 (0.5)	
Missing	17 (16.2)	37 (18.3)		12 (12.4)	20 (10.4)	
Educational level: no. (%)						
⩽9 years of schooling	27 (25.7)	65 (32.2)	0.49	32 (33.0)	45 (23.4)	0.19
⩾10 years of schooling	56 (53.3)	100 (49.5)		46 (47.4)		
Missing	22 (21.0)	37 (18.3)		19 (19.6)	37 (19.3)	
Salty-food preference: no. (%)						
Dislike	10 (9.5)	31 (15.3)	0.57	24 (24.7)	29 (15.1)	0.11
Neutral	39 (37.1)	71 (35.1)		36 (37.1)	97 (50.5)	
Like	39 (37.1)	69 (34.2)		19 (19.6)	36 (18.8)	
Missing	17 (16.2)	31 (15.3)		18 (18.6)	30 (15.6)	
Tomatoes: no. (%)						
⩽1–2 times/week	52 (49.5)	105 (52.0)	0.89	47 (48.5)	94 (49.0)	0.61
⩾3–4 times/week	42 (40.0)	75 (37.1)		44 (45.4)	80 (41.7)	
Missing	11 (10.5)	22 (10.9)		6 (6.2)	18 (9.4)	
Citrus fruits: no. (%)						
⩽1–2 times/week	48 (45.7)	79 (39.1)	0.49	29 (29.9)	65 (33.9)	0.78
⩾3–4 times/week	44 (41.9)	91 (45.0)		56 (57.7)	106 (55.2)	
Missing	13 (12.4)	32 (15.8)		12 (12.4)	21 (10.9)	
Spinach and green vegetables: no. (%)						
⩽1–2 times/week	29 (27.6)	64 (31.7)	0.55	30 (30.9)	60 (31.3)	0.95
⩾3–4 times/week	65 (61.9)	112 (55.4)		58 (59.8)	112 (58.3)	
Missing	11 (10.5)	26 (12.9)		9 (9.3)	20 (10.4)	
Carrots and pumpkins: no. (%)						
⩽1–2 times/week	44 (41.9)	86 (42.6)	0.85	35 (36.1)	78 (40.6)	0.70
⩾3–4 times/week	52 (49.5)	95 (47.0)		52 (53.6)	98 (51.0)	
Missing	9 (8.6)	21 (10.4)		10 (10.3)	16 (8.3)	
Green tea: no. (%)						
⩽1–2 times/week	11 (10.5)	13 (6.4)	0.32	11 (11.3)	21 (10.9)	1.00
⩾3–4 times/week	93 (88.6)	184 (91.1)		83 (85.6)	165 (85.9)	
Missing	1 (1.0)	5 (2.5)		3 (3.1)	6 (3.1)	

Table 2 Multivariate conditional logistic regression models examining the relation between family history and the risk of stomach cancer

	Men (105 cases/202 controls)			Women (97 cases/192 controls)		
Variables adjusted for	**RR**	**95% CI**	***P*-value**	**RR**	**95% CI**	***P*-value**
Univariate	0.96	0.48–1.91	0.907	1.92	1.02–3.64	0.044
Model 1	0.99	0.49–2.03	0.985	1.78	0.92–3.46	0.065
Model 2	0.89	0.40–1.97	0.768	1.73	0.82–3.65	0.153

CI = onfidence interval. Model 1: Adjusted for *H. pylori* infection and the number of siblings (0–2, 3–5, 6+). Model 2: Adjusted for *H. pylori* infection, the number of siblings (0–2, 3–5, 6+), smoking status (never, past, current), drinking habit self-rated preference of salty foods (dislike, neutral, like), consumption of green–yellow vegetables, citrus fruits and green tea (≥3–4 times a week, ≤1–2 times a week), and educational level (≤9 years of schooling, ≥10 years of schooling). Missing values in each variable were treated as an additional category.

Table 3 Association between family history of stomach cancer and *H. pylori* infection, and joint contribution of family history and *H. pylori* infection on the risk of stomach cancer

		No. of subjects	*P* for 2×2 χ^2 test	No. of cases	OR[a]	95% CI	OR[b]	95% CI
Men								
FH negative	*Hp negative*	46 (17.8%)	1.00	12	1	(reference)	1	(reference)
	Hp positive	212 (82.2%)		77	1.63	0.80–3.33	1.81	0.79–4.15
FH positive	*Hp negative*	9 (18.4%)		2	0.76	0.13–4.44	0.72	0.11–4.86
	Hp positive	40 (81.6%)		14	1.64	0.62–4.32	1.66	0.54–5.12
Women								
FH negative	*Hp negative*	44 (18.6%)	0.234	7	1	(reference)	1	(reference)
	Hp positive	192 (81.4%)		66	2.92	1.22–6.99	2.98	1.10–8.02
FH positive	*Hp negative*	6 (11.3%)		2	2.02	0.30–13.6	1.84	0.17–19.9
	Hp positive	47 (88.7%)		22	5.30	1.87–15.0	5.10	1.58–16.5

CI = confidence interval; FH = family history; Hp = *H. pylori*. OR[a]: Crude odds ratio. OR[b]: Odds ratio adjusted for the number of siblings (0–2, 3–5, 6+), smoking status (never, past, current), drinking habit self-rated preference of salty foods (dislike, neutral, like), consumption of green–yellow vegetables, citrus fruits and green tea (≥3–4 times a week, ≤1–2 times a week), and educational level (≤9 years of schooling, ≥10 years of schooling). Missing values in each variable were treated as an additional category

in this study sample, especially in men and to a lesser degree in women (*P*-value for χ^2 test 1.00 and 0.23 for men and women, respectively).

In another logistic regression analysis comparing the risk of the disease among the four subgroups created by the combination of presence or absence of a family history and *H. pylori* infection, significantly increased risk (multivariate adjusted OR 5.10, 95% CI 1.58–16.5) was observed in women with a family history of stomach cancer and *H. pylori* infection compared with those without these risk factors. In men, however, no significant associations were observed.

DISCUSSION

In this case–control study nested within a large-scale cohort of Japanese, we found that women with a family history of stomach cancer were associated with an increased risk of the disease independent of *H. pylori* infection. Women with both a family history and *H. pylori* infection had a greater than five-fold increased risk of the disease compared to those without these factors. The combined effect of these factors on the final risk of stomach cancer is approximately equivalent to the multiplicative product of the risks from the separate factors. Some biologic interaction between these two factors has been reported previously (Sepulveda *et al*, 2002). In a study of familial gastric cancer kindred, Rocco *et al* (2003) observed genetic abnormalities in the stomach of the first-degree relatives only in the presence of *H. pylori* infection, suggesting an interplay between the infection and the genetic profile of the host.

We did not find a significant association in men. This did not seem to be caused by a confounding of *H. pylori* infection. Some previous studies found stronger impact of family history on the disease risk in women than in men, which may partly be consistent with the present finding (Nagase *et al*, 1996; Yatsuya *et al*, 2002). Family history of stomach cancer was associated with a significantly increased risk of the disease (OR 4.5, 95% CI 1.3–15.2) in women, whereas it was related to a nonsignificant increased risk in men (OR 1.2, 95% CI 0.6–2.5) in a hospital-based case–control study in Japan (Nagase *et al*, 1996). The relative risk associated with a positive family history adjusted for age and the size of the family was 1.28 (95% CI 0.95–1.72) in men and 1.92 (95% CI 1.33–2.77) in women in a prospective study of Japanese (Yatsuya *et al*). However, other studies did not necessarily find the effect restricted to women (Palli *et al*, 1994; Inoue *et al*, 1998), which would suggest that the gender difference observed in the present study may be related to the study limitations.

First, the present study is based on about one-third of the cohort members who donated residual serum sample used for the general health checkup. Due to the fact that our previous study found an increased risk associated with a family history in men, though the increase was of borderline strength (Yatsuya *et al*, 2002), it might be possible that the male sample for this nested case–control study may potentially be biased. Future study with more cases with blood sample or with another indicator of *H. pylori* infection is needed to elucidate this issue.

Second, we did not classify cases by the location of cancer within the stomach or the histological type because the relevant information was not available in all cases. Stomach cancer in

cardia was not associated with a family history of the disease in a case-control study conducted in Japan (Inoue *et al*, 1998). In addition, cancer in gastric cardia is reported to be associated more to environmental exposures, such as smoking or alcohol drinking (Inoue *et al*, 1994; Sasazuki *et al*, 2002), and environmental exposures in men were more diverse than in women, which may contribute to mask or exceed the effect of family history.

Third, recall of family cancer history is reported to differ between men and women, that is, women provided the history more accurately than men in a validation study (Kerber and Slattery, 1997); several studies have indicated the possibility of gender bias in recall as an explanation for the gender-specific association found in women (Wu *et al*, 1996). The lack of association in men in this study sample may be caused by a misclassification of subjects due to inaccurate reporting of family history, which would have attenuated the association.

Unexpectedly, family history of stomach cancer and serological prevalence of *H. pylori* infection assessed at the time of enrollment were not related in the combined sample of cases and controls in the present study. This may be due to a higher prevalence of *H. pylori* infection in the present sample than in the previous studies that found positive associations (45–70%) (Kikuchi *et al*, 1998; Brenner *et al*, 2000). Clearance of the infection in the course of development of stomach cancer via chronic atrophic gastritis may possibly explain the lack of association because such clearance is of likely relevance for some proportion of cases even when blood samples have been taken several years before diagnosis.

In conclusion, a family history of stomach cancer was associated with an increased risk of the disease in women. In addition, we observed that women with both a family history and *H. pylori* infection were associated with a greater than five-fold increased risk of the disease compared to those without these risk factors. Measurements of *H. pylori* infection together with the family history allow meaningful refinement of risk stratification beyond that provided by either factor alone. The study thereby partly confirms and extends the still quite limited empirical evidence on an issue that might well be relevant for potential screening strategies at least in women.

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Appendix A

Japan Collaborative Cohort Study Group

The present members of the JACC Study and their affiliations are as follows: A Tamakoshi (present chairman of the study group, Nagoya University Graduate School of Medicine); M Mori (Sapporo Medical University School of Medicine); Y Motohashi (Akita University School of Medicine); I Tsuji (Tohoku University Graduate School of Medicine); Y Nakamura (Jichi Medical School); H Iso (Institute of Community Medicine, University of Tsukuba); H Mikami (Chiba Cancer Center); Y Inaba (Juntendo University School of Medicine); Y Hoshiyama (Showa University School of Medicine); H Suzuki (Niigata University Graduate School of Medical and Dental Sciences); H Shimizu (Gifu University School of Medicine); H Toyoshima (Nagoya University Graduate School of Medicine); S Tokudome (Nagoya City University Graduate School of Medicine); Y Ito (Fujita Health University School of Health Sciences); S Hashimoto (Fujita Health University School of Medicine); S Kikuchi (Aichi Medical University School of Medicine); A Koizumi (Graduate School of Medicine and Faculty of Medicine, Kyoto University); T Kawamura (Kyoto University Center for Student Health); Y Watanabe and T Miki (Kyoto Prefectural University of Medicine Graduate School of Medical Science); C Date (Faculty of Human Environmental Sciences, Mukogawa Women's University); K Sakata (Wakayama Medical University); T Nose (Tottori University Faculty of Medicine); N Hayakawa (Research Institute for Radiation Biology and Medicine, Hiroshima University); T Yoshimura (Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan); K Fukuda (Kurume University School of Medicine); N Okamoto (Kanagawa Cancer Center); H Shio (Moriyama Municipal Hospital); Y Ohno (former chairman of the study group, Asahi Rosai Hospital); T Kitagawa (Cancer Institute of the Japanese Foundation for Cancer Research); T Kuroki (Gifu University); and K Tajima (Aichi Cancer Center Research Institute).

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A nested case–control study of stomach cancer in relation to green tea consumption in Japan

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To evaluate whether green tea consumption provides protection against stomach cancer, the relative risks (RRs) were calculated in the Japan Collaborative Study for Evaluation of Cancer Risk, sponsored by the Ministry of Health and Welfare (JACC Study). The study was based on 157 incident cases and 285 controls aged 40–79 years. Cox proportional hazards regression analysis was used to estimate the RRs for stomach cancer. It was found that green tea consumption had no protective effect against stomach cancer. After adjustment for age, smoking status, *H. pylori* infection, history of peptic ulcer, and family history of stomach cancer along with certain dietary elements, the risks associated with drinking one or two, three or four, five to nine, and 10 or more cups of green tea per day, relative to those of drinking less than one cup per day, were 1.3 (95% confidence interval (CI): 0.6–2.8), 1.0 (95% CI: 0.5–1.9), 0.8 (95% CI: 0.4–1.6), and 1.2 (95% CI: 0.6–2.5), respectively (*P* for trend = 0.899). We found no inverse association between green tea consumption and the risk of stomach cancer.
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Keywords: green tea; stomach cancer; JACC Study

Stomach cancer is the second most common cancer worldwide (Parkin *et al*, 1999). In Japan, this cancer is the leading cause of cancer death among women and the second among men (Statistics and Information Department, Minister's secretariat, Ministry of Health and Welfare Japan, 2000). It has recently been reported that green tea consumption is inversely associated with the risk of stomach cancer; in other words, it has a protective effect. Green tea polyphenols have various anticarcinogenic effects, such as strong antioxidant activity, and inhibition of nitrosation and cell proliferation.

Although case–control studies (Kono *et al*, 1988; Yu and Hsieh, 1991; Yu *et al*, 1995; Ji *et al*, 1996; Inoue *et al*, 1998; Setiawan *et al*, 2001) have found a reduced risk of stomach cancer in association with green tea consumption, prospective studies (Galanis *et al*, 1998; Nakachi *et al*, 2000; Nagao *et al*, 2001; Tsubono *et al*, 2001) have not. A recent prospective study found that green tea had a protective effect against stomach cancer. Urinary tea polyphenols have been associated with protection from the risk of stomach cancer, while controlling *Helicobacter pylori* infection. Past studies did not consider the presence or absence of a history of infection with *H. pylori*, a strong risk factor for stomach cancer (Asaka *et al*, 1997). Assuming that green tea consumption is related to *H. pylori* infection, when a subject has a history of infection with *H. pylori* and consumes a large quantity of green tea, the protective effect, if any, would be masked. The present nested case–control study aimed to examine the association between green tea consumption and the risk of stomach cancer, while controlling *H. pylori* infection and other potential confounders, using data from the Japan Collaborative Cohort (JACC) Study, a Japan-wide population-based prospective study. This is the first study to analyse the effects of green tea consumption while controlling *H. pylori* infection.

MATERIAL AND METHODS

JACC Study

This study was part of the Japan Collaborative Cohort Study for Evaluation of Cancer Risk sponsored by the Ministry of Education, Science, Sports and Culture of Japan (the JACC Study), a nationwide multicentre collaborative study to prospectively evaluate various risk or protective factors for cancer mortality and

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incidence. Details of the study design were reported previously. Briefly, the cohort included 110 792 men and women (46 465 and 64 327, respectively), aged 40–79 years at recruitment, who were enrolled from 1988 to 1990. Enrollment was based on the participants of a general health checkup periodically provided by the 45 municipalities involved. The informed consent procedures were approved by the Ethics Committee of Medical Care and Research, University of Occupational and Environmental Health, Kitakyushu, and the Ethical Board of the Nagoya University School of Medicine, Japan.

At the time of recruitment, baseline characteristics were gathered by a self-administered questionnaire, which covered the medical history and included items such as drinking and smoking, level of education, and family history of several medical conditions including cancer. About one-third of the cohort members ($n = 39\,293$) also donated a residual serum sample (about 2 ml) to be used for the general health checkup. This sample was partitioned into 0.3–0.5 ml aliquots and stored at −80°C, until laboratory analyses were performed. The *H. pylori* antibody level was measured in the serum using HM-CAPTM (Enteric Products, Westbury, NY, USA) with an antigen from Japanese (J-HM-CAP). The cutoff value was determined at 2.3, which was recommended in the manufacturer's instructions.

Follow-up and identification of stomach cancer cases, and selection of control subjects

The vital status of each participant was checked annually by each regional research centre, with permission from the Ministry of Public Management, Home Affairs, Post and Telecommunications to review their population register sheets. The incidence of cancer was ascertained in 24 study areas ($n = 65\,184$) and coded according to the tenth revision of the International Classification of Disease and the second edition of the International Classification of Diseases for Oncology. These data were collected at the central office of the Research Committee.

We first restricted the subjects to those who lived in the study areas where cancer incidence was ascertained. We then excluded 857 participants with a self-reported history of cancer. From the remaining 64 327 subjects, diagnosis of stomach cancer at 12 or more months after cohort recruitment was documented in 804 subjects until the end of 1997. Serum had been obtained from 218 cases of the initial 804 cases. However, seven cases without sufficient serum for the laboratory analyses and one case without an eligible control subject were excluded. Thus, the study reported here included 210 cases in total. There were no differences between those selected for the case–control study nested within the cohort and those who were not selected in terms of the variables included in the multivariate model. The lag time between blood sampling and stomach cancer diagnosis varied between 12 and 113 months (median, 50 months). Each of these subjects was matched with two control subjects with respect to sex, age at recruitment (as near as possible), and study area, who had also provided an adequate baseline blood sample, and who were alive and free of confirmed cancer by the end of 1997. Owing to a lack of eligible subjects, a few sets ($n = 10$) contained only one control, and thus there was a total of 410 controls.

Since questions on the daily consumption of green tea were not included in the questionnaire in seven areas (four rural areas and three urban/rural areas), we excluded these data (49 cases and 88 controls). Of the 161 cases and 322 controls remaining, eight cases (5.0%) and 38 controls (11.8%) had green tea consumption data missing from the questionnaire; so these too were excluded. Owing to a lack of eligible subjects, 16 sets were further excluded. The remaining 151 cases and 265 controls were included in the present analysis.

Data processing

Cox proportional hazard regression analysis was used. The relative risk (RR) and its 95% confidence interval (CI) were calculated based on the regression coefficient and its standard error (Cox, 1972), for an indicator term corresponding to the level of an independent variable. For multivariate analysis, several factors were listed as potential confounders according to epidemiological studies (Boeing, 1991; Hoshiyama and Sasaba, 1992; World Cancer Research Fund, 1997; Hoshiyama *et al*, 2002; Yatsuya *et al*, 2002). Trends of association were assessed by the regression model assigning scores (0–4) to the levels of the independent variables. Statistical significance (two-sided) was based on the ratio of the regression coefficient and its standard error. Statistical analysis (PHREG procedure) was performed using the Statistical Analysis System (SAS Institute, 1983).

RESULTS

Table 1 compares the characteristics of the cases and the controls. The consumption of green tea varied substantially. The proportion with a history of *H. pylori* infection was higher for the cases than for the controls. The proportion with a family history of stomach cancer was also higher for the cases than for the controls. The proportion of current smokers was also higher for the cases than for the controls. The cases consumed rice, miso soup, green-yellow vegetables, and fruit more frequently than the controls.

Table 2 shows the RR and its CI for stomach cancer according to green tea consumption. The age/sex-adjusted RRs associated with drinking one or two, three or four, five to nine, and 10 or more cups of green tea per day, relative to those of drinking less than one cup per day, were 1.2 (95% CI: 0.5–2.9), 0.9 (95% CI: 0.4–1.9), 0.7 (95% CI: 0.3–1.5), and 1.0 (95% CI: 0.4–2.4), respectively. Multivariate RRs were similar to age/sex-adjusted and age/sex- and *H. pylori* infection-adjusted RRs.

Table 1 Characteristics of cases and controls

No	Case 151	Control 265
Age (years)	61.7	61.5
Green tea (cups per day)		
<1	18	31
1 or 2	19	23
3 or 4	41	73
5–9	50	105
⩾10	23	33
H. pyroli infection (%)	88.7	77.7
History of peptic ulcer (%)	19.7	18.2
Family history of stomach cancer (%)[a]	20.5	16.2
⩽9 years of schooling (%)[b]	37.1	32.0
Smoking (%)		
Current	32.2	28.7
Past	18.5	18.0
Daily dietary consumption (%)		
Rice (⩾4 bowls day^{-1})	36.5	31.8
Miso soup (⩾1 cup day^{-1})	83.7	77.9
Preference for salty foods (yes)	33.8	29.6
Green-yellow vegetables (⩾1 day^{-1})	46.7	42.3
White vegetables (⩾1 day^{-1})	38.6	40.5
Fruits (⩾3 week^{-1})	44.4	39.3

[a]We defined a positive family history of stomach cancer as when the subject had a least one first-degree relative (parents or siblings) with a history of stomach cancer. [b]Information on educational level was measured as the age of formal schooling completed and was classified into two categories: ⩽15 years old (corresponds to ⩽9 years of schooling) and ⩾16 years old (corresponds to ⩾10 years of schooling).

Table 2 Relative risk of stomach cancer according to green tea consumption

	Green tea consumption (cups day^{-1})					
	<1	**1 or 2**	**3 or 4**	**5–9**	**≥10**	**P for trend**
Case/controls	18/31	19/23	41/73	50/105	23/33	
Age/sex-adjusted RR	1.0	1.2 (0.5–2.9)	0.9 (0.4–1.9)	0.7 (0.3–1.5)	1.0 (0.4–2.4)	0.515
Age/sex- and *H. pylon*-adjusted RR	1.0	1.1 (0.4–2.9)	0.9 (0.4–1.9)	0.7 (0.3–1.5)	1.1 (0.4–2.5)	0.628
Multivariate RR[a]	1.0	1.3 (0.6–2.8)	1.0 (0.5–1.9)	0.8 (0.4–1.6)	1.2 (0.6–2.5)	0.899

[a]Adjusted for age (four classes), smoking status (never, past, current), sex, *H. pylori* infection, history of peptic ulcer, family history of stomach cancer, educational level (two levels), consumption of rice, miso soup, green–yellow vegetables, white vegetables, fruits, and preference for salty foods (two categories). Values in parantheses are 95% confidence intervals. RR = relative risk.

Table 3 Relative risk of *H. pylori* infection positive according to green tea consumption among controls

	Green tea (cups day^{-1})					
	<1	**1 or 2**	**3 or 4**	**5–9**	**≥10**	**P for trend**
Age–sex-adjusted RR of *H. pylon* infection positive	1.0	1.0 (0.2–3.8)	1.0 (0.3–2.8)	1.1 (0.4–3.1)	0.7 (0.2–2.5)	0.901

Table 3 shows the age/sex-adjusted RRs of *H. pylori* infection positivity according to green tea consumption. *H. pylori* infection did not differ with the consumption of green tea.

DISCUSSION

This nested case–control study is the first study to investigate any association between green tea consumption and the risk of stomach cancer while controlling *H. pylori* infection. Among the possible limitations of the present study was incomplete data. About 10% of subjects were excluded from the analysis because they had not provided information concerning their daily consumption of green tea. We could not fully evaluate the effects of the exclusion of these data. Nevertheless, there was no difference between the percentages of smokers in the whole data (53.1% of men and 2.9% of women) and those in the included data (51.9% and 3.7%, respectively), as examined by the Cochran–Mantel–Haenszel χ^2 test ($P = 1.000$ and 0.843, respectively). The missing information therefore seemed to occur randomly.

The second possible problem with the present study was in the questionnaire. The original words of the question on green tea were: Do you drink Japanese tea (green tea)? There are various kinds of Japanese tea, although for Japanese people green tea is the one that most often comes to mind. About 89% of the total production of Japanese tea in 1999 was ordinary green tea (The Ministry of Agriculture, Forestry, and Fisheries, 1999). We believe that a slight misclassification could have derived from the idiosyncrasy of our questionnaire pertaining to Japanese tea (green tea).

Green tea is widely consumed in Japan and other Asian countries. If drinking green tea protects against stomach cancer, it would be an inexpensive and convenient method of primary prevention. Tsubono *et al* reported that there was no association between green tea consumption and the risk of stomach cancer, consistent with the finding of this prospective study. Little other evidence is available from prospective studies (Galanis *et al*, 1998; Tsubono *et al*, 2001). Past studies did not consider the influence of *H. pylori* infection. Subjects with chronic gastritis caused by *H. pylori* infection might have limited their consumption of green tea. If so, the prevalence of infection would have been lower in the subjects with higher intakes of green tea. If not, the prevalence of infection would have been higher among the subjects with higher intakes of green tea. This condition would have masked an inverse association between the risk of stomach cancer and green tea consumption. We examined the association of *H. pylori* infection and green tea consumption, and found that *H. pylori* infection did not differ with the consumption of green tea (see Table 3).

Our findings are in general agreement with those of four prospective studies which found no inverse association between green tea consumption and the risk of stomach cancer (Galanis *et al*, 1998; Nakachi *et al*, 2000; Nagao *et al*, 2001; Tsubono *et al*, 2001). The number of cases of stomach cancer was relatively large in three studies. Recently, another prospective study was conducted in Shanghai, China. Urinary EGC positivity showed a statistically significant inverse association with stomach cancer (OR = 0.52, 95% CI = 0.28–0.97) after adjustment for *H. pylori* seropositivity, smoking, alcohol drinking, and the level of serum carotenes (Sun *et al*, 2002). Cumulative excretion of EGC increased with increasing green tea consumption in human subjects (Yang *et al*, 1998). It might be important to evaluate biomarkers of tea polyphenol exposure.

In summary, we found no inverse association between the consumption of green tea and the risk of stomach cancer in Japan in a nested case–control study.

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Appendix

JAPAN COLLABORATIVE COHORT STUDY GROUP

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Serum midkine concentrations and gastric cancer

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Midkine (MK) is one of a family of heparin-binding growth factors, and increased MK expression is reported in various types of human carcinomas. To clarify the association between serum MK (S-MK) concentrations and gastric cancer, we examined S-MK concentrations of gastric cancer patients (n = 275) and healthy controls (n = 275). S-MK concentrations of all subjects were measured by enzyme-linked immunosorbent assay (ELISA). The medians (25th and 75th percentiles) of S-MK were 192 (123 and 314) pg/mL in the cases and 170 (81 and 273) pg/mL in the controls ($P < 0.01$). We also compared S-MK concentrations in each group divided by the progression stage or histological type of cancer. A difference was observed in the median S-MK concentrations between early and advanced cancers [182 (105 and 301) pg/mL *vs* 203 (139 and 331) pg/mL, $P = 0.07$], but not between intestinal and diffuse type cancers [185 (121 and 306) pg/mL *vs* 198 (127 and 323) pg/mL, $P = 0.51$]. We found that those progression stages affect S-MK concentration more strongly than the histological types in gastric cancer patients. Because S-MK seems to reflect the progression stage of gastric cancer, it may serve as a useful marker in the clinical follow-up of gastric cancer patients. (*Cancer Sci* 2005; 96: 54–56)

Midkine (MK) is a heparin-binding growth factor which is the product of a retinoic acid-responsive gene whose expression increases during the early differentiation stage in embryonal carcinoma cells.[1–3] MK exhibits various activities such as vascularization, promoting the growth of fibroblast, suppressing apoptosis, and inducing cell migration, and is considered to be involved in carcinogenesis and tumor progression.[4–9] Increased MK mRNA and protein expressions are reported in many human carcinomas such as gastric, pancreatic, bile duct, colorectal, hepatocellular, esophageal cancers, and in lung, breast, bladder, ovarian, and prostate carcinomas as well as in Wilms' tumors and neuroblastomas.[10–20] As it is a secreted protein, MK can be detected in the blood. Increased concentrations of blood MK have been reported in esophageal cancer and neuroblastoma.[21,22] Although there are several reports indicating that serum MK (S-MK) is elevated in other cancers, those studies involved only a few subjects.[23–25] To gain a better understanding of S-MK concentrations in gastric cancer, we investigated S-MK concentrations in each group divided by the progression stage or histological type of gastric cancer.

Materials and methods

Subjects. The case subjects were patients who were newly diagnosed as having gastric cancer at one of nine hospitals in the Tokyo Metropolitan Area between 1993 and 1995. Patients who had undergone treatment for gastric cancer were excluded at entry. An endoscopy was performed on all eligible cases, and the diagnosis was confirmed by an examination of resection or biopsy specimens. Data on pathological findings, including the type and stage of the cancer, were then recorded. Gastric cancer was subdivided by progression stage (early or advanced) and histological type (intestinal or diffuse) based on the criteria proposed by the Japanese Research Society for Gastric Cancer (JRSGC). The control subjects were recruited from a group of apparently healthy people who underwent medical checkups at a health promotion center in the same area. The cases and the controls were asked to provide sera, and written informed consent was obtained from all subjects. All sera of the cases were provided within 2 months from diagnosis; and before surgery between 1993 and 1995 we enrolled 788 gastric cancer patients and 1007 apparently healthy controls. From this group, we randomly selected 275 cases considering sex and age. Between case and control subjects, sex and age (±2 years) were matched. From the control subjects with the same age and sex, one whose date of phlebotomy was the nearest to that of each case subject was selected.

Serum samples of the subjects were collected using the same methods and frozen at −80°C until analysis.

ELISA assay. Serum *Helicobacter pylori* IgG antibodies were measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (J-HM-CAP; Kyowa Medex, Japan). Intra-assay coefficient of variation in J-HM-CAP (three different concentration samples in eight intra-assays) was 5.7% (range, 1.2–14.3%). Inter-assay coefficient of variation in J-HM-CAP (10 different concentration samples in three interassays) was 0.5% (range, 0.0–2.0%). The assay was performed according to the manufacturer's instructions. The *H. pylori* IgG level of ≥2.7 EV (the appropriate cut-off value of this kit established in our previous study) was determined as positive.[26] S-MK concentrations were measured with solid-phase human MK immunoassay ELISA kit systems as previously described.[25] Intra-assay coefficient of variation in S-MK kit (5 different concentration samples in 2 intra-assays) was 3.1% (range, 0.0–8.6%). Inter-assay coefficient of variation in S-MK kit (5 different concentration samples in 5 interassays) was 16.5% (range, 11.0–24.5%). During the measurements, serum samples were analyzed in randomly ordered duplicates in order to reduce systematic and interassay errors. All assays were performed by laboratory personnel who were blinded to each case/control status.

Statistics analysis. Because skewness and kurtosis of the distribution in S-MK concentrations was not improved though logarithmic transformation of S-MK concentrations was performed, we used non-parametric analysis. A comparison of the median S-MK concentrations between different groups was made using the Mann–Whitney U-test. The other characteristics were compared between cases and controls by using the χ^2 test. Cut-off points of the quartiles for smoking and drinking doses were described in our previous study.[27] All analyses were carried out using HALWIN (Gendaisugakusha, Kyoto, Japan).

Results

The age distribution of the cases was as follows: 20–29 years, 4 cases (1.4%); 30–39, 36 (13.1%); 40–49, 47 (17.1%); 50–59,

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Table 1. Characteristics of cases and controls

	Cases (n = 275)		Controls (n = 275)		P for difference
	N	%	N	%	
Sex					
Male	142	51.6	142	51.6	
Female	133	48.4	133	48.4	
Mean age ± SD	53.5 ± 10.5		53.6 ± 10.5		
Mean S-MK ± SD (pg/mL)	265 ± 367		190 ± 146		$P < 0.01$
H. pylori infection					$P < 0.01$
No	18	6.5	105	38.2	
Yes	257	93.5	170	61.8	
Smoking dose (cigarette-years[†])					$P = 0.31$
0 (Never-smoker)	127	46.2	141	51.3	
1–399	39	14.2	44	16.0	
400–799	52	18.9	42	15.3	
800+	51	18.5	35	12.7	
unknown	6	2.2	13	4.7	
Drinking dose (alcohol-years[‡])					$P < 0.05$
0 (Never-drinker)	92	33.5	74	26.9	
Occasional and 0.1–134.9	46	16.7	63	22.9	
135.0–1349.9	51	18.5	57	20.7	
1350.0+	68	24.7	45	16.4	
unknown	18	6.6	36	13.1	

[†]Cigarettes/day multiplied by years of smoking. [‡]Pure alcohol intake (ml)/day multiplied by years of drinking.

Table 2. S-MK concentrations in cases and controls

	N	Median of S-MK[†] (pg/ml)	$P^{\ddagger}$
Controls	275	170 (81 and 273)	<0.01
Cases	275	192 (123 and 314)	
Progression stage[§]			
Ealy	123	182 (105 and 301)	0.07
Advanced	151	203 (139 and 331)	
Histological type			
Intestinal	120	185 (121 and 306)	0.51
Diffuse	155	198 (127 and 323)	

[†]The numbers in parentheses indicate 25th and 75th percentiles.
[‡]The Mann-Whitney U test was used for group comparison.
[§]One case lacked data on the progression stage for cancer.

94 (34.2%); 60–69, 94 (34.2%). The cases included 142 males (51.6%) and 133 females (48.4%).

The characteristics of the cases and controls are shown in Table 1. The differences were observed in the distribution of *H. pylori* infection and drinking dose between cases and controls, but we found that these factors didn't affect S-MK values significantly for multiple regression analysis with log-transformation of S-MK values (data not shown). The distributions of all characteristics between early and advanced cases, and between intestinal and diffuse type cancer cases were not significantly different (data not shown). The medians (25th and 75th percentiles) of S-MK were 192 (123 and 314) pg/mL in the cases and 170 (81 and 273) pg/mL in the controls (Table 2). We also calculated the median S-MK concentrations of each group divided by the progression stage or histological type of cancer (Table 2). A difference was observed in the median (25th and 75th percentiles) S-MK concentrations between early and advanced cancers [182 (105 and 301) pg/mL *vs* 203 (139 and 331) pg/mL, $P = 0.07$], but not between intestinal and diffuse type cancers [185 (121 and 306) pg/mL *vs* 198 (127 and 323) pg/mL, $P = 0.51$].

Discussion

Increased MK expression has been reported in various human carcinomas.[10–20] Our study showed that the S-MK levels in gastric cancer patients were significantly higher than those in age- and gender-matched control subjects. A recent study has also reported that the S-MK levels in esophageal cancer patients were elevated.[21] Given the inherent limitations of any case-control study, it is not clear whether the high S-MK levels in their case subjects were the cause or the result of gastric cancer. Previous studies have demonstrated that S-MK levels decreased after surgical resection of the tumor in several carcinomas, including gastric cancer.[24] This indicates that the high level of S-MK in our case subjects may be the result rather than the cause of their gastric cancer. MK protein overexpressed in carcinoma tissue may be secreted into the circulation, thus inducing those S-MK high levels. However, there still remains the possibility that up-regulated MK accelerates tumor progression. A prospective study will be needed to clarify whether MK produces such an effect.

The median S-MK concentrations in the advanced cancer group was higher than that in the early cancer group, but there was no significant difference in S-MK concentrations between the intestinal and diffuse types. Therefore, it would seem that the progression stage of cancer rather than the histological type of cancer affects S-MK concentrations more strongly. In neuroblastoma patients, plasma MK levels were found to be higher among patients in the more advanced stages of the disease.[22] Those results suggested that the amount of MK produced may increase with advancing cancer stages. We also analyzed the association between tumor volume and gastric cancer, but could find no clear association between the two (data not shown).

In conclusion, S-MK may be more useful as a marker in the clinical follow-up of patients rather than in the early diagnosis of gastric cancer.

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LOWER DIGESTIVE TRACT EMR

ENDOSCOPIC SUBMUCOSAL DISSECTION FOR THE RELIABLE EN BLOC RESECTION OF COLORECTAL MUCOSAL TUMORS

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ABSTRACT

Scheduled piecemeal resection has been actively conducted for granular type laterally spreading tumor (LST-G) in Japan, as long as a definite preoperative diagnosis is made. However, en bloc resection is desirable for depressed lesions (e.g. IIc lesion) as well as non-granular type laterally spreading tumor (LST-NG) since they have considerable high risk for submucoasl invasion and require precise histopathological evaluation. Endoscopic submucosal dissection (ESD) has been developed for the en bloc resection of mucosal tumors of gastrointestinal tract and widely applied especially in gastric lesions. Although the large intestine involves structural and technical difficulties, we conducted en bloc resection by ESD while exercising sorts of ingenuity for preparation; endoscopes, instruments, local injections, and others. ESD is a reliable technique that allows en bloc resection of gastrointestinal mucosal lesions, and even has a splendid possibility for the treatment of early stage colorectal cancer.

Key words: endoscopic submucosal dissection (ESD), colorectal mucosal tumor, LST-NG, en bloc resection, Flex knife.

INTRODUCTION

Progress has been made in determining the malignancy and extent of local invasion of colorectal tumor through a magnifying endoscope[1] and other instruments, allowing a considerably definite preoperative evaluation of the lesion. Considering granular type laterally spreading tumor (LST-G), scheduled piecemeal resection has been actively conducted even for considerably large-sized tumors,[2] as long as a definite preoperative diagnosis is made, since its malignant potential is not so high. Alternatively, depressed lesions (e.g. IIc lesion) as well as non-granular type laterally spreading tumor (LST-NG) frequently involve submucoasl invasion. Therefore, a precise histopathological evaluation is essential with respect to tumor depth and vascular infiltration with resected specimen in an en bloc fashion. Endoscopic submucosal dissection (ESD) has been developed for the en bloc resection of mucosal tumors of the gastrointestinal tract and widely applied, especially to gastric lesions.[3,4] However, the large intestine involves the following issues that are not seen in the upper gastrointestinal tract:

1. Very thin walls present a high risk of perforation;
2. Enterobacterium-induced, serious peritonitis may develop in the event of perforation; and
3. Lumen is narrow and angulated, causing poor operability of an endoscope and generating higher difficulty in endoscopic procedure.

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To overcome these issues, we exercise sorts of ingenuity for preparation, endoscopes, instruments, local injections, and others and conduct en bloc resection by ESD while ensuring operational safety.

INGENUITIES TO ENSURE SAFETY

(1) Preparation

The patient is instructed to avoid fiber-rich meals on the day before endoscopy and to take a 10-mL bottle of picosulfate after dinner. A mixture of Niflec® 2 L and 10 mL of dimeticon is used as the intestinal lavage on the day of endoscopy. Mixing of dimeticon markedly reduces adhesive residues, which makes it easier to wash the lumen in case a few residues remained.

(2) Endoscopic system

For incision and dissection, an endoscope with a diameter as small as possible is recommended to obtain good maneuverability in the narrow lumen. We use a water-jet system-furnished, ultra-slim endoscope (outer diameter: 9.8 mm). Retroflex manipuration is neccesary, especially at the oral end of large-sized lesions, and for lesions overstride a fold. Therefore, it is essential to use an endoscope where the diameter is as small as possible and provides good operability. Furthermore, use of a transparent disposable attachment (Olympus, Japan) facilitates good visual field and allows stable dissection.