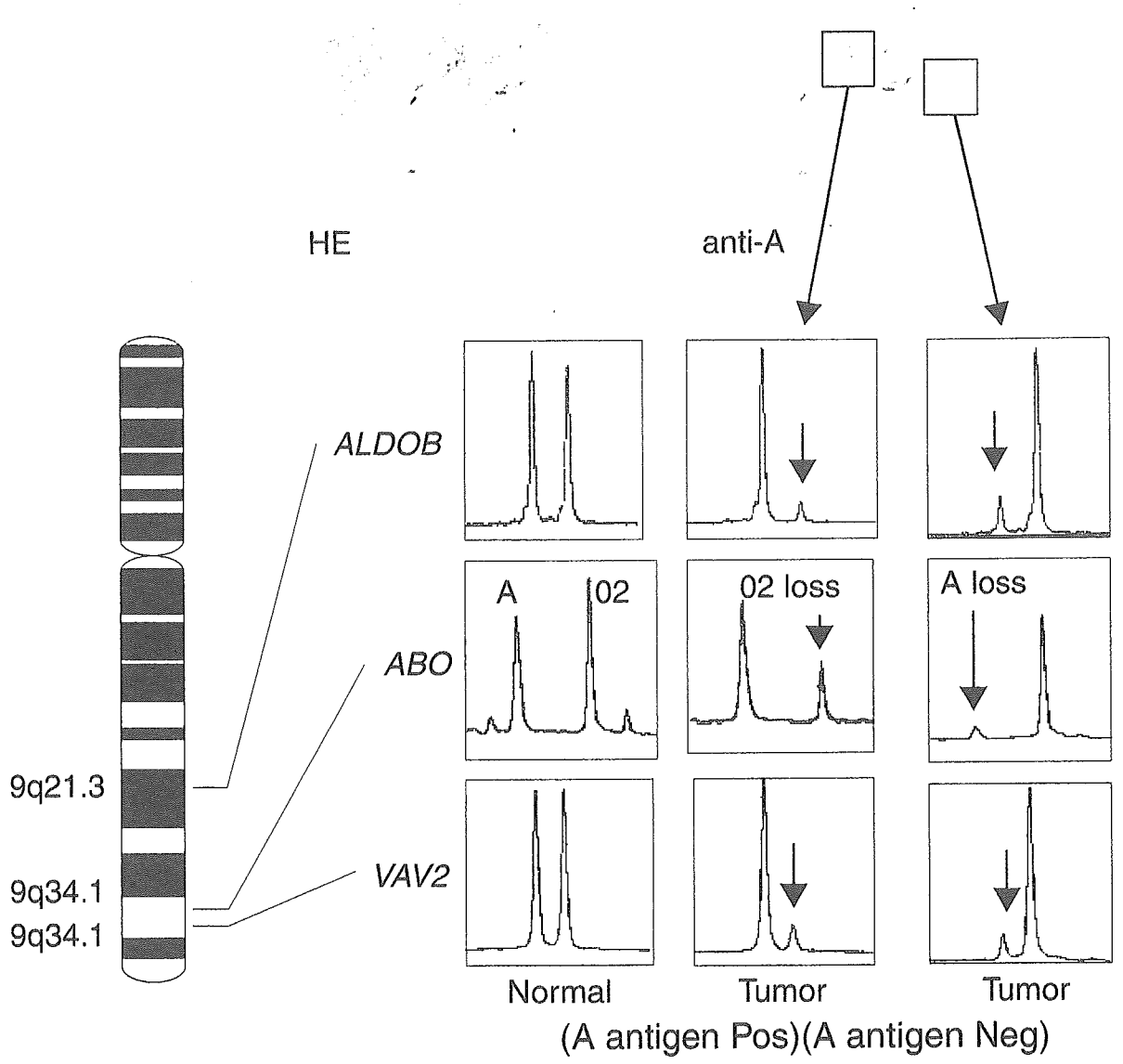


**Figure 4** A case of bladder cancer showing chimeric expression of the A antigen. DNA was extracted from areas showing positive or negative A antigen expression and subjected to blunt-end SSCP analysis using three single nucleotide polymorphic markers (*ALDOB*, *ABO* and *VAV2*) on 9q. The patient's genotype was A/O2. A allele was lost in the sample taken from the area showing negative A antigen expression, while O2 allele was lost in the sample taken from the area showing positive A antigen expression. Note that two polymorphic loci (*ALDOB*, 9q21.3 centromeric to the *ABO* locus and *VAV2*, 9q34.1 telomeric to the *ABO* locus) also showed LOH and suggested a large regional chromosome deletion, while the parental origin of the lost allele in these two loci was different between areas showing A-antigen positive or negative expression.

which six cases showed negative and three cases showed heterogenous expression of the A antigen (Table 5). Cases homozygous for A allele were regarded as retaining at least one copy of the *ABO* gene. No statistical association was found between the expression level of the A antigen and A allelic loss ($P=0.26$). In BiPS analysis, expression of the A antigen was negative in all of the four cases with full methylation and statistical association was shown between the expression of the A antigen and methylation status ($P=0.035$). Taking A allelic loss or full methylation in combination, 76.9% (10/13) cases with A allelic loss and/or full methylation showed negative A antigen expression, while the expression of the A antigen was negative in 38.7% (12/31) of cases that retained A allele and showed partial or no methylation. Cases with A allelic loss and/or full methylation showed significant correlation with negative A antigen expression ($P=0.02$) (Table 5). In analysis of 37 cases that underwent radical cystectomy, A allelic loss was observed in seven cases and they all showed negative A antigen expression in the tumor (Table 6). Compared with 30 cases that retained the A allele (including A/A

**Table 3** Methylation status in the *ABO* gene promoter region and expression of A antigen

| *No.* | *Case* | *Genotype* | *LOH*[a] | *Methylation status*[b] | *Methylation status (%)*[c] | | | | | | *RE 7*[d] | *Expression of A antigen* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *re* 1 | *re* 2 | *re* 3 | *re* 4 | *re* 5 | *re* 6 | | |
| 1 | 37 | A/O2 | O2 | Full | +(100) | +(100) | +(100) | +(100) | +(100) | +(71) | + | − |
| 2 | 65 | A/B | B | Full | +(100) | +(100) | +(100) | +(86) | +(100) | +(97) | + | − |
| 3 | 72 | A/O1 | O1 | Full | +(70) | +(62) | +(85) | +(57) | +(87) | +(61) | + | − |
| 4 | 228 | A/B | Ret | Full | +(100) | +(100) | +(100) | +(100) | +(100) | +(100) | + | − |
| 5 | 85 | A/O1 | O1 | Partial | − | +(85) | +(69) | − | +(100) | +(100) | + | − |
| 6 | 235 | A/O1 | O1 | Partial | − | +(85) | +(100) | − | − | − | + | − |
| 7 | 10 | A/O2 | Ret | Partial | − | − | +(23) | − | − | − | − | − |
| 8 | 186 | A/O2 | Ret | Partial | − | +(62) | +(38) | − | − | − | − | − |
| 9 | 220 | A/O1 | O1 | Partial | − | − | +(62) | − | − | − | − | − |
| 10 | 229 | A/O1 | O1 | Partial | − | − | +(46) | − | − | − | − | − |
| 11 | 226 | A/O2 | Ret | Partial | − | − | +(62) | − | − | − | − | +/− |
| 12 | 40 | A/O1 | Ret | Partial | +(100) | +(100) | +(100) | − | − | − | − | + |
| 13 | 141 | A/O2 | O2 | Partial | +(100) | +(100) | +(100) | − | − | − | − | + |
| 14 | 5 | A/O2 | O2 | No | − | − | − | − | − | − | − | − |
| 15 | 43 | A/O1 | O1 | No | − | − | − | − | − | − | − | − |
| 16 | 77 | A/O2 | O2 | No | − | − | − | − | − | − | − | − |
| 17 | 97 | A/O2 | O2 | No | − | − | − | − | − | − | − | − |
| 18 | 195 | A/O1 | Ret | No | − | − | − | − | − | − | − | − |
| 19 | 7 | A/A | NI | No | − | − | − | − | − | − | − | − |
| 20 | 71 | A/B | B | No | − | − | − | − | − | − | + | +/− |
| 21 | 184 | A/O2 | O2 | No | − | − | − | − | − | − | + | +/− |
| 22 | 183 | A/B | Ret | No | − | − | − | − | − | − | + | +/− |
| 23 | 212 | A/O2 | Ret | No | − | − | − | − | − | − | − | +/− |
| 24 | 225 | A/B | Ret | No | − | − | − | − | − | − | − | +/− |
| 25 | 3 | A/A | NI | No | − | − | − | − | − | − | − | +/− |
| 26 | 98 | A/A | NI | No | − | − | − | − | − | − | − | +/− |
| 27 | 78 | A/O2 | Ret | No | − | − | − | − | − | − | − | + |
| 28 | 79 | A/O2 | Ret | No | − | − | − | − | − | − | − | + |
| 29 | 94 | A/O1 | Ret | No | − | − | − | − | − | − | − | + |
| 30 | 185 | A/O2 | Ret | No | − | − | − | − | − | − | − | + |
| 31 | 193 | A/B | Ret | No | − | − | − | − | − | − | − | + |
| 32 | 221 | A/O1 | Ret | No | − | − | − | − | − | − | − | + |
| 33 | 222 | A/O2 | Ret | No | − | − | − | − | − | − | − | + |
| 34 | 45 | A/A | NI | No | − | − | − | − | − | − | − | + |
| 35 | 80 | A/A | NI | No | − | − | − | − | − | − | − | + |

[a]The cases in which A allele was retained were shown.

[b]Full methylation indicates all the regions were methylated, Partial; at least one regions were methylated, No; all the regions were ummethylated by SSCP analysis.

[c]Numbers in parentheses indicate the proportion of CpG sites methylated in the amplified DNA fragments.

[d]Methylation was analyzed using MSP.

**Table 4** Correlation of the expression of A antigen with methylation status in 35 cases underwent TUR

| *Expression of A antigen* | *Each locus (Nos. methylated/nos. unmethylated)* | | | | | | *All loci* | | | P |
|---|---|---|---|---|---|---|---|---|---|---|
| | *re* 1 | *re* 2 | *re* 3 | *re* 4 | *re* 5 | *re* 6 | *Full* | *Partial* | *None* | |
| Positive/Hetero | 2/17 | 2/17 | 3/16 | 0/16 | 0/16 | 0/16 | 0 | 3 | 16 | 0.0093 |
| Negative | 4/12 | 8/8 | 9/7 | 4/12 | 5/11 | 4/12 | 4 | 6 | 6 | |

Among 44 cases that underwent TUR, nine cases showing loss of A allele were not included in Table 4.
Hetero: heterogenous expression.

homozygotes), the frequency of A antigen expression was significantly low in those showing A allelic loss ($P=0.003$) (Table 6). MSP of *RE 7* showed methylation in seven cases (18.9%) in which the expression of the A antigen was negative in six cases. Methylation status was significantly correlated with negative expression of the A antigen ($P=0.03$). Taking A allelic loss and methylation in combination, 91% (10/11) of cases with A allelic loss and/or methylation were negative for the A antigen expression, while the expression of the A antigen was negative in 23.8% (5/21) of cases

**Table 5** Correlation of the expression of A antigen with A alleleic loss and hypermethylation of the *ABO* gene promoter region in 44 cases that underwent TUR

| *Expression of A antigen* | *A allele* | | P | *Full methylation* | *Partial or no methylation* | P | *A loss and/or full methylation*[a] | *A retained and partial/ no methylation* | P |
|---|---|---|---|---|---|---|---|---|---|
| | *Loss* | *Retain* | | | | | | | |
| Positive/ Hetero | 3 | 19 | 0.26 | 0 | 22 | 0.035 | 3 | 19 | 0.02 |
| Negative | 6 | 16 | | 4 | 18 | | 10 | 12 | |

[a]The cases that showed loss of A allele and/or full and partial methylation.
Hetero: heterogenous expression.

**Table 6** Correlation of the expression of A antigen with A allele loss and/or hypermethylation of the *ABO* gene promoter region in 37 cases that underwent radical cystectomy

| *Expression of A antigen* | *A allele* | | P | *MSP (RE 7)* | | P | *A loss and/or methylated* | *A retain and unmethylated* | P |
|---|---|---|---|---|---|---|---|---|---|
| | *Loss* | *retain* | | *M* | *UM* | | | | |
| Positive/heterogenous | 0 | 19 | 0.003 | 1 | 16[a] | 0.03 | 1 | 16 | 0.0005 |
| Negative | 7 | 11 | | 6 | 9[b] | | 10 | 5 | |

[a]Two cases were not available.
[b]Three cases were not available.
M, methylated; UM, unmethylated.

showing retained A allele and no methylation. A allelic loss and methylation were significantly correlated with the expression level of the A antigen ($P = 0.0005$) (Table 6). In one case, the expression of the A antigen was chimeric and the tumor was divided into areas showing positive or negative expression (Figure 4). This case was an A/O2 heterozygote, and the allelic status was determined from the dissected specimen. O2 allele was lost in the area showing positive staining, while the A allele was lost in the area showing negative staining. Allelic status was also examined in the *ALDOB* and *VAV2* loci, where the parental origin of the lost allele was different between positively and negatively stained areas, indicating that allelic loss in the tumor involved large chromosomal region between 9q21.3 and 9q34.1.

### Expression of the A Antigen in Dysplasia and Normal Urothelium

A total of 23 cases that underwent radical cystectomy were examined for expression of the A antigen in concomitant dysplastic lesions and normal urothelium (Table 7). In analysis of 13 cases showing positive A antigen expression in the tumor, A allele was retained in all cases and only one case showed hypermethylation together with normal expression of the A antigen in the dysplasia specimen. In analysis of 10 cases showing negative expression of the A antigen in the tumor, eight showed A allelic loss and/or methylation. Abnormal expression of the A antigen was observed only in one case (A-9), in which dysplasia specimen showed heterogeneous expression but A allelic loss and methylation were not observed in the tumor.

## Discussion

Previously, we reported that LOH on chromosome 9 was a frequent genetic event in TCCs of the bladder and its detection in urine samples would be an useful indicator for tumor recurrence in patients with TCC that underwent TUR.[4] Frequencies of LOH of the *ABO* locus examined in this study seems higher than those reported previously.[17,18] In previous studies, allelic status of the *ABO* gene was examined by PCR/RFLP; however, LOH is barely detectable by PCR/RFLP if the proportion of tumor cells in the sample is below 60%, due to the formation of heteroduplex dimers that are resistant to the restriction enzyme digestion.[30] Blunt-end SSCP analysis is a sensitive method to detect an LOH from clinical samples, of which the proportion of tumor cells is as low as 10–20%.[26] However, LOH study from small lesions such as concomitant dysplasia was still difficult due to technical problems. Slebos *et al*[31] reported that the lower the amount of DNA in the PCR, the greater the risk for allele ratios that were abnormal due to a chance distribution of alleles in the reaction and the DNA equivalent of a minimum of about 100 cells is required for a full representation of both alleles in the analysis. Furthermore, DNAs extracted from formalin-fixed paraffin-embedded sections often harbor degradation and fail in the PCR amplifica-

**Table 7** Correlation of the expression of A antigen in the tumor, dysplasia and normal urothelium specimens with the genetic and epigenetic changes in the primary tumor

| *Case* | *Genotype* | *Tumor* | | | *Dysplasia expression* | *Urothelium expression* |
|---|---|---|---|---|---|---|
| | | *Expression* | *LOH* | *Methylation status* | | |
| A-22 | A/O1 | Positive | O1 | M | Positive | Positive |
| A-1 | A/O2 | Positive | O2 | UM | Positive | Positive |
| A-10 | A/O2 | Positive | O2 | UM | Positive | Positive |
| A-3 | A/O2 | Positive | O2 | UM | Positive | Positive |
| A-46 | A/O2 | Positive | O2 | UM | Positive | Positive |
| A-5 | A/O1 | Positive | Ret | UM | Positive | Positive |
| A-43 | A/O1 | Positive | Ret | UM | Positive | Positive |
| AB-5 | A/B | Positive | Ret | UM | Positive | Positive |
| A-5 | A/O1 | Positive | Ret | UM | Positive | Positive |
| A-18 | A/O1 | Positive | Ret | NA | Positive | Positive |
| A-29 | A/A | Positive | NI | UM | Positive | Positive |
| A-47 | A/A | Positive | NI | UM | Positive | Positive |
| A-14 | A/A | Positive | NI | NA | Positive | Positive |
| A-15 | A/O2 | Negative | A | M | Positive | Positive |
| A-16 | A/O2 | Negative | A | M | Positive | Positive |
| A-48 | A/O2 | Negative | A | M | Positive | Positive |
| A-2 | A/O2 | Negative | A | UM | Positive | Positive |
| A-31 | A/O1 | Negative | A | UM | Positive | Positive |
| A-6 | A/O1 | Negative | A | NA | Positive | Positive |
| A-13 | A/O1 | Negative | O1 | M | Positive | Positive |
| A-9 | A/O1 | Negative | O1 | UM | Hetero | Positive |
| A-33 | A/O2 | Negative | O2 | NA | Positive | Positive |
| AB-2 | A/B | Negative | B | M | Positive | Positive |

UM and M indicate whether the *RE 7* sequences were unmethylated and methylated, respectively;
Hetero: heterogenous expression; NA: not applicable.

tion, suggesting potential difficulty in assessing the allelic status of small lesions from archival materials. The aim of the present study was to elucidate the underlying mechanisms of reduced expression of the histo-blood group A antigen in bladder cancer, and to determine if IHC of the A antigen expression could be available as a hallmark to determine the allelic loss and/or epigenetic alterations of the *ABO* gene on a cell-to-cell basis.

In cases with radical cystectomy, allelic status was examined using DNAs extracted from histological slides and directly comparable with the A antigen expression in the same specimen and expression of the A antigen was negative in all cases showing A allelic loss. In cases that underwent TUR, three cases showed heterogenous expression of the A antigen, regardless of A allelic loss in the sample. In TUR cases, DNAs were extracted from fresh frozen samples obtained by cold-cup biopsies, while the expression of the A antigen was examined in formalin-fixed paraffin-embedded sections of the resected tumors. Discrepancies between A allelic loss and A antigen expression in TUR cases may be explained by the difference of materials subjected to analysis. As we indicated in Figure 4, some tumors show polyclonal development as to the allelic loss of chromosome 9 and direct comparison between biopsies and resected specimen may be difficult in such cases. In BiPS analysis, full methylation was observed in four cases and they all showed negative expression of the A antigen (Table 5). CpG islands were densely methylated in full methylation and they were closely correlated with the transcriptional silencing of the *ABO* gene. In cases with partial methylation, A antigen expression was also negative in 66.7% (6/9) of cases. Although partial methylation may play some role in transcriptional silencing, we used full methylation as an indicator of methylation in this study. As methylation extended to the most downstream of the *ABO* gene promoter region (*re 6*) in full methylation, we designed a primer set for MSP spanning region 7, which overlapped the downstream of region 6. The size of the amplified DNA fragment in MSP was as short as 96 bp and we used it as an indicator of full methylation in analysis of DNAs extracted from formalin-fixed paraffin-embedded sections. As MSP amplifies methylated DNA sequences selectively, its sensitivity is much higher than that of BiPS analysis and may have a risk of overestimation. In fact, MSP showed methylation in three cases that showed no methylation in BiPS analysis and the expression of the A antigen in these three cases were heterogenous. This may indicate the heterogeneity of the methylation status, suggesting only small number of cells harbored methylation (Table 3). In cases that underwent TUR, negative A antigen expression was signifi-

cantly correlated with full methylation ($P=0.035$), but not with A allelic loss ($P=0.26$) (Table 5). In cases that underwent radical cystectomy, both methylation and A allelic loss were significantly correlated with the expression of the A antigen ($P=0.003$ for A allelic loss, $P=0.03$ for MSP, respectively). Using these two indices in combination, 29.5% (13/44) of the cases that underwent TUR and 29.7% (11/37) of cases that underwent radical cystectomy showed loss of the A allele and/or hypermethylation of the *ABO* gene. They were significantly correlated with the expression of the A antigen ($P=0.02$ for TUR cases, $P=0.0005$ for radical cystectomy cases) (Tables 5 and 6). Negative A antigen expression was observed in 50.0% (22/44) in TUR cases and 48.6% (18/37) in cases that underwent radical cystectomy, which was attributable to genomic deletion and/or hypermethylation of the *ABO* gene in at least 45% (10/22) of cases that underwent TUR and 66.7% (10/15) of cases that underwent radical cystectomy. It is apparent that A allelic loss and/or hypermethylation of the *ABO* gene could not be the sole cause for negative A antigen expression. As the antigenic determinant of the A antigen is the terminal structure of the carbohydrate chains, incomplete synthesis of carbohydrate chains associated with oncogenesis may also be concerned with the reduced expression of the A antigen. Methylation seems to be more predominant than loss of the A allele in cases that underwent TUR. This might be explained by the observation that superficial papillary tumors such as pTa or pT1 stages comprised most of the TUR cases, while more than 70% of them were invasive cancers above Stage pT2 in cases that underwent radical cystectomy. In our previous study, frequencies of LOH on chromosome 9 were 67% in pTa, 71% in pT1 and 80% in tumors ≧pT2 stages.[4] As for the putative tumor suppressors found on chromosome 9, p16 and $p14^{ARF}$ are located on 9p21.[32,33] And an area on 9q31–34 is most prone to be deleted in TCC of the bladder,[34,35] which is also a candidate locus for a putative tumor suppressor gene. Reportedly, deletion of chromosome 9 is an early genetic event in the development of bladder cancers.[1] However, there is not enough evidence to support this hypothesis regarding the occurrence of chromosome 9 deletion in preneoplastic lesions. In a few studies using microsatellite markers from microdissected specimens, allelic loss on chromosome 9 was observed in bladder dysplasia.[3,7] We studied the expression of the A antigen on the dysplasia specimens by IHC, aiming at screening genetic alterations in precancerous lesions of the bladder. Expression of the A antigen was examined in 23 cases of bladder cancer comprising dysplasia, among which the numbers of tumors showing positive or negative expression were 13 and 10, respectively. All of the cases showing positive expression retained the A allele in the tumor and only one case showed hypermethylation, while the expression of the A antigen was preserved in dysplasia and normal urothelium in all cases. In the analysis of 10 cases showing negative A antigen expression in the tumor, loss of the A allele and/or the hypermethylation was observed in eight cases. Expression of the A antigen was preserved in normal urothelium and dysplasia in all but one case showing heterogenous expression in the dysplasia. This case did not exhibit LOH or hypermethylation in the tumor. These results suggested that LOH and/or hypermethylation of the *ABO* gene were infrequent genetic and epigenetic alterations in dysplasia and normal urothelium of the bladder bearing TCC. Furthermore, one case showed chimeric expression of the A antigen in the tumor, among which the expression of the A antigen coincided with loss or retention of the A allele (Figure 4). Analysis of two polymorphic markers in the vicinity of *ABO* gene locus also showed LOHs and the parental origin of the lost allele in these two loci was opposite as was shown in analysis of the *ABO* gene locus. Previously, we reported loss of chromosome 9 was observed in 71% of TCCs of the bladder and nearly 50% of them involved both 9p and 9q, suggesting monosomy or uniparental aneusomy of chromosome 9.[4] Thus, the deletion was considered to involve large chromosomal regions at least between 9q21.3 and 9q34.1 and possibly on the same allele. This finding may suggest the idea that the tumor showed polyclonal development as to the deletion of the 9q allele and that the loss of chromosome 9 might not be an early genetic event associated with tumorigenesis.

In conclusion, reduced expression of the A antigen in bladder cancer reflects allelic loss of the *ABO* gene assigned to 9q34.1 and/or hypermethylation of its promoter region, which is a specific marker for genetic and epigenetic alterations in bladder cancer but not in dysplasia.

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# A genetic variant in the gene encoding the stress70 protein chaperone family member *STCH* is associated with gastric cancer in the Japanese population

Masayuki Aoki [a], Ken Yamamoto [a], Shigekazu Ohyama [b], Yoshitaka Yamamura [c], Seiichi Takenoshita [d], Kokichi Sugano [e], Toshinari Minamoto [f], Masaki Kitajima [g], Haruhiko Sugimura [h], Shinya Shimada [i], Hirokazu Noshiro [j], Masahiro Hiratsuka [k], Motonori Sairenji [l], Itasu Ninomiya [m], Masahiko Yano [n], Katsuhiko Uesaka [o], Seiki Matsuno [p], Yoshihiko Maehara [q], Takashi Aikou [r], Takehiko Sasazuki [s,*]

[a] *Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan*
[b] *Department of GI Surgery, Cancer Institute Hospital, Tokyo 170-8455, Japan*
[c] *Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya 464-8681, Japan*
[d] *Second Department of Surgery, Fukushima Medical University, Fukushima 960-1295, Japan*
[e] *Oncogene Research Unit/Cancer Prevention Unit, Tochigi Cancer Center Research Institute, Utsunomiya 320-0834, Japan*
[f] *Division of Diagnostic Molecular Oncology and Surgical Oncology, Cancer Research Institute, Kanazawa University, Kanazawa 920-0934, Japan*
[g] *Department of Surgery, School of Medicine, Keio University, Tokyo 160-8582, Japan*
[h] *Department of Pathology, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan*
[i] *Second Department of Surgery, Kumamoto University School of Medicine, Kumamoto 860-8556, Japan*
[j] *Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan*
[k] *Department of Surgery, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka 537-8511, Japan*
[l] *Department of Gastrointestinal Surgery, Kanagawa Cancer Center, Yokohama 241-0815, Japan*
[m] *Gastroenterologic Surgery, Department of Oncology, Division of Cancer Medicine, Graduate School of Medical Science, Kanazawa University, Kanazawa 920-8641, Japan*
[n] *Department of Surgery and Clinical Oncology, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan*
[o] *Surgical Oncology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan*
[p] *First Department of Surgery, Tohoku University School of Medicine, Sendai 980-8575, Japan*
[q] *Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan*
[r] *First Department of Surgery, Kagoshima University School of Medicine, Kagoshima 890-8520, Japan*
[s] *International Medical Center of Japan, Tokyo 162-8655, Japan*

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**Abstract**

Association analysis, based on linkage disequilibrium between specific alleles in the candidate loci and nearby genetic markers, has been proposed to identify genes conferring susceptibility to multifactorial diseases. Using the affected sib-pair method, we previously mapped four candidate chromosomal regions, 1p32, 2q33–q35, 11p13–p14, and 21q21, for gastric cancer by linkage analysis. To identify genes involved in the disease, we performed a gene-based association analysis of 66 genes, located on 21p11–21q22, using 126 single nucleotide polymorphisms (SNPs) as genetic markers in 373 patients with 250 controls. We found a significant association of five SNPs in the stress70 protein chaperon family member *STCH* gene with gastric cancer, especially with the non-cardia localization subgroup ($P = 0.0005$–$0.02$, odds ratio = 1.44–1.72). Comparisons of haplotype frequency showed significant association

* Corresponding author. Fax: +81 3 5273 0113.
*E-mail address:* sasazuki@nciryo.hosp.go.jp (T. Sasazuki).

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between *TTGGC* haplotype and gastric cancer ($P = 0.0001$, odds ratio = 1.59). These results suggest that, in the Japanese population, *STCH* might be a new candidate for conferring susceptibility to this disease.


*Keywords:* Gastric cancer; Polymorphism; Single nucleotide polymorphism; Linkage disequilibrium; Association study; STCH; Chromosome 21q

Despite a continuous decline in gastric cancer (GC) incidence and mortality over the past decades, GC still remains the second leading cause of cancer death worldwide [1]. The pathogenesis of GC is multifactorial; several genetic and environmental factors, including lifestyle, diet, and *Helicobacter pylori* (*H. pylori*) infection, probably contribute to its development and progression, although the precise mechanisms are still unclear [2–4]. Because cumulative epidemiological studies have provided evidence that genetic factors play an important role in the pathogenesis of GC, efforts to identify genetic susceptibility to GC have been extensive [5–10]. These studies focused on candidate genes that encode proteins thought to be involved in carcinogenesis, such as those involved in cell cycle, apoptosis, carcinogen metabolism, DNA repair or those known to be somatically altered in cancer. However, the approach is limited by its reliance on current knowledge in selecting candidate genes based on function.

Sequencing of the entire human genome has been completed and information on the location of almost all genes is now available [11]. Hence, physical mapping by linkage analysis enables the efficient search for genes responsible for a given human trait. We recently conducted a genome-wide linkage analysis, using affected sib-pairs with GC, and found these candidate chromosomal regions: 1p32, 2q33–q35, 11p13–p14, and 21q21 which showed nominal evidence for linkage with the multipoint LOD sore ($P < 0.01$), 1.26, 1.74, 1.74, and 1.39, respectively [12]. Among these regions, 21q was reported to show frequent loss of heterozygosity (LOH) in the Japanese population [13–15]. In addition, chromosome 21 is relatively short in physical length and contains a countable number of genes in the candidate region 21p11–21q22. Thus, we attempted to search for GC-susceptible gene(s), by an association study, in this chromosomal region.

In this report, we show the results of gene-based single nucleotide polymorphism (SNP) analyses, using a panel of Japanese GC patients, for 66 genes located in the region 21p11–21q22. Our data suggest that the gene encoding *STCH*, a microsome-associated member of the stress70 protein chaperone family (MIM601100), is likely to contribute, in part, to genetic susceptibility to GC.

## Materials and methods

*Study subjects and SNP genotyping.* This study was reviewed and approved by the Ethics Committee of Kyushu University, Faculty of Medicine, Fukuoka, Japan. Peripheral blood samples were obtained from unrelated Japanese individuals who provided written informed consent before enrolling in the study. The patients were recruited from the hospitals all over the country: Cancer Institute Hospital, Aichi Cancer Center Hospital, Fukushima Medical University, Tochigi Cancer Center Research Institute, Kanazawa University, Keio University, Hamamatsu University School of Medicine, Kumamoto University School of Medicine, Kyushu University, Osaka Medical Center Kanagawa Cancer Center, Osaka University, Nagoya University, Tohoku University, or Kagoshima University and health controls were recruited from the Fukuoka area. DNA extraction was performed using QIAamp DNA Blood kit (Qiagen, City, Country). The diagnosis of GC was confirmed pathologically, and 373 patients (mean age at diagnosis 49.6 years old; 55.9% male) and 250 controls (mean age 56.5 years old; 57.6% male) were enrolled.

Sixty-six genes on chromosome 21p11–21q22 were analyzed by SNPs, which were validated by the TaqMan Validated SNP Genotyping Assays (Applied Biosystems). A total of 126 SNPs, at least one SNP per gene, were genotyped by Assay-On-Demand primer and probe sets (Applied Biosystems), using ABI PRISM 7900HT (Applied Biosystems), according to the manufacturer's protocol.

*Statistics.* $\chi^2$ tests were employed to evaluate statistical differences in genotype distributions and allele frequencies of each SNP between GC and control groups. Genotype distributions of tested polymorphism were compatible with the Hardy–Weinberg equilibrium. A $P$ values less than 0.05 were considered statistical significant. Since several reports suggested different etiologies in subsites or histological subtypes [16–18], association analyses were also done for subgroups stratified on tumor localization and histology. Linkage disequilibrium (LD) was evaluated by Lewontin's $D'$ ($|D'|$), running all pairs of biallelic loci [19]. All statistical analyses were performed with SNPAlyze ver. 4.0 Pro software (Dynacom) [20].

## Results

A total of 126 SNPs, listed in (Table 1), were genotyped for 373 GC patients and 250 control subjects. Location of these SNPs in each gene was as follows: 98 SNPs in intron, five SNPs synonymous cSNP, five SNPs non-synonymous cSNP, one SNP in 5′ untranslated region (5′ UTR), eight SNPs in 3′ UTR, three SNPs upstream of the 5′-end of the first exon (5′ upstream), five SNPs downstream of the 3′-end of the last exon (3′ downstream), and one SNP in 5′ or 3′ UTR of different two genes. These SNPs covered 66 genes and frequencies of the minor allele observed in control subjects were between 0.01 and 0.50 (average was 0.27). Association analysis revealed that nine SNPs showed a significant difference ($P < 0.05$) in allele frequency between the two groups (Table 1). Among them, a series of SNPs locating in the *STCH* gene were significantly associated with GC ($P$ value of allele frequency: 0.002–0.020; genotype frequency: 0.025–0.036; Table 2).

Table 1
List of SNPs analyzed in this study

| Gene symbol | dbSNP ID | Location | Chromosome position[a] | Minor allele frequency[b] | *P* value[c] |
|---|---|---|---|---|---|
| *BAGE2* | rs209033 | Intron | 10079879 | 0.21 | 0.192 |
| *LIP1* | rs1573435 | Intron | 14422273 | 0.04 | 0.669 |
| *RBM11* | rs424021 | Intron | 14515879 | 0.16 | 0.200 |
| *ABCC13* | rs1012999 | Intron | 14581421 | 0.36 | 0.821 |
| *STCH* | rs9982492 | 3′ downstream | 14657546 | 0.46 | 0.0121* |
| | rs12479 | 3′ UTR | 14667140 | 0.45 | 0.0199* |
| | rs1882881 | Intron | 14676857 | 0.46 | 0.0024* |
| | rs2242661 | 5′ upstream | 14677692 | 0.44 | 0.0063* |
| | rs2142239 | 5′ upstream | 14686886 | 0.49 | 0.0661 |
| *SAMSN1* | rs2822698 | Intron | 14783504 | 0.34 | 0.548 |
| | rs760344 | Intron | 14815438 | 0.24 | 0.809 |
| *NRIP1* | rs2228507 | Coding, non-synonymous | 15261441 | 0.21 | 0.535 |
| *C21orf116* | rs1041404 | 3′ downstream | 15346738 | 0.35 | 0.374 |
| *USP25* | rs926612 | Intron | 16028181 | 0.07 | 0.428 |
| | rs2823491 | Intron | 16076627 | 0.11 | 0.094 |
| | rs2026853 | Intron | 16121160 | 0.17 | 0.578 |
| | rs1022457 | Intron | 16161602 | 0.24 | 0.177 |
| *CXADR* | rs211956 | Intron | 17814837 | 0.22 | 0.742 |
| | rs764657 | Intron | 17848524 | 0.45 | 0.557 |
| *BTG3* | rs2824389 | Intron | 17900047 | 0.41 | 0.100 |
| *C21orf91* | rs243556 | Intron | 18112298 | 0.10 | 0.352 |
| *CHODL* | rs1051520 | 3′ UTR | 18561134 | 0.03 | 0.681 |
| | rs1051526 | 3′ UTR | 18561362 | 0.02 | 0.773 |
| *PRSS7* | rs1540000 | Intron | 18588772 | 0.07 | 0.181 |
| | rs1023388 | Intron | 18639112 | 0.09 | 0.277 |
| | rs719521 | Intron | 18691902 | 0.11 | 0.458 |
| | rs2824806 | Intron | 18693121 | 0.14 | 0.684 |
| *C21orf42* | rs2829747 | Intron | 25721664 | 0.22 | 0.832 |
| *MRPL39* | rs1537087 | Intron | 25886699 | 0.44 | 0.316 |
| *JAM2* | rs2026222 | Intron | 25954499 | 0.32 | 0.258 |
| | rs2829877 | 3′ UTR | 26008915 | 0.11 | 0.367 |
| *GABPA* | rs7283438 | Intron | 26031386 | 0.03 | 0.831 |
| | rs1573269 | Intron | 26051631 | 0.41 | 0.412 |
| *APP* | rs2070653 | Intron | 26185464 | 0.15 | 0.246 |
| | rs380417 | Intron | 26194030 | 0.44 | 0.799 |
| | rs216773 | Intron | 26241805 | 0.11 | 0.735 |
| | rs2070657 | Intron | 26276888 | 0.40 | 0.885 |
| | rs2830012 | Intron | 26319478 | 0.45 | 0.786 |
| | rs2070655 | Intron | 26345432 | 0.44 | 0.686 |
| | rs2234988 | Intron | 26398958 | 0.40 | 0.419 |
| | rs400154 | Intron | 26455793 | 0.18 | 0.829 |
| *CYYR1* | rs966410 | Coding, synonymous | 26774595 | 0.22 | 0.925 |
| *ADAMTS1* | rs436525 | Coding, synonymous | 27134631 | 0.43 | 0.659 |
| *ADAMTS5* | rs229076 | 3′ UTR | 27216730 | 0.01 | 0.499 |
| *C21orf23* | rs933154 | Intron | 28038046 | 0.17 | 0.476 |
| *C21orf127* | rs2293996 | Intron | 29176499 | 0.04 | 0.346 |
| *ZNF294* | rs2250574 | Coding, synonymous | 29240078 | 0.08 | 0.020* |
| | rs2247324 | Intron | 29247801 | 0.43 | 0.208 |
| *C21orf6* | rs11170 | 3′ UTR | 29299961 | 0.24 | 0.908 |
| *USP16* | rs2832156 | Intron | 29336473 | 0.19 | 0.876 |
| *CCT8* | rs2070610 | 5′ UTR | 29367819 | 0.08 | 0.331 |
| *C21orf7* | rs2832231 | Intron | 29458817 | 0.45 | 0.443 |
| *BACH1* | rs1153292 | Intron | 29611066 | 0.12 | 0.432 |
| *GRIK1* | rs1011794 | Intron | 29839001 | 0.26 | 0.104 |
| | rs2253443 | Intron | 29879378 | 0.27 | 0.507 |
| | rs363582 | Intron | 29945050 | 0.05 | 0.529 |
| | rs2070395 | Intron | 29987968 | 0.18 | 0.752 |
| | rs2284462 | Intron | 30042996 | 0.16 | 0.971 |
| | rs458685 | Intron | 30099382 | 0.22 | 0.658 |
| | rs1882826 | Intron | 30138972 | 0.36 | 0.607 |
| | rs455477 | Intron | 30192745 | 0.21 | 0.787 |
| *CLDN17* | rs681521 | 3′ downstream | 30453595 | 0.14 | 0.466 |

Table 1 (*continued*)

| Gene symbol | dbSNP ID | Location | Chromosome position[a] | Minor allele frequency[b] | *P* value[c] |
|---|---|---|---|---|---|
| *CLDN8* | rs686364 | Coding, non-synonymous | 30509664 | 0.42 | 0.519 |
| *TIAM1* | rs723470 | Intron | 31447173 | 0.43 | 0.013* |
| | rs723469 | Intron | 31447216 | 0.40 | 0.310 |
| | rs2833334 | Intron | 31496283 | 0.38 | 0.459 |
| | rs2833347 | Intron | 31527251 | 0.41 | 0.045* |
| | rs2833350 | Intron | 31544529 | 0.41 | 0.070 |
| | rs2251496 | Intron | 31583846 | 0.38 | 0.095 |
| | rs845937 | Intron | 31595078 | 0.37 | 0.714 |
| | rs13045983 | Intron | 31608700 | 0.12 | 0.838 |
| | rs1783006 | Intron | 31645060 | 0.47 | 0.782 |
| | rs550710 | Intron | 31681143 | 0.30 | 0.796 |
| | rs743414 | Intron | 31737828 | 0.29 | 0.045* |
| | rs502986 | Intron | 31740639 | 0.06 | 0.898 |
| | rs2284518 | Intron | 31751123 | 0.15 | 0.535 |
| | rs2833439 | Intron | 31828941 | 0.12 | 0.979 |
| *SOD1* | rs2070424 | Intron | 31961191 | 0.47 | 0.898 |
| *SFRS15* | rs1544 | Intron | 32007456 | 0.34 | 0.239 |
| *HUNK* | rs2211781 | Intron | 32171165 | 0.32 | 0.114 |
| | rs1892650 | Intron | 32219677 | 0.12 | 0.262 |
| | rs2032084 | Intron | 32276635 | 0.44 | 0.950 |
| *C21orf45* | rs3818 | 3′ downstream | 32554058 | 0.40 | 0.564 |
| *C21orf61, C21orf108* | rs2833761[d] | 3′ UTR, 5′ UTR | 32606286 | 0.19 | 0.084 |
| *C21orf108* | rs2010203 | Intron | 32654580 | 0.38 | 0.728 |
| *C21orf63* | rs2833813 | Intron | 32720360 | 0.41 | 0.737 |
| | rs762174 | Intron | 32754813 | 0.47 | 0.017* |
| | rs958342 | Intron | 32787774 | 0.40 | 0.966 |
| *C21orf77* | rs2833906 | Intron | 32867392 | 0.30 | 0.193 |
| *TCP10L* | rs6517105 | Coding, non-synonymous | 32872939 | 0.30 | 0.590 |
| *C21orf59* | rs2070382 | Intron | 32898484 | 0.06 | 0.968 |
| *SYNJ1* | rs844978 | Intron | 32940308 | 0.50 | 0.966 |
| | rs844975 | Intron | 32981078 | 0.50 | 0.906 |
| *C21orf49, C21orf62* | rs1557264[e] | Intron | 33089514 | 0.10 | 0.313 |
| *OLIG2* | rs1005573 | Intron | 33320586 | 0.36 | 0.939 |
| *IFNGR2* | rs2070386 | Intron | 33709508 | 0.11 | 0.091 |
| | rs2834229 | Intron | 33756597 | 0.16 | 0.959 |
| *GART* | rs9984077 | Coding, non-synonymous | 33818983 | 0.04 | 0.230 |
| *SON* | rs2070389 | Intron | 33851292 | 0.12 | 0.064 |
| *ITSN1* | rs2070391 | Intron | 33949012 | 0.24 | 0.260 |
| | rs2834251 | Intron | 34006791 | 0.25 | 0.197 |
| | rs2834259 | Intron | 34054961 | 0.38 | 0.424 |
| *ATP5O* | rs2834301 | 5′ upstream | 34211116 | 0.32 | 0.570 |
| *C21orf51* | rs8742 | 3′ UTR | 34696768 | 0.39 | 0.812 |
| *KCNE1* | rs1012944 | Intron | 34747136 | 0.15 | 0.651 |
| *DSCR1* | rs1012599 | Intron | 34817502 | 0.21 | 0.386 |
| | rs2070366 | Intron | 34868072 | 0.24 | 0.577 |
| *CLIC6* | rs2070368 | Intron | 35002268 | 0.38 | 0.434 |
| *RUNX1* | rs2268276 | Intron | 35102884 | 0.39 | 0.225 |
| | rs2268282 | Intron | 35139672 | 0.46 | 0.206 |
| | rs2248734 | Intron | 35154352 | 0.16 | 0.152 |
| | rs8130963 | Intron | 35189288 | 0.14 | 0.675 |
| | rs1892687 | Intron | 35233892 | 0.30 | 0.376 |
| | rs743342 | Intron | 35282303 | 0.29 | 0.066 |
| *C21orf18* | rs880221 | Intron | 36342655 | 0.30 | 0.865 |
| *CBR3* | rs879892 | Intron | 36430748 | 0.46 | 0.605 |
| *C21orf5* | rs1023367 | Intron | 36541594 | 0.45 | 0.742 |
| *ZCWCC3* | rs2236433 | Coding synonymous | 36614459 | 0.46 | 0.383 |
| *CHAF1B* | rs13049605 | Intron | 36694708 | 0.38 | 0.411 |
| | rs218634 | 3′ downstream | 36716791 | 0.03 | 0.271 |
| *CLDN14* | rs128494 | Intron | 36756128 | 0.46 | 0.237 |
| *SIM2* | rs743428 | Intron | 37012878 | 0.45 | 0.763 |
| | rs2073601 | Coding non-synonymous | 37039178 | 0.04 | 0.917 |

(*continued on next page*)

Table 1 (*continued*)

| Gene symbol | dbSNP ID | Location | Chromosome position[a] | Minor allele frequency[b] | *P* value[c] |
|---|---|---|---|---|---|
| *HLCS* | rs14407 | 3′ UTR | 37045795 | 0.42 | 0.597 |
| | rs1009778 | Intron | 37190729 | 0.45 | 0.373 |
| | rs1065758 | Coding synonymous | 37230781 | 0.06 | 0.679 |

[a] Chromosome position of SNP is from the dbSNP build 124 in the database of National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).
[b] Minor allele frequencies observed in control samples are shown.
[c] *P* values of $\chi^2$ test in allele frequency difference are shown.
[d] This SNP locates in 3′ UTR of *C21orf61* and 5′ UTR of *C21orf108*.

The intronic SNP (rs1882881) was significantly associated with the disease on the assumption of recessive mode of inheritance (Table 2).

Subsequent LD analysis of five SNPs spanning 30 kb of the *STCH* gene showed nearly complete LD ($D' = 0.99$) (data not shown). To investigate if particular a haplotype, constituted by these SNPs, was associated with the disease, haplotype frequencies were estimated and association analysis carried out. As shown in Table 3, the most frequent haplotype *TTGGC* was significantly associated with risk of GC (OR = 1.59, $P = 0.0001$). Since genotype information for the two SNPs in *STCH* (rs2242661/rs12479) was available in the International HapMap Project database (http://www.hapmap.org), haplotype frequencies for the two loci in different ethnic groups were calculated and compared with our subjects. The frequency of the TG haplotype of rs2242661/rs12479 in the GC group (52.3%) was significantly higher than the control group (43.5%) (OR = 1.40, $P = 0.005$), in addition to the frequencies in CEU (Utah residents with ancestry from northern and western Europe, 48.9%), HCB (Han Chinese in Beijing, China, 45.6%), and YRI (Yoruba in Ibadan, Nigeria, 33.9%), supporting the association of this *STCH* haplotype with GC.

To investigate if a particular subgroup of GC is associated with *STCH*, GC samples were stratified on subsites and histological subtypes, and association analyses with *STCH* were done. As shown in Table 4, SNPs in *STCH* showed a significant association with GC ($P = 0.0005$–$0.02$) in allele frequency for the non-cardia tumor localization group, whereas none of the five SNPs achieved a statistically significant level in the cardia group. On the other hand, the diffuse-type histology subgroup showed an association with *STCH* ($P = 0.02$–$0.04$); however, this association was less significant than that observed in the non-cardia group.

## Discussion

In a gene-based case-control study, which focused on chromosomal region 21p11–q22, using SNPs as genetic markers, we identified the *STCH* gene as a candidate for conferring susceptibility to GC in the Japanese population. Since direct association analysis, using functional variants, is currently limited by incomplete knowledge about functional variation, indirect association mapping, using marker SNPs, has been considered to identify genes involved in common diseases, such as gastric cancer [21–23]. Recent studies employing large-scale gene-based association analysis have revealed genes conferring susceptibility to common diseases, such as myocardial infarction, rheumatoid arthritis, and diabetic nephropathy [24–27]. We have recently shown candidate chromosomal regions for GC by family-based linkage analysis [12], and subsequently applied gene-based SNPs mapping to a promising candidate region, 21p11–q22, in this study. In contrast to genome-wide SNP mapping, this strategy was more cost-effectively designed.

The *STCH* gene belongs to the *HSP70*-like family, which encodes highly conserved ATPase molecules essential for viability of all living organisms, and its mRNA is constitutively expressed in all human cell types [28,29]. Different from other *HSP70* family genes, *STCH* is induced by the $Ca^{2+}$ ionophore A23187, but not by heat shock and, structurally, it lacks a C-terminal peptide-binding domain, well-conserved in the *HSP70* family [28,29]. This truncated structure is conserved in *Caenorhabditis elegance*, rat and human tissue, and is similar to a proteolytically cleaved N-terminal ATPase fragment of *Hsp70/Hsc70* [29]. This cleaved fragment of *Hsp70/Hsc70* was shown to be regulated by *Bag1*, which binds to *Bcl-2* as its cellular target, and is highly expressed in gastrointestinal epithelial cells [30,31]. Moreover, the N-terminal ATPase domain of this gene was reported to interact with ubiquitin-like proteins, *Chap1* and *Chap2*, which can modulate transit through the G2/M phase of the cell cycle or the apoptotic mechanism, respectively [32]. Although our current knowledge on the molecular function of *STCH* is limited, these findings suggest a broader role of *STCH* in regulating cell cycle and apoptosis-related events leading to tumorigenesis.

Our present study showed association of *STCH* with the non-cardia subsite group of GC, but not the cardia group. The precise mechanism accounting for this asso-

Table 2
Genotype and allele frequencies of five SNPs in *STCH* in GC cases and controls

| SNP | Location | Genotype | | | | Allele | | | | | Recessive model | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Control (%) | GC (%) | *P* value | | Control (%) | GC (%) | OR [95% CI] | *P* value | | Control | GC | OR [95%CI] | *P* value |
| rs2142239 | 5′ upstream | C/C | 67 (28) | 78 (21) | 0.130 | C | 239 (51) | 331(45) | 1.24 [0.98–1.56] | 0.066 | CC + CT | 67 | 78 | 1.20 [0.83–1.72] | 0.32 |
| | | C/T | 105 (44) | 175 (48) | | T | 233 (49) | 401 (55) | | | TT | 169 | 288 | | |
| | | T/T | 64 (27) | 113 (31) | | | | | | | | | | | |
| rs2242661 | 5′ upstream | C/C | 76 (32) | 86 (23) | 0.025* | C | 270 (56) | 353 (48) | 1.38 [0.98–1.56] | 0.006* | CC + CT | 194 | 267 | 1.56 [1.05–2.32] | 0.03* |
| | | C/T | 118 (49) | 181 (49) | | T | 210 (44) | 379 (52) | | | TT | 46 | 99 | | |
| | | T/T | 46 (19) | 99 (27) | | | | | | | | | | | |
| rs1882881 | Intron 1 | G/G | 73 (29) | 77 (21) | 0.025* | G | 270 (54) | 330 (45) | 1.42 [1.13–1.78] | 0.002* | GG + GT | 197 | 253 | 1.65 [1.12–2.39] | 0.009* |
| | | G/T | 124 (50) | 176 (48) | | T | 230 (46) | 400 (55) | | | TT | 53 | 112 | | |
| | | T/T | 53 (21) | 112 (31) | | | | | | | | | | | |
| rs12479 | 3′ UTR | G/G | 71 (29) | 135 (38) | 0.051 | G | 272 (55) | 439 (61) | 1.32 [1.04–1.66] | 0.020* | GG + GA | 201 | 304 | 1.34 [0.87–2.06] | 0.17 |
| | | G/A | 130 (52) | 169 (47) | | A | 226 (45) | 277 (39) | | | AA | 48 | 54 | | |
| | | A/A | 48 (19) | 54 (15) | | | | | | | | | | | |
| rs9982492 | 3′ downstream | C/C | 70 (28) | 136 (38) | 0.036* | C | 269 (54) | 444 (62) | 1.35 [1.06–1.69] | 0.012* | CC + CT | 199 | 308 | 1.43 [0.92–2.19] | 0.10 |
| | | C/T | 129 (52) | 172 (48) | | T | 225 (46) | 276 (38) | | | TT | 48 | 52 | | |
| | | T/T | 48 (19) | 52 (14) | | | | | | | | | | | |

* *P* value < 0.05.

Table 3
Frequencies of major haplotypes constituted by five or two SNPs in *STCH* in GC cases and controls

| Haplotype: five loci[a] | Control (%, *n* = 466) | GC (%, *n* = 680) | OR [95%CI][b] | *P* value | Haplotype: two loci[c] | Control (%, *n* = 478) | GC (%, *n* = 702) | OR [95%CI] | *P* value | CEU[d] (%, *n* = 180) | HCB[d] (%, *n* = 90) | YRI[d] (%, *n* = 180) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *TTTGC* | 40.6 | 52.2 | 1.59 [1.25–2.01] | 0.0001* | *TG* | 43.5 | 52.3 | 1.40 [1.10–1.76] | 0.005* | 48.9 | 45.6 | 33.9 |
| *CCGAT* | 41.4 | 37.6 | 1.16 [0.91–1.47] | 0.23 | *CA* | 44.7 | 38.6 | 1.28 [1.01–1.62] | 0.04* | 27.2 | 40.0 | 15.6 |
| *CCGGC* | 4.8 | 4.3 | 1.11 [0.63–1.96] | 0.71 | *CG* | 11.5 | 8.9 | 1.27 [0.87–1.86] | 0.21 | 23.9 | 14.1 | 50.6 |
| *TCGGC* | 4.5 | 3.1 | 1.48 [0.78–2.78] | 0.22 | | | | | | | | |
| *CCTGC* | 2.4 | 1.7 | 1.47 [0.63–3.42] | 0.37 | | | | | | | | |

[a] Five major haplotypes constituted by rs2142239, rs2242661, rs1882881, rs12479, and rs9982492 are shown.
[b] OR of each haplotype is relative value to other haplotypes.
[c] Three major haplotypes constituted by rs2242661 and rs12479 are shown.
[d] Frequencies of three haplotypes constituted by rs2242661 and rs12479 in CEU (Utah residents with ancestry from northern and western Europe), HCB (Han Chinese in Beijing, China), and YRI (Yoruba in Ibadan, Nigeria) were calculated based on the database of International HapMap Project (http://www.hapmap.org).
* *P* value < 0.05.

Table 4
Allele frequencies of five SNPs in *STCH* in subgroups of GC

| SNP | Controls (%) | | Tumor localization subgroup | | | | | | Histological subtypes | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cardia | | | Non-cardia | | | Diffuse type | | | Intestinal type | | |
| | | | (%) | OR [95%CI] | *P* value | (%) | OR [95%CI] | *P* value | (%) | OR [95%CI] | *P* value | (%) | OR [95%CI] | *P* value |
| rs2142239 | C | 239 (51) | 30 (44) | 1.30 [0.77–2.16] | 0.31 | 110 (42) | 1.44 [1.05–1.94] | 0.02* | 206 (45) | 1.23 [0.95–1.59] | 0.11 | 109 (48) | 1.12 [0.81–1.53] | 0.48 |
| | T | 233 (49) | 38 (56) | | | 154 (58) | | | 248 (55) | | | 119 (52) | | |
| rs2242661 | C | 270 (56) | 33 (49) | 1.36 [0.81–2.26] | 0.23 | 117 (44) | 1.62 [1.19–2.18] | 0.0018* | 221 (49) | 1.36 [1.04–1.75] | 0.02* | 115 (50) | 1.26 [0.92–1.73] | 0.15 |
| | T | 210 (44) | 35 (51) | | | 147 (56) | | | 233 (51) | | | 113 (50) | | |
| rs1882881 | G | 270 (54) | 30 (45) | 1.41 [0.84–2.35] | 0.19 | 103 (41) | 1.72 [1.26–2.33] | 0.0005* | 206 (46) | 1.37 [1.05–1.76] | 0.02* | 109 (47) | 1.35 [0.98–1.83] | 0.06 |
| | T | 230 (46) | 36 (55) | | | 151 (59) | | | 240 (54) | | | 125 (53) | | |
| rs12479 | G | 272 (55) | 40 (61) | 1.28 [0.75–2.15] | 0.36 | 161 (64) | 1.47 [1.07–2.00] | 0.02* | 268 (61) | 1.29 [0.99–1.67] | 0.05 | 137 (60) | 1.25 [0.90–1.71] | 0.17 |
| | A | 226 (45) | 26 (39) | | | 91 (36) | | | 172 (39) | | | 91 (40) | | |
| rs9982492 | C | 269 (54) | 40 (61) | 1.29 [0.76–2.17] | 0.35 | 166 (65) | 1.54 [1.12–2.10] | 0.0063* | 269 (61) | 1.32 [1.01–1.70] | 0.04* | 139 (60) | 1.28 [0.92–1.75] | 0.13 |
| | T | 225 (46) | 26 (39) | | | 90 (35) | | | 171 (39) | | | 91 (40) | | |

* *P* value $< 0.05$.

ciation is unclear. It has been reported that *H. pylori* infection is a GC risk factor, especially for tumors developing in the non-cardia subsite [33]. *H. pylori* infection affects the expression of the *Hsp70* gene family in mice, human, and rat gastric mucosa, which eventually causes to gastric tissue injury via *H. pylori*-induced inducible nitric oxide synthase (iNOS) [34,35]. In addition, it has been reported that the *H. pylori* infection activates NF-κB signaling pathway and, subsequently, interleukin-8 production, mainly through the $Ca^{2+}$/calmodulin system [36], suggesting that expression of *STCH* is affected by *H. pylori* infection as this gene is induced by the $Ca^{2+}$ ionophore A23187. Therefore, it may be hypothesized that the change in expression level of *STCH* by *H. pylori* infection is also involved in the gastric tissue injury pathway, hence, this gene is associated with the development of GC in the non-cardia subsite.

Although public databases such as the international HapMap project are currently available and expected to play a key role in trait and disease association studies, there were limited resources for selecting a subset of informative SNPs covering all LD blocks in 21p11–q22 when this study was designed. In addition, the SNPs locating in other genes such as *ZNF294*, *TIAM1*, and *C21orf63* showed weak association with GC ($P = 0.013$–$0.045$ in Table 1). Therefore, it should be considered that we could not detect other gene(s) than STCH which contribute to development of GC because of the limited number of SNPs and patients. Large number of SNPs covering all LD blocks and thousands of patients would be required to increase statistical power for a future association analysis. Regarding *STCH* it was not possible to determine which SNP of *STCH* was directly related to GC susceptibility in the present study. We should consider that another unidentified polymorphism, in strong LD with the analyzed SNPs, may contribute to functional differences among the haplotypes. Further analyses to test reproducibility are important in minimizing statistical type I errors and the significance of associated variants with GC should then be confirmed by functional studies.

In summary, we have attempted a gene-based association analysis for genes locating on the candidate chromosomal region of GC, 21p11–21q22. We identified the *STCH* gene as a candidate for conferring susceptibility to GC, especially to non-cardiac GC. These results suggest that *STCH* itself, as well as molecules involved in biological pathway related to its function, might be targets for prophylaxis or disease treatment.

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**Table 3.** Operative Details

| | Group A (n = 263) | Group B (n = 260) | Total (N = 523) | P |
|---|---|---|---|---|
| Gastrectomy, No. of patients | | | | .62 |
| Total | 102 | 97 | 199 | |
| Distal subtotal | 160 | 160 | 320 | |
| Proximal subtotal | 1 | 3 | 4 | |
| Splenectomy, No. of patients | 98 | 93 | 191 | .79 |
| Pancreatectomy, No. of patients | 9 | 13 | 22 | .39 |
| Operation time, minutes | | | | < .001 |
| Median | 237 | 300 | 270 | |
| Range | 127-625 | 153-600 | 127-625 | |
| Blood loss, mL | | | | < .001 |
| Median | 430 | 660 | 530 | |
| Range | 32-1,810 | 60-2,885 | 32-2,885 | |
| Blood transfusion | | | | < .001 |
| No. of cases | 37 | 78 | 115 | |
| % | 14.1 | 30.0 | 22.0 | |
| No. of retrieved nodes | | | | < .001 |
| Median | 54 | 74 | 61 | |
| Range | 14-161 | 30-235 | 14-235 | |

section before registration. In another case, the surgeon performed para-aortic dissection despite the allocation to group A because after randomization, he found a positive node behind the common hepatic artery, believed to be strongly suggestive of metastasis in the para-aortic area. The postoperative course of this patient, who was allocated to group A but treated as group B, was uneventful, and analyzing this patient as either group A or group B had no effect on the results in this study. We left this case in group A based on intention-to-treat analysis. In the other eight patients, nodal stations No.13 and/or No.14v were not dissected in distal third tumors.

In another case, the initial histological diagnosis following endoscopic biopsy was poorly differentiated adenocarcinoma but the final histology of the resected stomach revealed gastric lymphoma. We included this patient in the morbidity/mortality analysis, but will exclude their data from the final survival analyses.

### Operative Morbidity

The overall operative morbidity rate was 24.5%. The morbidity for group B patients was higher than group A (28.1% and 20.9%, respectively), but the difference did not reach statistical significance ($P = .067$). The incidence of the four major surgical complications was not different between the two groups (Table 4).

There were various other complications reported, and the incidence was significantly higher in group B than group A patients. Paralytic ileus causing significant delay of recommencement of oral feeding, abdominal and/or left pleural lymphorrhea requiring prolonged drainage for more than 1 week, and severe diarrhea, were specific to the extended para-aortic dissection group (Table 4). Reoperation was needed in 12 patients (2.3%), and there was no difference in the reoperation rate between the two groups. Median hospital stay after surgery was 21 days in group A, and 24 days in group B ($P < .01$).

### Hospital Mortality

There were four hospital deaths (0.8%)—two in each group. Each group had one patient who died of postoperative complications, and one died of rapidly progressive cancer. All other patients recovered from surgery and were discharged from hospital.

In this randomized controlled trial, the role of para-aortic dissection will be evaluated in terms of survival benefit,

**Table 4.** Operative Morbidity and Hospital Mortality

| | Group A (n = 263) | | Group B (n = 260) | | |
|---|---|---|---|---|---|
| | No. of Patients | % | No. of Patients | % | P |
| Any complication | 55 | 20.9 | 73 | 28.1 | .067 |
| Anastomotic leak | 6 | 2.3 | 5 | 1.9 | .99 |
| Pancreatic fistula | 14 | 5.3 | 16 | 6.2 | .71 |
| Abdominal abscess | 14 | 5.3 | 15 | 5.8 | .85 |
| Pneumonia | 12 | 4.6 | 4 | 1.5 | .072 |
| Others | 24 | 9.1 | 52 | 20.0 | < .001 |
| Obstruction or ileus | 5 | | 11 | | |
| Lymphorrhea | 0 | | 10 | | |
| Left pleural effusion | 1 | | 6 | | |
| Severe diarrhea | 0 | | 3 | | |
| Reoperation | 5 | 1.9 | 7 | 2.7 | .57 |
| Hospital death | 2 | 0.8 | 2 | 0.8 | .99 |

operative morbidity/mortality, and quality of life. The results will provide important information and should guide decision making regarding the choice of operative methods. The quality of life and survival among these patients are still in the follow-up phase, and the analyses will take place in 2004 and 2006, respectively. This report compares the morbidity and mortality rates of D2 plus para-aortic node dissection with standard D2 dissection.

There is a wide variation in operative morbidity and mortality following gastric cancer surgery among countries and institutions. The presence of comorbid disease that affects patient fitness for surgery, surgical experience of the operator, and the workload volume seem to be important factors.[17,18] The mortality for gastrectomy in Western countries often exceeds 5% and approaches 16% in some series.[19-21] Conversely, Japanese studies have consistently reported a mortality rate of lower than 2% in retrospective observations. To date, the present study is the first large-scale prospective randomized controlled trial in Japan to compare surgical techniques under strict quality control and data management. The extremely low hospital death rate after extended para-aortic lymphadenectomy (0.8%) in this multi-institutional setting confirms the findings from previous retrospective reports.

This trial is a striking contrast to the the Dutch[4] and British[5] D1/D2 trials, in which D2 lymphadenectomy was associated with operative mortality rates of 10% and 13%, respectively. One important criticism of the European randomized trials was the issue of learning curve, as many British and Dutch surgeons participating in the trials were new to the D2 procedure. Surgical experience, specific anatomic knowledge, and careful postoperative managements by experienced teams are crucial to the success of this type of surgery. An Italian group appropriately carried out a phase 2 study of D2 lymphadenectomy in selected institutions[22] until an acceptable operative mortality rate was achieved, before conducting a randomized controlled trial comparing D1 and D2 gastrectomies.

The D2 gastrectomy procedure is known as "extended lymphadenectomy" in Western countries, while Japanese surgeons employ D2 as a standard technique, and reserve the term "extended" for para-aortic dissection. Lymphatic drainage from the stomach flows to the perigastric nodes and then to the nodes around the celiac axis and its main branches. From here it enters the para-aortic nodes before joining the systemic circulation via the thoracic duct. Hence, the para-aortic nodes may be regarded as the final station of nodes that can be dissected to remove the threat of systemic metastases originating from the lymphatic system. Many Japanese surgeons in specialized centers who performed para-aortic dissection found microscopic metastases in this region, and believe that this type of surgery may be potentially worthwhile. However, the risk associated with para-aortic dissection dictates advanced operative skills and intensive postoperative care. Therefore, scientific evidence supporting a survival benefit must be obtained before employing this technique in routine gastric cancer surgery.

The very low operative morbidity and mortality achieved in this JCOG trial can be attributed to several factors: (1) we selected a group of fit patients who could tolerate para-aortic dissection in the study. (2) Only specialist surgeons with an established track record of extended lymphadenectomy participated in the trial. (3) High-throughput centers were selected for their operative skills and standardized postoperative management. (4) Pancreatectomy was avoided whenever possible, while splenectomy accompanied total gastrectomy in most cases. We report that there was no significant difference in the overall complications between the two groups; however, the para-aortic dissection group had significantly higher "other" complications (on free format) compared with standard D2. Lymphorrhea and paralytic ileus were more specific to this operation. This observation may be biased because of the surgeon's awareness of the patient's randomization arm of para-aortic dissection.

In the British and Dutch trials, splenectomy with or without distal pancreatectomy was highlighted as a major risk factor for operative morbidity and mortality.[5,23] Total gastrectomy for proximal tumor requires more advanced surgical skill and is associated with a higher morbidity compared to distal gastrectomy. Proximal gastric tumors are rapidly increasing in number in the western countries,[24,25] while the incidence remains stable in Japan,[26] and this may partly explain the superior results obtained in Japanese studies. However, no difference was observed in the distribution of the primary tumor location between the Dutch[4] and the Japanese cohort. The proportion of total to distal gastrectomy was also very similar. Therefore, variation in tumor location and type of gastrectomy could not account for the difference in morbidity/mortality, at least between these trials. JCOG recently launched a randomized controlled trial to evaluate the role of splenectomy combined with total gastrectomy in proximal tumors.[27]

Gastric cancer, though decreasing in incidence worldwide, remains a major health problem in many countries. R0 (no residual disease) resection is the only curative measure; but the more extended the surgery, it is believed the greater is the risk of operative morbidity and mortality. The type of gastrectomy and the extent of lymphadenectomy must be carefully planned for each individual patient with gastric cancer. The Japanese guidelines clearly define D2 gastrectomy as standard surgery[28] based on the excellent results in Japanese studies, while the British cancer guidance[6] discourages D2 based on the poor results of their randomized trial. This contrast should be addressed by surgeons' efforts, such as establishment of specialized standard training systems or production of evidence by high-quality randomized trials in specialized centers.

In conclusion, this study has shown that specialized surgeons could safely perform gastrectomy with D2 lymphadenectomy in patients with low operative risks. Extending the surgery to para-aortic lymphadenectomy did not increase the major operative complications and hospital deaths. However, compared with the D2 procedure, para-aortic dissection requires a longer operation time, leads to a larger volume of blood loss, and longer hospital stay. Until survival benefits are clarified when the data mature sufficiently, para-aortic lymphadenectomy for gastric cancer should be regarded as experimental surgery[28] and only performed in specialized institutions within the context of a well-designed clinical trial.

## Appendix

The appendix is included in the full-text version of this article, available on-line at www.jco.org. It is not included in the PDF (via Adobe® Acrobat Reader®) version.

## Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.


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Short Communication

# No association between fruit or vegetable consumption and the risk of colorectal cancer in Japan

**Y Tsubono*[,1], T Otani[2], M Kobayashi[2], S Yamamoto[3], T Sobue[3] and S Tsugane[2] for the JPHC Study Group[4]**

*[1]Division of Health Policy, Tohoku University School of Public Policy, Kawauchi, Sendai 980-8576, Japan; [2]Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045, Japan; [3]Statistics and Cancer Control Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045, Japan*

In a pooled analysis of two prospective studies with 88 658 Japanese men and women, fruit and vegetable consumptions, were not associated with a lower risk of colorectal cancer (705 cases); multivariate relative risk (95% confidence interval) for the highest *vs* the lowest quartile of intake being 0.92 (0.70–1.19) and 1.00 (0.79–1.27), respectively.
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Although fruit and vegetables have been suggested to confer protection against colorectal cancer, recent prospective studies in Western populations found no or limited associations (Michels *et al*, 2000; Voorrips *et al*, 2000). In Japan, mortality from colorectal cancer increased during 1950–2000, especially in men (age-adjusted rate per 100 000 of 2.9–14.4 for colon and 5.6–9.3 for rectum in men; 3.3–9.5 for colon and 4.2–4.1 for rectum in women) (Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labor, and Welfare of Japan, 2003). Dietary factors may play a part in this increase, but the role of fruit and vegetables remains unclear. We therefore examined the association between fruit and vegetable consumption and the risk of colorectal cancer in the Japan Public Health Center (JPHC) prospective study on cancer and cardiovascular disease.

## MATERIALS AND METHODS

The JPHC study has two population-based cohorts, and study designs are described in detail elsewhere (Otani *et al*, 2003). Briefly, Cohort I started in 1990 and included 40 106 subjects (19 345 men and 20 761 women) who were 40–59 years of age, lived in four Public Health Center districts, responded sufficiently to a self-administered questionnaire, and had no history of cancer (73.7% of the eligible subjects). Cohort II started in 1993 and included 48 552 subjects (23 180 men and 25 372 women) who were 40–69 years of age, lived in five Public Health Center districts, responded sufficiently to a self-administered questionnaire, and had no history of cancer (77.9% of the eligible subjects).

Cohort I questionnaire asked about the average consumption during the previous month of 44 food items including two fruit (fruit and fruit juice) and five vegetables (green leafy vegetables, yellow vegetables, white vegetables, pickled vegetables, and vegetable juice). Cohort II questionnaire asked about the average consumption during the previous month of 52 food items including three fruit (apples, oranges, and fruit juice) and six vegetables (green vegetables, carrot, tomatoes, green pickled vegetables, other pickled vegetables, and vegetable juice). The questionnaires had six frequency categories for fruit juice and vegetable juice that ranged from 'rarely' to '5 glasses day$^{-1}$', and four (Cohort I) or five (Cohort II) categories for other items that ranged from 'never' or 'rarely' to 'almost everyday'. The amount of consumption of total fruit and total vegetables (g day$^{-1}$) were calculated from these responses. We documented the questionnaire assessment of fruit and vegetable consumption to be reasonably valid (Kobayashi *et al*, 2002).

We followed up vital and residential status of subjects and incidence of cancer until the end of 1999. During 694 074 person-years of follow-up from the two cohorts, 705 cases of histologically confirmed colorectal cancer (456 colon and 249 rectum) were identified. Five percent of the subjects moved out of the study regions and 0.04% were lost to follow-up.

We used Cox's regression to compute from each cohort relative risk (RR) and 95% confidence interval (CI) of colorectal cancer according to quartiles of total fruit or vegetable consumption with adjustment for potential confounders. We pooled these estimates to obtain summary measures using inverse-variance weighting. As we observed no differential findings between the two cohorts, we present the pooled results only. This study has approximately 80% statistical power, with the two-sided $\alpha$-error level of 5%, in detecting a true RR of 0.75 among the highest *vs* lowest quartiles of total vegetable consumption.

## RESULTS

Compared with men in Cohort I in the lowest quartile of total vegetable consumption, men in the highest quartile were more likely to engage in sports and use vitamin supplements, less likely to be current smokers, and consumed higher amount of meats and fish, but lower amount of cereals. The men in the two groups did not differ with respect to age, body mass index, or the prevalence

*Correspondence: Dr Y Tsubono; E-mail: ytsubono@metamedica.com
[4]Study group members are listed in Appendix A at the end of this article
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**Table 1** Pooled multivariate RR and 95% CI of colorectal cancer for total fruit and total vegetable consumption[a]

| | Quartiles of total fruit consumption | | | | | Quartiles of total vegetable consumption | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Lowest** | **Second** | **Third** | **Highest** | **Trend *P*** | **Lowest** | **Second** | **Third** | **Highest** | **Trend *P*** |
| Person-years in Cohort I | 94 449 | 95 035 | 94 925 | 95 901 | | 94 394 | 94 936 | 95 360 | 95 620 | |
| Person-years in Cohort II | 78 632 | 78 285 | 78 545 | 78 303 | | 78 581 | 78 766 | 78 467 | 77 950 | |
| *Men and women* | | | | | | | | | | |
| Colorectum | | | | | | | | | | |
| No. of cases | 114/94 | 102/81 | 97/73 | 64/80 | | 100/85 | 91/84 | 95/78 | 91/81 | |
| RR (95% CI) | 1.00 | 0.89 | 0.88 | 0.92 (0.70–1.19) | 0.40 | 1.00 | 0.98 | 0.92 | 1.00 (0.79–1.27) | 0.80 |
| Colon | | | | | | | | | | |
| No. of cases | 77/56 | 70/51 | 66/48 | 43/45 | | 67/50 | 60/53 | 68/44 | 61/53 | |
| RR (95% CI) | 1.00 | 0.89 | 0.93 | 0.92 (0.66–1.28) | 0.61 | 1.00 | 0.99 | 0.96 | 1.08 (0.80–1.45) | 0.73 |
| Rectum | | | | | | | | | | |
| No. of cases | 37/38 | 32/30 | 31/25 | 21/35 | | 33/35 | 31/31 | 27/34 | 30/28 | |
| RR (95% CI) | 1.00 | 0.88 | 0.78 | 0.91 (0.59–1.40) | 0.47 | 1.00 | 0.95 | 0.84 | 0.87 (0.58–1.31) | 0.37 |
| *Men* | | | | | | | | | | |
| Colorectum | | | | | | | | | | |
| No. of cases | 90/80 | 81/61 | 61/43 | 10/28 | | 83/66 | 62/55 | 60/45 | 37/46 | |
| RR (95% CI) | 1.00 | 0.86 | 0.79 | 1.06 (0.70–1.61) | 0.34 | 1.00 | 0.95 | 0.82 | 1.18 (0.88–1.59) | 0.86 |
| Colon | | | | | | | | | | |
| No. of cases | 59/51 | 57/36 | 42/31 | 8/16 | | 57/40 | 42/36 | 41/27 | 26/31 | |
| RR (95% CI) | 1.00 | 0.83 | 0.86 | 1.02 (0.61–1.70) | 0.57 | 1.00 | 0.96 | 0.84 | 1.24 (0.86–1.79) | 0.69 |
| Rectum | | | | | | | | | | |
| No. of cases | 31/29 | 24/25 | 19/12 | 2/12 | | 26/26 | 20/19 | 19/18 | 11/15 | |
| RR (95% CI) | 1.00 | 0.91 | 0.68 | 1.19 (0.59–2.36) | 0.42 | 1.00 | 0.91 | 0.81 | 1.06 (0.63–1.78) | 0.81 |
| *Women* | | | | | | | | | | |
| Colorectum | | | | | | | | | | |
| No. of cases | 24/14 | 21/20 | 36/30 | 54/52 | | 17/19 | 29/29 | 35/33 | 54/35 | |
| RR (95% CI) | 1.00 | 1.02 | 1.15 | 0.93 (0.61–1.42) | 0.77 | 1.00 | 1.03 | 1.08 | 0.88 (0.57–1.35) | 0.48 |
| Colon | | | | | | | | | | |
| No. of cases | 18/5 | 13/15 | 24/17 | 35/29 | | 10/10 | 18/17 | 27/17 | 35/22 | |
| RR (95% CI) | 1.00 | 1.07 | 1.19 | 0.87 (0.49–1.52) | 0.86 | 1.00 | 1.09 | 1.25 | 1.01 (0.58–1.76) | 0.96 |
| Rectum | | | | | | | | | | |
| No. of cases | 6/9 | 8/5 | 12/13 | 19/23 | | 7/9 | 11/12 | 8/16 | 19/13 | |
| RR (95% CI) | 1.00 | 0.77 | 0.95 | 0.84 (0.43–1.65) | 0.77 | 1.00 | 0.96 | 0.84 | 0.71 (0.36–1.38) | 0.27 |

RR = relative risk; CI = confidence interval. [a]RRs have been adjusted for sex, age (5-year groups), Public Health Centre area, body mass index in kg m$^{-2}$ (less than 19, 19–22.9, 23–26.9, and 27 or more), frequency of sports (never or 1 day/month or more), smoking (never, past, and current), alcohol consumption (non, occasional, 1–149, 150–299, and 300 g week or more), vitamin supplement use, quartiles of energy, cereals, meats, and fish by each cohort. The lowest quartile serves as reference category. The numbers of colon and rectal cancers are from Cohort I/Cohort II.

of regular drinkers. We observed similar tendencies for women in Cohort I, and for men and women in Cohort II.

We found no significant association between fruit or vegetable intakes and the risk of colorectal cancer (Table 1). Multivariate RRs (95% CI) for the highest *vs* the lowest quartile of intake were 0.92(0.70–1.19) and 1.00(0.79–1.27), respectively, based on 705 cases. We observed no association whether or not colon and rectal cancers were separated, or men and women were separated. Exclusion of colorectal cancer cases diagnosed in the first 3 years of follow-up did not change the findings materially. Stratified analyses by covariates included in multivariate models did not reveal remarkable effect modifications. Analyses based on the octiles of total fruit or vegetable consumption did not show significant associations. No individual fruit or vegetables showed significant relations with risk.

## DISCUSSION

This is the first prospective cohort study of fruit and vegetable consumption and incident risk of colorectal cancer in Japan. Our results are consistent with the recent prospective studies in Western populations showing no substantial protective associations (Michels *et al*, 2000; Voorrips *et al*, 2000).

Our food frequency questionnaires had relatively small number of fruit and vegetable items and limited range of frequency categories. Nevertheless, we had observed in Cohort I an inverse association between fruit and vegetable intakes and the risk of gastric cancer (Kobayashi *et al*, 2002). It is therefore unlikely that failure to observe protective association was due to the crude designs of our questionnaires.

While mortality from colorectal cancer in Japan increased during 1950–2000, the average consumption of fruit and vegetables also increased during this period (42–117 and 242–311 g day$^{-1}$, respectively) (Kenko Eiyo Joho Kenkyukai, 2002). Our results, along with these time trends, suggest that low consumption of fruit and vegetables is not primarily responsible for the increased rate of colorectal cancer in Japan.

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## Appendix A

The members of the Japan Public Health Center-based Prospective Study (JPHC Study) Group are as follows: S Tsugane, M Inoue, T Sobue, T Hanaoka, National Cancer Center, Tokyo; J Ogata, S Baba, T Mannami, A Okayama, National Cardiovascular Center, Suita; K Miyakawa, F Saito, A Koizumi, Y Sano, I Hashimoto, Iwate Prefectural Ninohe Public Health Center, Ninohe; Y Miyajima, N Suzuki, S Nagasawa, Y Furusugi, Akita Prefectural Yokote Public Health Center, Yokote; H Sanada, Y Hatayama, F Kobayashi, H Uchino, Y Shirai, T Kondo, R Sasaki, Y Watanabe, Nagano Prefectural Saku Public Health Center, Saku; Y Kishimoto, E Takara, T Fukuyama, M Kinjo, M Irei, Okinawa Prefectural Chubu Public Health Center, Okinawa; K Imoto, H Yazawa, T Seo, A Seiko, F Ito, Katsushika Public Health Center, Tokyo; A Murata, K Minato, K Motegi, T Fujieda, Ibaraki Prefectural Mito Public Health Center, Mito; K Matsui, T Abe, M Katagiri, Niigata Prefectural Kashiwazaki Public Health Center, Kashiwazaki; M Doi, A Terao, Y Ishikawa, Kochi Prefectural Chuo-higashi Public Health Center, Tosayamada; H Sueta, H Doi, M Urata, N Okamoto, F Ide, Nagasaki Prefectural Kamigoto Public Health Center, Arikawa; H Sakiyama, N Onga, H Takaesu, Okinawa Prefectural Miyako Public Health Center, Hirara; F Horii, I Asano, H Yamaguchi, K Aoki, S Maruyama, M Ichii, Osaka Prefectural Suita Public Health Center, Suita; S Matsushima, S Natsukawa, Saku General Hospital, Usuda; S Watanabe, M Akabane, Tokyo University of Agriculture, Tokyo; M Konishi, K Okada, Ehime University, Matsuyama; H Iso, Y Honda, Tsukuba University, Tsukuba; H Sugimura, Hamamatsu University, Hamamatsu; Y Tsubono, Tohoku University, Sendai; M Kabuto, National Institute for Environmental Studies, Tsukuba; S Tominaga, Aichi Cancer Center Research Institute, Nagoya; M Iida, W Ajiki, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka; S Sato, Osaka Medical Center for Health Science and Promotion, Osaka; N Yasuda, Kochi Medical School, Nankoku; S Kono, Kyushu University, Fukuoka; K Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita; Y Takashima, Kyorin University, Mitaka; E Maruyama, Kobe University, Kobe; the late M Yamaguchi, Y Matsumura, S Sasaki, National Institute of Health and Nutrition, Tokyo; and T Kadowaki, Tokyo University, Tokyo, Japan.