

日本人における transporter associated with antigen processing 遺伝子多型と大腸がん感受性との関連性の検討

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1 **Title page**

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3 *Title:* The genotype of the transporter associated with antigen processing gene affects susceptibility
4 to colorectal cancer in Japanese

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18 *Keywords:* immune escape, TAP1, genetic polymorphism, colorectal cancer, Japanese

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25 Abstract

26 *Objective* Although colorectal cancer is one of the most frequent malignancies in Japan, the
27 associated genetic factors remain to be elucidated. Functional loss of the transporter associated with
28 antigen processing (TAP) 1 gene induces carcinogenesis. We investigated whether single nucleotide
29 polymorphisms (SNPs) in the *TAP1* gene (rs735883) are associated with susceptibility to colorectal
30 cancer in a Japanese population.

31 *Methods* The study participants were 143 cases and 243 clinical controls. After extracting DNA from
32 their peripheral blood cells, genotyping was conducted by the polymerase chain reaction-restriction
33 fragment length polymorphism method.

34 *Results* Participants with a mutated allele had an increased risk for colorectal cancer. The adjusted
35 odds ratios for the C/T, T/T, and the mutation type (C/T + T/T) compared to that of wild type (C/C)
36 were 2.27 (95% confidence interval [CI], 1.43–3.67), 1.95 (95% CI, 0.88–4.30), and 2.22 (95% CI,
37 1.42–3.55), respectively. Furthermore, a significant trend in the rate of cases was observed with an
38 increasing number of mutated alleles (P for trend = 0.0068).

39 *Conclusions* The genotype of the *TAP1* gene is associated with susceptibility to colorectal cancer.

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49 **Introduction**

50 According to a report by the International Agency for Research on Cancer (IARC),
51 colorectal cancer (CRC) is one of the most lethal malignant neoplasms worldwide despite the
52 decreasing mortality and incidence of CRC in developed countries. The age-standardized incidence
53 and mortality due to CRC per 100,000 are 17.2 and 8.2, respectively [1]. In Japan, the
54 age-standardized incidence of CRC, which accounts for 16.5% of all malignant neoplasms, is the
55 third highest among all kinds of cancer. The age-standardized mortality of CRC is 14.8 in men and
56 8.4 in women [2].

57 CRC carcinogenesis is a complex and multifactorial process. As is often true with
58 malignancies, the development of CRC is the result of interactions between environmental and
59 genetic factors. Many epidemiological studies have confirmed the effects of environmental factors. A
60 diet containing red or processed meats can markedly increase the risk of CRC [3]. Heavy alcohol
61 intake and smoking are also associated with CRC prognosis [4]. As a consequence, the IARC
62 reported that diet, exercise, and obesity are associated with CRC carcinogenesis. In contrast, genetic
63 factors are also associated with susceptibility to CRC. Gender differences have been reported in both
64 age-standardized incidence and mortality of CRC [1]. A personal or familial history of specific
65 diseases (e.g., a personal history of chronic inflammatory bowel disease and a familial or personal
66 history of adenomatous polyps) has also been considered a risk factor for CRC [4–9]. Although the
67 biological mechanisms underlying the carcinogenesis of CRC are not fully understood, one of the
68 proposed mechanisms is immune escape, which allows tumor cells to escape from immune
69 surveillance.

70 The transporter associated with antigen processing (TAP) protein, a heterodimer of TAP1
71 and TAP2 belonging to the major histocompatibility complex (MHC) class I, is responsible for
72 immune escape [10,11]. TAP translocates antigen peptides from the cytosol to the endoplasmic

73 reticulum (ER) lumen and helps MHC class I molecules bind to antigen peptides [12,13]. Several
74 *TAP1* gene polymorphisms have been identified, and antigen processing ability has been evaluated in
75 many studies. Some of the polymorphisms were found to decrease the efficacy of antigen processing
76 [14]. Recent studies have suggested that *TAP1* gene polymorphisms may increase the risk for vitiligo
77 [15], nasopharyngeal carcinoma [16], and CRC via downregulation of MHC class I molecules [17].
78 However, few epidemiological studies have focused on the *TAP1* gene polymorphisms (rs735883). In
79 this case-control study, we investigated the association of the *TAP1* genotype with susceptibility to
80 CRC in relation to gender and smoking status.

81

82 **Materials and Methods**

83 A total of 143 Japanese CRC cases and 243 Japanese non-cancer clinical controls were
84 recruited. The cases were consecutive patients treated at the University of Miyazaki (UOM) Hospital
85 and the University of Occupational and Environmental Health (UOEH) Hospital in Japan from
86 September 1992 to December 2006. The controls were recruited from patients suffering from
87 non-cancerous diseases in the hospitals near UOEH Hospital between September 1996 and
88 September 2001. All cases were histologically diagnosed with CRC including ascending, transverse,
89 and descending colon cancer as well as rectal cancer. The subjects' history of illness, residence,
90 occupation, and smoking status were examined by a self-questionnaire. No patients who had been
91 exposed to carcinogens, heavy metals, or radiation in their occupational history were included. All
92 subjects were classified into two groups according to smoking status: the "never" group, composed
93 of non-smokers; and the "smoker" group, composed of both current smokers and ex-smokers. All
94 cases and controls were given an explanation of the nature of the study, and written informed consent
95 was obtained from all participants. The Ethical Committee of UOM approved this study procedure
96 on December 7, 2005 (approval number: 239).

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98 *Polymerase chain reaction (PCR) amplification and genotyping*

99 Genomic DNA was extracted from peripheral blood lymphocytes with a DNA Extractor WB
100 Kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's protocol. The
101 single nucleotide polymorphisms (SNPs) (rs735883) are located on the intron 7 region of the *TAP1*
102 gene, and the analysis of this SNP was carried out using a PCR-fragment length polymorphism
103 (PCR-RFLP) assay, as described previously [15]. Briefly, samples were subjected to 35 cycles of
104 30-s denaturing at 95° C, 30-s annealing at 55° C, and 30-s extension at 72° C, followed by a
105 5-min final extension with PCR primers 5' -GTGCTCTCACGTTCCAAGGA-3' and 5'
106 -AGGAGTAGAGATAGAAGAACC-3' . Subsequently, a 183-bp PCR product was digested with
107 the *MspI* restriction enzyme and the restriction fragments were separated by agarose gel
108 electrophoresis in TAE buffer. The wild-type C allele was digested into fragments of 161 and 22 bp,
109 and the mutated type T allele was not digested.

110

111 *Statistical analysis*

112 Results are presented as means \pm standard deviation (SD) for continuous variables.
113 Pearson's chi-square tests were used for a categorical comparison of the data and for evaluating the
114 probability of Hardy–Weinberg equilibrium. The prevalence of each genotype was examined with the
115 Cochran–Armitage trend test. Welch's two-sample t-tests were used for numerical comparisons.
116 Multivariate analysis was conducted using a multiple logistic regression model after adjusting for age,
117 gender, or smoking status. A P-value < 0.05 (two-tailed) was considered significant. Smoking status
118 is associated with increasing risk of CRC [18], and there are many more male smokers than female
119 smokers among the Japanese population. As gender and smoking status could be confounding factors,
120 stratified analyses by gender and smoking status were conducted to exclude the effect of each factor.

121 Power analysis was performed to determine the statistical power of chi-square tests. All statistical
122 analyses were performed using R ver. 2.15.1.

123

124 **Results**

125 The general characteristics of the cases and the controls are shown in Table 1. The mean age
126 (\pm SD) was 65.8 (\pm 16.6) years for the controls and 63.9 (\pm 10.9) years for the cases ($P = 0.18$). The
127 frequencies of gender and smoking status were not significantly different between the cases and the
128 controls ($P = 0.12, 0.18$, respectively). No significant difference was observed between the cases and
129 the controls in terms of age, gender, and smoking status. The frequencies of the *TAP1* genotypes are
130 shown in Table 2. The allele frequencies in the cases were allele C: 0.58 and allele T: 0.42, and allele
131 C: 0.67 and allele T: 0.33 in the controls. The odds ratio (OR) for allele T compared to allele C was
132 1.47 (95% confidence interval [CI], 1.08–1.98, $P = 0.013$). The observed frequencies of the *TAP1*
133 allele in the controls were consistent with the allele frequencies in Japanese. Hardy–Weinberg
134 equilibrium was confirmed for the *TAP1* genotype in the controls ($P = 0.31$). The adjusted ORs for
135 the C/T and T/T genotypes compared to the C/C genotype were estimated to be 2.27 (95% CI, 1.43–
136 3.67) and 1.95 (95% CI, 0.88–4.30), respectively. Furthermore, that for the mutation type (C/T +
137 T/T) was 2.22 (95% CI, 1.42–3.55). Although a significant difference was not observed in the T/T
138 genotype, a significant trend on the rate of the cases was observed (P for trend = 0.0068).

139 The results of the stratified analysis by smoking status are shown in Table 3. In the “never”
140 group, the adjusted ORs were estimated to be 1.94 (95% CI, 1.02–3.75) for the C/T genotype and
141 2.04 (95% CI, 0.62–6.58) for the T/T compared to the C/C genotype. Those in the “smoker” group
142 were calculated to be 2.84 (95% CI, 1.43–5.92) for C/T and 1.98 (95% CI 0.64–5.83) for T/T. For the
143 mutated type (C/T + T/T) the ORs were 1.95 (95% CI 1.05–3.72) in the “never” group and 2.69
144 (95% CI, 1.37–5.51) in the “smoker” group, respectively. The P for the trend was 0.079 in the “never”

145 group and 0.027 in the “smoker” group.

146 The results of the analysis stratified by gender are shown in Table 4. In the male group, the
147 adjusted ORs were estimated to be 2.04 (95% CI, 1.11–3.84) for the C/T genotype and 2.38 (95% CI,
148 0.86–6.61) for the T/T genotype compared to the C/C genotype. The ORs for the C/T and T/T were
149 2.91 (95% CI, 1.40–6.32) and 1.45 (95% CI, 0.36–5.10), respectively, in the female group. For the
150 mutated type, the ORs were 2.09 (95% CI, 1.15–3.87) in the male group and 2.63 (95% CI, 1.29–
151 5.62) in the female group. *P* for the trend was 0.065 in the male group and 0.052 in the female group.

152

153 **Discussion**

154 A significant association was observed between the *TAPI* genotype and CRC (C/C vs. C/T +
155 T/T, adjusted OR, 2.22, *P* < 0.01). Although the C/T genotype was significantly associated with CRC
156 (adjusted OR, 2.27, *P* < 0.01), a significant association was not observed for the T/T genotype
157 (adjusted OR, 1.95, *P* = 0.093). Stratified analyses were conducted to exclude the effects of gender
158 and smoking status, (Tables 3 and 4) and a significant association was observed between the *TAPI*
159 genotype and CRC.

160 However, a significant association with CRC was observed only in the C/T genotype, not in
161 the T/T genotype. It is likely that there were not enough participants to ensure the statistical power to
162 detect an association between the T/T genotype and CRC. The statistical power of the chi-square test
163 between the C/C and T/T genotypes was calculated to be 0.31, although it was recommended to be
164 larger than 0.8 [19,20]. In contrast, the statistical powers of the chi-square test among all genotypes
165 (C/C, C/T, and T/T), and between the C/C and C/T genotypes, were estimated to be 0.92 and 0.92,
166 respectively. It is possible that the T/T genotype shows a stronger immunity than the C/T genotype
167 and thus immune escape occurs less often in that genotype. Recent studies on *TAPI*-deficient mice
168 indicate the existence of a compensatory mechanism. CD8⁺ T cells have been reported to play an

169 important role in tumor surveillance by the immune system [21,22]. Although the population of
170 CD8⁺ T cells was diminished in the *TAP1*-deficient mice, compensatory increases in CD3⁺ and CD4⁺
171 T cell populations were observed [23]. Furthermore, the *TAP1*-independent pathway compensates for
172 antigen processing and CD8⁺ T cells functioned normally even in the *TAP1*-deficient mice *in vivo*
173 [24]. It is likely that some compensatory function would work only in the case of the T/T genotype as
174 the functional deficit of the C/T genotype in the *TAP1* gene might not be enough to drive a
175 compensating network. As a result, the immune escape of tumor cells could more likely be tolerated
176 by the C/T genotype.

177 The TAP protein plays an important role in antigen presentation mediated by MHC class I
178 and this process is considered essential for immune surveillance against tumors and pathogens. The
179 impairment of TAP function in tumor cells that induces loss of downregulation of class I molecules
180 on the cell surface is considered one of the main mechanisms of immune escape in a variety of
181 tumors [13,25–31]. Furthermore, *TAP1* gene polymorphisms lead to the loss of MHC class I antigen
182 processing ability [32]. The *TAP* gene is also considered a member of the ATP-binding cassette
183 superfamily, which is associated with membrane transportation of solutes such as ions. Molecules
184 belonging to the ATP-binding cassette family have nucleotide-binding domains and interact with
185 other molecules involved in genetic events, such as chromosome maintenance and DNA repair [33–
186 35]. Our results indicate that the polymorphisms (rs735883) located on the intron 7 of the *TAP1* gene
187 were associated with CRC but the functional mechanism remains to be elucidated. One likely
188 mechanism is exon skipping. According to a previous study, the SNP located on the intron could
189 cause exon skipping and aberrant RNA splicing [36]. Furthermore, the E2F8 binding motif
190 (TTTGCCGC) is located on intron 7 in the *TAP1* gene. E2F8 is a transcription factor that belongs to
191 the E2F superfamily and regulates the expression of genes related to the cell cycle and apoptosis [37].
192 In the case of the T allele, the cytosine at the 3' end is substituted to thymine and the aberrant binding

193 site (TTTGCCGT) is generated. E2Fs generally bind to the binding site located on the promoter
194 region or intron 1 [38] but it may be that the efficacy of splicing is affected by decreasing frequencies
195 of E2F8 binding to the aberrant binding site. Further study is essential to clarify the functional
196 mechanism that associates the polymorphisms (rs735883) with CRC.

197 The adjusted OR of the alcohol dehydrogenase enzyme (*ADH2*) gene was 1.92 (95% CI,
198 1.06–3.46) in a Japanese population [39]. Another study reported that the adjusted OR of the *RAD18*
199 gene, which is associated with DNA repair, was 2.10 (95% CI, 1.00–4.40) [40]. An increased number
200 of SNPs associated with CRC were identified recently in a genome-wide association study [41–43].
201 It is likely that a genetic difference may not be observed when exposure to a carcinogen is great
202 [44,45]. In fact, the differences among genotypes are easily observed in non-smokers or light
203 smokers compared to smokers [46,47]. In the present study, a significant difference was observed in
204 the C/T genotype and the mutation type (C/T + T/T) despite stratification by smoking status. Thus,
205 the *TAP1* genotype was strongly associated with susceptibility to CRC. As the adjusted ORs for the
206 C/T genotype and the mutation type (C/T + T/T) were larger in the “smoker” group than in the
207 “never” group, it is possible that the *TAP1* gene polymorphism interacts with smoking status. The
208 interaction between the *TAP1* genotype and smoking status was introduced into a logistic regression
209 model. However, the interaction was not significant. Similarly, the adjusted ORs for the C/T
210 genotype and the mutation type (C/T + T/T) were larger in the female group than in the male group
211 although the *TAP1* gene is located not on the sex chromosomes but on chromosome 6. Although the
212 interaction between the *TAP1* genotype and gender was also introduced into a logistic regression
213 model, it was not statistically significant either.

214 One of the limitations of our study was smoking status. The percentage of “smokers”
215 (46.9%) was less than that of controls (53.9%) although it was not a significant difference. The rate
216 of cigarette smoking decreases every year in Japan. The rate among males of age 60–69 years was

217 48.1% in 1992 and 34.8% in 2006. Higher rates may be found in certain regions. It is likely that there
218 were fewer smokers in the present study because the recruitment period was longer for the cases than
219 for the controls. It is also possible that the exact information about smoking status was not acquired
220 for the cases. To adjust and exclude the effects of smoking, we conducted a logistic regression model
221 analysis and a stratification analysis. The other limitation of our study was the diagnostic accuracy.
222 The period for recruitment was 14 years, and the diagnostic accuracy is likely to have changed with
223 marked improvements in medical examinations. Hence, the potential cases that were not diagnosed at
224 that time might be included in the controls. However, CRC screening by the local authority began in
225 1992, at which point recruitment of cases also started. Furthermore, the controls were not healthy
226 controls but clinical controls in our study. This limitation had less of an impact on the results of our
227 study.

228 A significant association was found between the *TAP1* gene polymorphisms (rs735883) and
229 CRC. This suggests that those with the risk allele (T) have a higher susceptibility to CRC. In order to
230 promote the high risk approach against the onset of CRC, additional studies of the association
231 between rs735883 and other SNPs and between rs735883 and other carcinogens are required.

232

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239

240 **Conflict of interest**

241 We declare that none of the authors hold any financial or personal relationship with other
242 people or organizations that could have inappropriately influenced this study.

243

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360 **Tables**

361 Table 1. General characteristics of the controls and the colorectal cancer patients.

		Controls	Cases
Age (years)		65.8 ± 16.6	63.9 ± 10.9
Gender (%)	Female	110 (45.3)	53 (37.1)
	Male	133 (54.7)	90 (62.9)
Smoking Status (%)	Never	112 (46.1)	76 (53.1)
	Smoker	131 (53.9)	67 (46.9)
Total		243	143

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363 Age is presented as mean ± standard deviation.

364 Gender and smoking status are presented as number of subjects.

365 No significant difference was observed between cases and controls.

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368 Table 2. Associations between the *TAPI* genotype and colorectal cancer.

Genotype	Controls	Cases	Crude OR (95% CI)	Adjusted OR (95% CI)
C/C (%)	106 (43.6)	38 (26.6)	—	—
C/T (%)	115 (47.3)	91 (63.6)	2.21 (1.39–3.49)**	2.27 (1.43–3.67)**
T/T (%)	22 (9.05)	14 (9.79)	1.78 (0.83–3.78)	1.95 (0.88–4.30)
Total	243	143		
C/T + T/T (%)	137 (56.4)	105 (73.4)	2.14 (1.37–3.34)**	2.22 (1.42–3.55)**

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370 95% CI: 95% confidence interval; OR: odds ratio.

371 Crude OR and adjusted OR for age, gender and smoking status were estimated using chi-square statistic and multivariate logistic regression,
 372 respectively.

373 *: $P < 0.05$; **: $P < 0.01$.

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377 Table 3. Associations between the *TAP1* genotype and CRC when stratified by smoking status.

Smoking status	Genotype	Controls	Cases	Crude OR (95% CI)	Adjusted OR (95% CI)
Never	C/C (%)	51 (45.5)	24 (31.6)	—	—
	C/T (%)	53 (47.3)	45 (59.2)	1.80 (0.97–3.36)	1.94 (1.02–3.75)*
	T/T (%)	8 (7.14)	7 (9.21)	1.86 (0.62–5.55)	2.04 (0.62–6.58)
	Total	112	76		
	C/T + T/T (%)	61 (54.5)	52 (68.4)	1.81 (0.99–3.32)	1.95 (1.05–3.72)*
Smoking status	Genotype	Controls	Cases	Crude OR (95% CI)	Adjusted OR (95% CI)
Smoker	C/C (%)	55 (42.0)	14 (20.9)	—	—
	C/T (%)	62 (47.3)	46 (68.7)	2.91 (1.46–5.82)**	2.84 (1.43–5.92)**
	T/T (%)	14 (10.7)	7 (10.4)	1.96 (0.68–5.64)	1.98 (0.64–5.83)
	Total	131	67		
	C/T + T/T (%)	76 (58.0)	53 (79.1)	2.74 (1.39–5.38)**	2.69 (1.37–5.51)**

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379 95% CI: 95% Confidence interval; OR: Odds ratio.

380 Crude OR and adjusted OR for age and gender were estimated using chi-square statistic and multivariate logistic regression, respectively.

381 *: $P < 0.05$; **: $P < 0.01$.

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395 Table 4. Associations between the *TAPI* genotype and CRC when stratified by gender.

Gender	Genotype	Controls	Cases	Crude OR (95% CI)	Adjusted OR (95% CI)
Male	C/C (%)	55 (41.4)	25 (27.8)	—	—
	C/T (%)	67 (50.4)	55 (61.1)	1.81 (1.00–3.25)*	2.04 (1.11–3.84)*
	T/T (%)	11 (8.27)	10 (11.1)	2.00 (0.77–5.22)	2.38 (0.86–6.61)
	Total	133	90		
	C/T + T/T (%)	78 (58.6)	65 (72.2)	1.83 (1.03–3.25)*	2.09 (1.15–3.87)*
Gender	Genotype	Controls	Cases	Crude OR (95% CI)	Adjusted OR (95% CI)
Female	C/C (%)	51 (46.4)	13 (24.5)	—	—
	C/T (%)	48 (43.6)	36 (67.9)	2.94 (1.41–6.15)**	2.91 (1.40–6.32)**
	T/T (%)	11 (10.0)	4 (7.55)	1.42 (0.41–4.95)	1.45 (0.36–5.10)
	Total	110	53		
	C/T + T/T (%)	59 (53.6)	40 (75.5)	2.66 (1.29–5.46)**	2.63 (1.29–5.62)**

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397 95% CI: 95% Confidence interval; OR: Odds ratio.

398 Crude OR and adjusted OR for age and smoking status were estimated using chi-square statistic and multivariate logistic regression,
399 respectively.

400 *: $P < 0.05$, **: $P < 0.01$.