Female

All sites (incl. CIS)	C00–C96 D05–D06	249 643	319	142	207	334	796	1956	4567	6109	8786	12 984	20 548	20 418	23 540	27 927	31 515	31 219	26 212	32 064
All site	C00–C96	237 569	319	142	207	319	633	1339	3121	4699	7532	11 866	19 495	19 599	22 563	27 097	30 626	30 521	25 781	31 710
Lip, oral cavity and pharynx	C00–C14	2752	0	0	0	11	36	18	34	70	70	80	216	203	286	335	352	345	308	388
Esophagus	C15	2554	0	0	0	0	0	0	1	17	10	65	163	207	244	338	369	321	337	482
Stomach	C16	35 126	0	0	0	2	21	95	220	405	778	1108	2100	2458	3151	4179	5157	5366	4409	5677
Colon	C18	29 382	0	2	0	16	21	40	134	218	367	714	1659	2149	2928	3773	4508	4435	3796	4622
Rectum and anus	C19–C21	13 843	0	0	0	4	5	21	73	144	266	467	1132	1410	1737	1869	1941	1822	1394	1558
Liver	C22	12 728	13	0	5	8	6	22	11	7	51	83	254	565	1091	2057	2511	2316	1915	1813
Gallbladder and bile ducts	C23–C24	9385	0	0	0	1	0	5	2	10	47	63	238	354	568	843	1194	1676	1819	2565
Pancreas	C25	9721	0	0	1	0	0	3	13	24	65	147	357	532	806	1047	1455	1655	1577	2039
Larynx	C32	221	0	0	1	0	0	0	2	0	0	10	5	20	25	38	34	35	24	27
Lung, bronchus and trachea	C33–C34	21 647	2	0	0	10	4	17	36	80	249	450	1112	1469	2018	2654	3415	3456	2969	3706
Skin	C43–C44	4480	12	0	7	8	24	20	46	53	54	92	143	183	272	451	496	664	654	1301
Breast	C50 D05	41 960	0	0	0	6	55	233	896	1730	3370	5604	6655	5133	4858	4162	3431	2789	1719	1319
Uterus (incl. CIS)	C53–C55 D06	23 306	0	0	0	12	201	928	2306	2447	2084	2164	2781	2491	1826	1651	1494	1154	874	893
Uterus (only invasive)	C53–C55	16 572	0	0	0	1	50	335	938	1170	1064	1451	2241	2148	1544	1449	1352	1086	857	886
Cervix uteri	C53	8779	0	0	0	1	47	294	803	960	781	925	923	771	663	628	599	535	426	423
Corpus uteri	C54	6625	0	0	0	0	3	32	125	195	261	462	1187	1292	813	720	648	445	276	166
Ovary	C56	7418	0	2	25	37	85	151	176	217	381	654	1228	951	743	703	678	527	392	468
Bladder	C67	3823	0	0	0	0	2	7	5	10	26	38	135	150	269	452	589	628	640	872
Kidney, Renal pelvis, Ureter and others	C64–C66 C68	4062	9	14	3	2	1	9	13	24	75	112	272	279	401	563	599	623	504	559
Brain and nervous system	C70–C72	1754	39	22	36	30	24	16	67	51	72	64	109	141	147	212	221	201	147	155
Thyroid	C73	5645	4	0	10	25	99	136	233	261	344	441	750	658	627	603	589	383	231	251
Malignant lymphoma	C81–85 C96	6823	11	24	24	50	86	65	72	100	174	216	458	455	643	762	990	1031	846	816
Multiple myeloma	C88–C90	2016	0	0	0	0	0	0	6	12	13	13	76	115	182	235	328	354	297	385
All leukemias	C91–C95	3638	100	48	39	42	55	64	86	94	130	137	254	224	346	402	440	424	347	406

Table 3. Age-specific incidence rate per 100 000 population in Japan according to sex and primary site, 2002

Primary sites	ICD-10	All ages	Age group (years)																	
			0–4	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–59	80–84	85+
Male																				
All sites (incl. CIS)	C00–C96 D05–D06	545.6	13.2	6.4	5.8	8.7	11.9	17.4	29.5	50.3	100.6	198.6	373.4	653.8	1036.8	1560.5	2306.7	2829.4	3250.5	3737.7
All sites	C00–C96	535.0	13.2	6.4	5.8	8.5	11.8	17.2	29.3	49.7	97.5	194.6	364.3	637.3	1010.6	1525.5	2266.2	2776.5	3207.1	3702.8
Lip, oral cavity and pharynx	C00–C14	13.2	0.0	0.0	0.1	0.3	0.6	0.8	1.6	2.3	3.3	8.4	15.0	21.5	32.9	41.3	47.7	44.0	47.6	51.7
Esophagus	C15	22.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.5	1.7	7.6	16.4	38.0	58.9	67.6	93.7	98.0	96.1	86.9
Stomach	C16	115.1	0.0	0.0	0.0	0.4	0.6	1.1	4.4	9.8	23.0	48.7	90.5	153.2	231.2	343.3	474.5	566.4	626.1	710.8
Colon	C18	59.5	0.0	0.0	0.1	0.2	0.7	0.8	2.1	5.1	11.4	22.7	41.6	76.3	125.1	181.8	250.0	295.4	316.4	371.0
Rectum and anus	C19–C21	40.0	0.0	0.0	0.0	0.2	0.1	0.5	2.3	4.3	10.4	19.7	37.3	65.3	98.8	130.6	150.5	163.1	159.1	168.4
Liver	C22	44.8	0.7	0.2	0.0	0.0	0.2	0.1	1.0	2.3	7.5	15.6	37.4	65.0	99.0	150.6	202.1	195.2	194.6	203.7
Gallbladder and bile ducts	C23–C24	13.6	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.9	3.1	6.6	10.6	20.9	30.6	54.0	78.5	131.8	178.6
Pancreas	C25	18.7	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.9	2.5	5.9	13.1	24.5	38.1	49.2	75.8	96.6	126.6	147.8
Larynx	C32	5.4	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.1	0.5	1.3	4.7	9.2	12.7	16.4	22.7	27.9	24.9	21.4
Lung, bronchus and trachea	C33–C34	83.5	0.0	0.1	0.0	0.0	0.1	0.5	1.5	3.5	8.9	21.1	41.6	76.0	120.3	210.2	395.0	540.3	658.9	684.8
Skin	C43–C44	6.0	0.1	0.0	0.1	0.1	0.3	0.7	1.0	0.8	1.2	2.3	3.0	5.0	9.4	12.7	22.0	33.5	43.6	83.6
Prostate	C61	47.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.9	5.9	21.0	68.8	139.4	253.1	330.9	375.6	454.6
Bladder	C67	19.4	0.2	0.0	0.0	0.1	0.0	0.4	0.4	1.6	3.0	6.7	12.9	20.8	31.1	49.4	81.1	111.6	134.5	180.1
Kidney, renal pelvis, ureter and others	C64–C66 C68	13.1	0.8	0.1	0.0	0.1	0.4	0.6	0.9	2.0	4.4	6.9	12.6	18.9	22.6	38.4	50.0	64.4	68.5	67.7
Brain and nervous system	C70–C72	3.5	1.4	2.1	1.4	1.2	1.2	1.4	1.4	1.4	2.3	3.4	3.7	4.6	5.3	6.6	9.4	9.7	9.8	14.4
Thyroid	C73	2.6	0.0	0.0	0.1	0.2	0.4	0.6	1.0	1.9	1.8	2.5	4.0	4.1	5.4	5.9	7.0	6.7	6.9	9.3
Malignant lymphoma	C81–85 C96	14.0	0.6	0.8	1.1	1.8	1.7	1.6	2.4	3.5	7.5	9.3	10.1	18.5	24.8	36.0	44.8	66.3	76.3	90.6
Multiple myeloma	C88–C90	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	1.0	1.5	3.4	5.6	8.4	14.0	17.8	30.9	35.4
All leukemias	C91–C95	8.1	3.4	2.4	1.2	1.5	1.8	2.1	2.2	2.4	3.9	4.6	6.5	8.8	11.6	20.9	26.8	34.7	37.5	46.5

Female

All sites (incl. CIS)	C00–C96 D05–D06	383.0	11.1	4.9	6.8	9.5	20.4	42.1	97.2	149.0	226.7	319.5	386.2	464.7	564.4	719.6	931.8	1137.7	1399.5	1791.3
All site	C00–C96	364.5	11.1	4.9	6.8	9.1	16.2	28.8	66.4	114.6	194.3	292.0	366.4	446.0	540.9	698.2	905.6	1112.3	1376.5	1771.5
Lip, oral cavity and pharynx	C00–C14	4.2	0.0	0.0	0.0	0.3	0.9	0.4	0.7	1.7	1.8	2.0	4.1	4.6	6.9	8.6	10.4	12.6	16.4	21.7
Esophagus	C15	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3	1.6	3.1	4.7	5.8	8.7	10.9	11.7	18.0	26.9
Stomach	C16	53.9	0.0	0.0	0.0	0.1	0.5	2.0	4.7	9.9	20.1	27.3	39.5	55.9	75.5	107.7	152.5	195.6	235.4	317.2
Colon	C18	45.1	0.0	0.1	0.0	0.5	0.5	0.9	2.9	5.3	9.5	17.6	31.2	48.9	70.2	97.2	133.3	161.6	202.7	258.2
Rectum and anus	C19–C21	21.2	0.0	0.0	0.0	0.1	0.1	0.5	1.6	3.5	6.9	11.5	21.3	32.1	41.6	48.2	57.4	66.4	74.4	87.0
Liver	C22	19.5	0.5	0.0	0.2	0.2	0.2	0.5	0.2	0.2	1.3	2.0	4.8	12.9	26.2	53.0	74.2	84.4	102.2	101.3
Gallbladder and bile ducts	C23–C24	14.4	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.2	1.2	1.6	4.5	8.1	13.6	21.7	35.3	61.1	97.1	143.3
Pancreas	C25	14.9	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.6	1.7	3.6	6.7	12.1	19.3	27.0	43.0	60.3	84.2	113.9
Larynx	C32	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.5	0.6	1.0	1.0	1.3	1.3	1.5
Lung, bronchus and trachea	C33–C34	33.2	0.1	0.0	0.0	0.3	0.1	0.4	0.8	2.0	6.4	11.1	20.9	33.4	48.4	68.4	101.0	125.9	158.5	207.0
Skin	C43–C44	6.9	0.4	0.0	0.2	0.2	0.6	0.4	1.0	1.3	1.4	2.3	2.7	4.2	6.5	11.6	14.7	24.2	34.9	72.7
Breast	C50 D05	64.4	0.0	0.0	0.0	0.2	1.4	5.0	19.1	42.2	86.9	137.9	125.1	116.8	116.5	107.2	101.4	101.6	91.8	73.7
Uterus (incl. CIS)	C53–C55 D06	35.8	0.0	0.0	0.0	0.3	5.1	20.0	49.1	59.7	53.8	53.2	52.3	56.7	43.8	42.5	44.2	42.1	46.7	49.9
Uterus (only invasive)	C53–C55	25.4	0.0	0.0	0.0	0.0	1.3	7.2	20.0	28.5	27.5	35.7	42.1	48.9	37.0	37.3	40.0	39.6	45.8	49.5
Cervix uteri	C53	13.5	0.0	0.0	0.0	0.0	1.2	6.3	17.1	23.4	20.1	22.8	17.3	17.5	15.9	16.2	17.7	19.5	22.7	23.6
Corpus uteri	C54	10.2	0.0	0.0	0.0	0.0	0.1	0.7	2.7	4.8	6.7	11.4	22.3	29.4	19.5	18.6	19.2	16.2	14.7	9.3
Ovary	C56	11.4	0.0	0.1	0.8	1.1	2.2	3.3	3.7	5.3	9.8	16.1	23.1	21.6	17.8	18.1	20.0	19.2	20.9	26.1
Bladder	C67	5.9	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.2	0.7	0.9	2.5	3.4	6.4	11.6	17.4	22.9	34.2	48.7
Kidney, renal pelvis, ureter and others	C64–C66 C68	6.2	0.3	0.5	0.1	0.1	0.0	0.2	0.3	0.6	1.9	2.8	5.1	6.3	9.6	14.5	17.7	22.7	26.9	31.2
Brain and nervous system	C70–C72	2.7	1.4	0.8	1.2	0.9	0.6	0.3	1.4	1.2	1.9	1.6	2.0	3.2	3.5	5.5	6.5	7.3	7.8	8.7
Thyroid	C73	8.7	0.1	0.0	0.3	0.7	2.5	2.9	5.0	6.4	8.9	10.9	14.1	15.0	15.0	15.5	17.4	14.0	12.3	14.0
Malignant lymphoma	C81–85 C96	10.5	0.4	0.8	0.8	1.4	2.2	1.4	1.5	2.4	4.5	5.3	8.6	10.4	15.4	19.6	29.3	37.6	45.2	45.6
Multiple myeloma	C88–C90	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.3	0.3	1.4	2.6	4.4	6.1	9.7	12.9	15.9	21.5
All leukemias	C91–C95	5.6	3.5	1.6	1.3	1.2	1.4	1.4	1.8	2.3	3.4	3.4	4.8	5.1	8.3	10.4	13.0	15.5	18.5	22.7

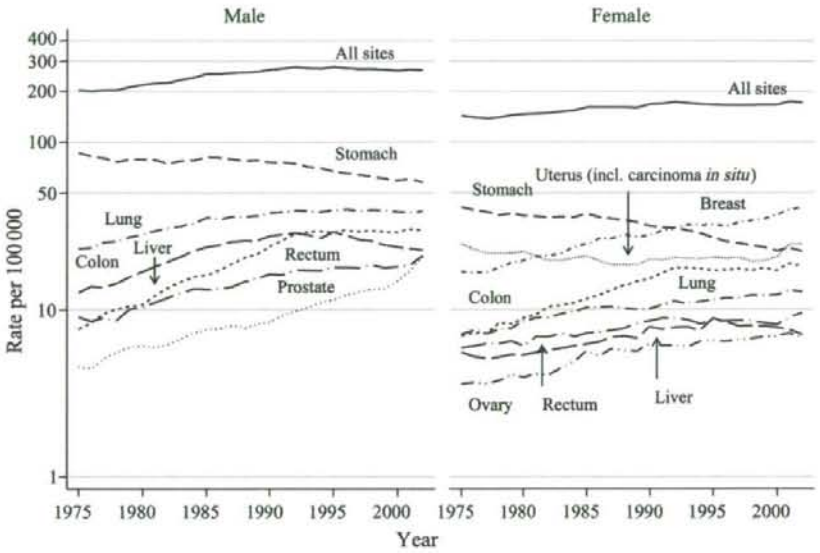

Figure 1. Trends of age-standardized cancer incidence rates for five major sites and male- and female-specific sites (standard population: world population).

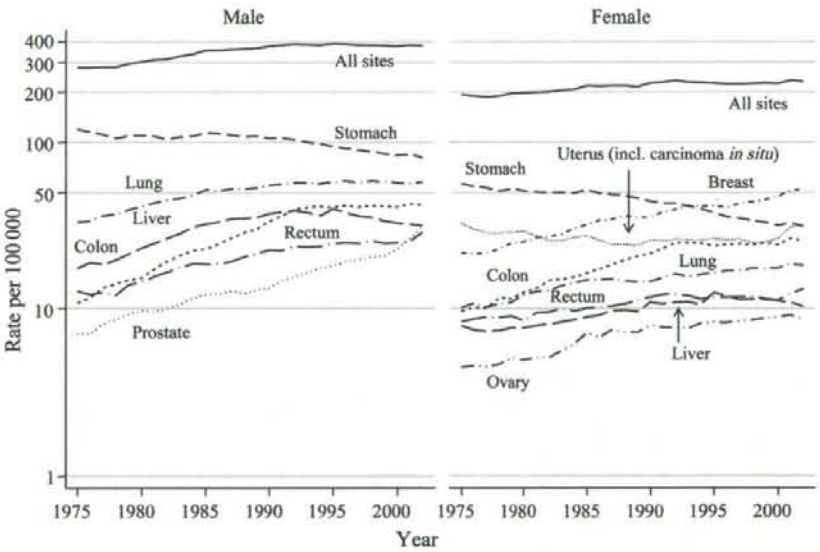

Figure 2. Trends of age-standardized cancer incidence rates for five major sites and male- and female-specific sites (standard population: 1985 Japanese model population).

Jpn J Clin Oncol 2008;38(8)571–576
doi:10.1093/jjco/hyn061

Epidemiology Note

Lifetime and Age-Conditional Probabilities of Developing or Dying of Cancer in Japan

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Received April 3, 2008; accepted June 18, 2008; published online July 29, 2008

The concepts of lifetime and age-conditional probabilities of developing and dying of cancer are introduced as indexes to understand the risk of cancer. In this paper, we estimated the lifetime and age-conditional probabilities of developing and dying of cancer in 2001 and 2005, respectively, in Japan. It is estimated that one in two Japanese males and one in three females will develop cancer, and one in four Japanese males and one in six females will die of cancer. Moreover, the probabilities of developing cancer within specific decades of age are obtained as the short-term risks.

Key words: lifetime probability – age-conditional probability – developing cancer – dying of cancer – life table.

BACKGROUND

Recently, lifetime and age-conditional probabilities of developing or dying of cancer were introduced as indexes to understand the risk of cancer (1,2). Lifetime probability of developing cancer is defined as the percentage of the population developing cancer at least once in a lifetime. Age-conditional probability of developing cancer is the percentage of the population developing cancer before a specific age, given that the individuals are cancer-free at the current age. Lifetime and age-conditional probabilities of dying of cancer are defined in the same manner as the probability of developing cancer. These indexes are useful for planning, monitoring and evaluating cancer control programs. The details of the mathematical derivation of these probabilities have been previously published by Wun et al. (1) Using the same procedure, the probabilities for the development of cancer in the Japanese population in 1999 were also published by Kamo et al. (2) The purpose of this paper is to update previous estimates by our research group for the development of cancer in 2001 and for dying of cancer in 2005.

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PATIENTS AND METHODS

Cross-sectional age-stratified incidence, mortality and population are needed to estimate lifetime and age-conditional probabilities of developing cancer. Cancer incidence data were provided by the National Cancer Center (3,4). Mortality and population data were originated from National Vital Statistics provided by the Ministry of Health, Labor and Welfare. These data are stratified by 5-year age intervals 0–4, 5–9, ..., 80–84 and the final open interval 85+. Incidence and mortality rates stratified these intervals were converted to the probabilities using an exponential model and were applied to a cohort of 100 000 live births. Using these probabilities, we estimate the number of cancer incidence, mortality, cancer free and survivor in life table. Then, lifetime probability of developing cancer is calculated dividing the number of incidence in life table by 100 000. Similarly, age-conditional probability of developing cancer

is calculated dividing the number of incidence during the considered age interval by the number of cancer free at the beginning of age interval in life table. In the estimation of cancer mortality, we used an ordinary life table, whereas for developing cancer, we used multiple life tables, as proposed by Wun et al. (1) and Kamo et al. (2) Note that our estimates for the probability were based on the assumption that the age-specific incidence or mortality rates will be the same in the future as observed in the calendar year of our source data (2001 for incidence, 2005 for mortality).

RESULTS AND CONCLUSION

The lifetime probability of developing cancer in 2001 was estimated to be 49.01% for men and 37.36% for women (Tables 1, 2 and 3). The lifetime probability of dying of cancer in 2005 was estimated to be 26.59% for men and 16.17% for women (Table 4). These probabilities mean that one in two males and one in three females will develop cancer during their lifetime, and one in four males and one in six females will die of cancer. Since the lifetime probability of developing cancer in 1999 was reported to be 46.35% for males and 34.81% for females (2), the increase in incidence was about 3% over 2 years. Furthermore, the lifetime probability of dying of cancer in 1999 was reported to be 29.44% for males and 20.52% for females, so there was a decrease of about 3% during those 6 years. We have reported that since 1975 the lifetime probability of dying of cancer increases and it becomes stable around 1995 (2). The decreasing trend for the lifetime probability of dying in comparison with 1999 and 2005, which is obtained in this paper, may be reflected in the slight decreasing trend of age-adjusted mortality rate. The reason why the lifetime probability corresponding to the age adjusted rate is that the numbers in life table are regarded as the rate per 100 000 adjusted by age distribution.

Age-conditional probabilities of developing and dying of cancer in 2001 and dying of cancer in 2005 are also shown in Tables 1 and 4, respectively. In these tables, 'Eventually' means the lifetime probability of those who were cancer-free in Table 1 (or alive in Table 4) at 'Current age'. For example, a male who was cancer free at 50-year old has 16.42% chance of developing cancer before 70, and such male (cancer free at 50) saddled with 49.79% chance of developing cancer during his lifetime. Table 2 shows the composite life table in 2001, which includes the results for both cancer mortality and incidence in 2001.

Table 3 shows the lifetime probability of developing cancer in 2001 and of dying of cancer in 2005, stratified by primary site. It also illustrates the probability of developing cancer within the following 10 years, which is a useful indication of the short-term risk of cancer (5,6). For example, a 60-year-old man has 2.85% (1 in 35) chance of developing

Table 1. Percent developing cancer before a specific age, for males and females who were cancer-free at current age in Japan in 2001

Current age	10	20	30	40	50	60	70	80	Eventually	1 in
Male										
0	0.12	0.20	0.35	0.76	2.22	6.93	17.65	34.52	49.01	2
10		0.08	0.23	0.64	2.11	6.86	17.64	34.62	49.20	2
20			0.15	0.56	2.04	6.80	17.62	34.65	49.28	2
30				0.41	1.90	6.70	17.60	34.76	49.50	2
40					1.50	6.36	17.40	34.79	49.71	2
50						5.02	16.42	34.37	49.79	2
60							12.46	32.08	48.93	2
70								24.28	45.14	2
80									35.29	3
Female										
0	0.09	0.17	0.48	1.67	4.26	8.32	14.44	23.34	37.36	3
10		0.08	0.39	1.59	4.19	8.27	14.42	23.36	37.46	3
20			0.31	1.51	4.12	8.20	14.37	23.33	37.45	3
30				1.21	3.83	7.94	14.13	23.14	37.35	3
40					2.66	6.83	13.13	22.28	36.70	3
50						4.31	10.81	20.26	35.17	3
60							6.88	16.89	32.66	3
70								11.09	28.56	4
80									22.10	5

Table 2. Probability of developing cancer in Japan in 2001

Age	Male							Female						
	Total alive at beginning of interval	Cancer free at beginning of interval	Cancer survival at beginning of interval	Number who die this interval	Number who die of cancer this interval	Number who develop cancer this interval	Cumulative probability of developing cancer from birth (%)	Total alive at beginning of interval	Cancer free at beginning of interval	Cancer survival at beginning of interval	Number who die this interval	Number who die of cancer this interval	Number who develop cancer this interval	Cumulative probability of developing cancer from birth (%)
0	100 000	100 000	0	455	10	81	0.08	100 000	100 000	0	386	9	68	0.07
5	99 545	99 473	71	68	11	37	0.12	99 614	99 556	59	50	9	26	0.09
10	99 476	99 379	97	60	12	36	0.15	99 565	99 489	76	42	9	32	0.13
15	99 416	99 296	120	221	18	45	0.20	99 523	99 425	98	92	12	45	0.17
20	99 195	99 048	147	313	23	59	0.26	99 431	99 300	131	134	15	103	0.27
25	98 882	98 700	182	334	28	94	0.35	99 297	99 078	219	152	28	207	0.48
30	98 548	98 301	247	398	49	155	0.51	99 144	98 747	397	206	59	469	0.95
35	98 150	97 797	353	584	91	252	0.76	98 938	98 132	806	296	125	724	1.67
40	97 566	97 055	511	846	189	498	1.26	98 642	97 239	1404	451	225	1077	2.75
45	96 720	95 905	815	1381	409	960	2.22	98 192	95 941	2251	717	393	1513	4.26
50	95 339	93 983	1356	2198	796	1902	4.12	97 475	94 113	3362	1088	604	1963	6.23
55	93 141	90 707	2434	3389	1419	2814	6.93	96 386	91 686	4700	1512	812	2091	8.32
60	89 752	85 989	3762	4661	2105	4372	11.30	94 874	88 933	5941	2085	1074	2712	11.03
65	85 090	79 203	5888	7087	3372	6345	17.65	92 789	85 282	7507	3309	1527	3408	14.44
70	78 004	69 475	8529	10 227	4552	8254	25.90	89 480	80 256	9223	5242	2073	4152	18.59
75	67 777	56 334	11 443	13 441	5056	8618	34.52	84 237	73 304	10 933	8655	2712	4745	23.34
80	54 336	41 057	13 279	17 347	5059	6456	40.98	75 583	63 477	12 106	14 197	3404	4438	27.77
85+	36 989	25 675	11 314	36 989	3875	8032	49.01	61 385	50 099	11 287	61 385	3473	9587	37.36
Total					27 073	49 008						16 565	37 361	

"Cancer survival at beginning of interval" is calculated as the difference between "Total alive at beginning of interval" and "Cancer-free at beginning of interval."

Table 3. Lifetime probability of developing and dying from cancer and probability of developing cancer, by age, in Japan

Primary sites	ICD-10th	Lifetime probability (%) of				Probability (%) of developing cancer in next 10 years by age group (2001)				
		Developing (2001)		Dying (2005)						
		%	1 in	%	1 in	30–39	40–49	50–59	60–69	70–79
Male										
All site	C00–C96	49.01	2	26.59	4	0.41	1.50	5.02	12.46	24.28
Stomach	C16	10.77	9	4.42	23	0.08	0.38	1.21	2.85	4.88
Lung	C33–C34	8.00	12	6.23	16	—	0.15	0.56	1.59	4.09
Colon	C18	5.44	18	1.84	54	—	0.19	0.60	1.49	2.45
Prostate	C61	4.11	24	1.39	72	—	—	0.11	0.77	2.11
Liver	C22	3.95	25	2.99	33	—	0.12	0.50	1.24	1.78
Rectum	C19–C21	3.04	33	1.15	87	—	0.13	0.43	0.94	1.25
Esophagus	C15	1.97	51	1.20	83	—	—	0.27	0.62	0.89
Bladder	C67	1.96	51	0.61	165	—	—	0.17	0.40	0.88
Pancreas	C25	1.76	57	1.61	62	—	—	0.18	0.41	0.75
Gallbladder	C23–C24	1.45	69	1.11	90	—	—	0.09	0.26	0.61
Kidney	C64–C66, C68	1.18	85	0.54	186	—	0.05	0.15	0.30	0.51
Malignant lymphoma	C81–C85, C96	1.15	87	0.66	151	—	0.06	0.13	0.25	0.45
Lip, oral cavity and pharynx	C00–C14	0.96	104	0.52	191	—	0.06	0.16	0.28	0.36
Hematopoietic tissue	C91–C95	0.73	137	0.57	177	—	—	0.08	0.16	0.27
Skin	C43–C44	0.69	146	0.09	1139	—	—	0.05	0.12	0.26
Larynx	C32	0.46	216	0.14	724	—	—	0.06	0.13	0.23
Brain and nervous system	C70–C72	0.34	291	0.11	872	—	—	—	0.06	0.10
Multiple myeloma	C88–C90	0.34	295	0.27	368	—	—	—	0.07	0.14
Thyroid	C73	0.23	432	0.06	1640	—	—	—	0.06	0.07
Colon/rectum	C18–C21	8.47	12	2.99	33	0.07	0.32	1.03	2.44	3.74
Female										
All site	C00–C96	37.36	3	16.17	6	1.21	2.66	4.31	6.88	11.09
Stomach	C16	5.79	17	2.21	45	0.07	0.26	0.49	0.96	1.75
Breast	C50, D05	5.08	20	1.26	80	0.31	1.10	1.20	1.09	0.96
Colon	C18	4.74	21	1.72	58	—	0.14	0.42	0.86	1.50
Lung	C33–C34	3.52	28	2.13	47	—	0.09	0.27	0.60	1.12
Liver	C22	2.08	48	1.42	70	—	—	0.09	0.43	0.79
Uterus (only invasive)	C53–C55	2.07	48	0.65	154	0.23	0.29	0.43	0.37	0.36
Rectum	C19–C21	1.95	51	0.64	157	—	0.09	0.24	0.41	0.56
Gallbladder	C23–C24	1.83	54	1.12	89	—	—	0.07	0.20	0.50
Pancreas	C25	1.67	60	1.34	74	—	—	0.09	0.25	0.50
Ovary	C56	1.03	97	0.53	187	0.05	0.13	0.22	0.21	0.19
Malignant lymphoma	C81–C85, C96	0.94	106	0.47	212	—	0.05	0.09	0.17	0.28
Skin	C43–C44	0.81	123	0.07	1424	—	—	—	0.09	0.18
Thyroid	C73	0.78	128	0.13	773	0.06	0.11	0.16	0.17	0.15
Bladder	C67	0.73	137	0.24	412	—	—	—	0.10	0.21
Kidney	C64–C66, C68	0.63	158	0.27	371	—	—	0.06	0.12	0.21
Hematopoietic tissue	C91–C95	0.55	182	0.37	267	—	—	0.05	0.09	0.14
Esophagus	C15	0.43	233	0.21	472	—	—	—	0.08	0.11

Continued

Table 3. *Continued*

Primary sites	ICD-10th	Lifetime probability (%) of				Probability (%) of developing cancer in next 10 years by age group (2001)				
		Developing (2001)		Dying (2005)						
		%	1 in	%	1 in	30–39	40–49	50–59	60–69	70–79
Lip, oral cavity and pharynx	C00–C14	0.42	241	0.19	525	—	—	—	0.07	0.11
Multiple myeloma	C88–C90	0.35	287	0.24	409	—	—	—	0.06	0.12
Brain and nervous system	C70–C72	0.33	302	0.09	1100	—	—	—	0.06	0.08
Larynx	C32	0.04	2759	0.01	9692	—	—	—	—	—
colon/rectum	C18–C21	6.68	15	2.35	43	0.07	0.23	0.66	1.28	2.07

ICD-10th, International Classification of Diseases, 10th Revision
—, Value less than 0.05
'Colon/rectum' denotes the probability of colon and rectum being combined.

Table 4. Percent dying from cancer by a specific age, for males and females who were alive at current age in Japan in 2005

Current age	Dying from cancer by age									
	10	20	30	40	50	60	70	80	Eventually	1 in
Male										
0	0.02	0.05	0.10	0.22	0.71	2.67	7.72	17.25	26.59	4
10		0.03	0.07	0.19	0.69	2.65	7.74	17.31	26.69	4
20			0.05	0.17	0.66	2.64	7.73	17.33	26.73	4
30				0.12	0.62	2.60	7.73	17.39	26.86	4
40					0.50	2.51	7.69	17.44	27.00	4
50						2.05	7.35	17.32	27.09	4
60							5.60	16.15	26.49	4
70								12.02	23.80	4
80									16.75	6
Female										
0	0.02	0.04	0.08	0.25	0.80	2.16	4.60	9.22	16.17	6
10		0.02	0.06	0.24	0.78	2.15	4.60	9.24	16.21	6
20			0.04	0.22	0.76	2.14	4.58	9.23	16.21	6
30				0.18	0.72	2.10	4.56	9.22	16.22	6
40					0.55	1.94	4.40	9.09	16.13	6
50						1.40	3.90	8.64	15.75	6
60							2.56	7.42	14.72	7
70								5.14	12.84	8
80									9.01	11

stomach cancer before age 70. This table is ordered by lifetime probability of developing cancer in 2001. The cancer site with the highest lifetime probability in 2001 was stomach for both sexes (males, 10.77%; females, 5.79%). The cancer site with the highest dying probability in 2005 was lung for males (6.23%) and stomach for females (2.21%). The following are the comparisons of the five sites with the highest probability of incidence between 1999 and 2001 and of mortality between 1999 and 2005. The probability of developing cancer for males in 1999 was highest for stomach (10.46%), followed by lung (7.44%), colon (5.25%), liver (3.76%) and prostate (3.26%), and for females, the probability was highest for stomach (5.59%), followed by breast (4.54%), colon (4.40%), lung (3.09%) and uterus (2.39%) (2). So, the sites with the highest probabilities were the same (stomach) between 1999 and 2001.

The probability of developing prostate cancer became higher than that of liver cancer for men, and the probability of developing liver cancer became higher than that of uterine cancer for women in 2001. The probability of dying cancer for males in 1999 was highest for lung (6.59%), followed by stomach (5.55%), liver (3.42%), colon (2.04%) and pancreas (1.66%), and for female the probability was highest for stomach (3.37%), followed by lung (2.61%), colon (2.18%), liver (1.76%) and gallbladder (1.60%) (2). Thus, the sites and their order did not change between 1999 and 2005. Here, we must note that the probability is not necessarily in proportion to the number, because the number depends on the current age distribution of population. Actually, the site with the largest number of incidence is breast for female in 2001, but it is the second highest in our estimate of probability of developing cancer (Table 3).

The primary aim of this paper was to estimate lifetime and age-conditional probabilities of developing and dying of cancer. Although the method for deriving these probabilities requires several assumptions (1), these probabilities express the current risk of cancer well. Lifetime or age-conditional probability is a very familiar index for nations, because the risk of cancer can be converted to a percentage for each situation. Especially, the interpretation using the reciprocal number of this probability, for example, one in two males will develop cancer and one in four males will die of cancer during their lifetime, makes the risk for cancer to an intuitively comprehensible form.

In many other countries, these indexes are reported as a useful summary of the cancer risk, for example (5) in Canada and (6) in USA, and quoted in popular press. Moreover by extending the method for estimating probability, we will be able to include several factors which influence on cancer risk, for example smoking status (7). So it is necessary to introduce these useful indexes in Japan, which will contribute Japanese cancer control program and prevention.

Acknowledgements

The authors give thanks to the Research Group for Population-Based Cancer Registration in Japan.

Funding

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare, Japan (8−2), and the Foundation for the Promotion of Cancer Research for the Third-Term Comprehensive 10-Year Strategy for Cancer Control. K.K.'s research was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan, Grant-in-Aid for Young Scientists (B), No. 18790398, 2006–2008.

Conflict of interest statement

None declared.

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Association between genetic polymorphisms of the base excision repair gene *MUTYH* and increased colorectal cancer risk in a Japanese population

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(Received May 28, 2007/Revised October6/October 17, 2007/Accepted October 24, 2007/Online publication February 4, 2008)

The *MUTYH* gene encodes a DNA glycosylase that can initiate the base excision repair pathway and prevent G:C > T:A transversion by excising adenine mispaired with 8-hydroxyguanine. Biallelic germline mutations of *MUTYH* have been shown to predict familial and sporadic multiple colorectal adenomas and carcinomas, however, whether there is an association between single nucleotide polymorphisms (SNPs) of *MUTYH* and sporadic colorectal cancer (CRC) risk has remained unclear. In this study we investigated four *MUTYH* SNPs, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G, and 972G > C (Gln324His), for an association with increased CRC risk in a population-based series of 685 CRC patients and 778 control subjects from Kyushu, Japan. A statistically significant association was demonstrated between IVS1+11T and increased CRC risk (odds ratio [OR]: 1.43; 95% confidence interval [CI]: 1.012–2.030; $P = 0.042$) and one of the five haplotypes based on the four SNPs, the IVS1+11T – IVS6+35G – IVS10–2A – 972C (TGAC) haplotype containing IVS1+11T, was demonstrated to be associated with increased CRC risk (OR, 1.43; 95% CI, 1.005–2.029; $P = 0.046$). Subsite-specific analysis showed that the TGAC haplotype was statistically significantly ($P = 0.013$) associated with an increased risk of distal colon, but not proximal colon or rectal cancer. Furthermore, IVS1+11C > T was found to be in complete linkage disequilibrium with –280G > A and 1389G > C (Thr463Thr). The results indicated that Japanese individuals with –280A/IVS1+11T/1389C genotypes or the TGAC haplotype are susceptible to CRC. (*Cancer Sci* 2008; 99: 355–360)

Intracellular DNA is at risk of damage by reactive oxygen species (ROS) generated by normal metabolism and environmental exposure, and 8-hydroxyguanine (8-ohG) is one of the products induced by ROS damage and is known to be a mutagenic lesion.[1,2] The base excision repair (BER) pathway plays an important role in repairing oxidative-damage-induced mutations, and the *MUTYH* gene encodes the glycosylase capable of initiating the BER pathway by catalyzing the removal of adenine residues mispaired with 8-ohG.[3–5] It has been indicated that defects in the BER pathway may contribute to tumorigenesis by increasing mutation frequency in oncogenes and tumor suppressor genes.[6] In fact, it has been reported that some cases of autosomal recessive inherited multiple colorectal adenomatous polyposis and carcinoma with an increased frequency of somatic G:C > T:A mutations in *APC* are attributable to biallelic germline mutations in the *MUTYH* gene.[7–10] The disease-causing mutations, Y165C, G382D, 466delE, E466X, and Y90X have been reported in Caucasians, Indian, Pakistani and other ethnic groups.[8,9,11,12] The frequencies of Y165C and G382D have been investigated in several colorectal cancer (CRC) case-control studies, and monoallelic carriers of these variants were found in 0.0–2.6% of the cases and 0.0–2.1% of the controls and biallelic carriers of these variants were found in 0.0–0.8% of the cases and 0% of the controls, respectively.[13–18] However, neither of these two variants has ever been detected in East Asians, including Japanese,[19–22] suggesting that they are ethnicity-specific alleles. Based on the above findings, we hypothesized that *MUTYH* variants other than Y165C and G382D act as low-penetrance susceptibility alleles in Japanese CRC, similar to a situation previously reported for the *APC* and *CHEK2* gene variants.[23,24]

We conducted a CRC case-control study to evaluate the significance of *MUTYH* variants in a Japanese population. In the single-nucleotide polymorphisms (SNPs) reported in the Japanese population,[19,20] four SNPs (IVS1+11C > T, IVS6+35G > A, IVS10–2A > G and 972G > C [Gln324His]), were selected, and all 685 cases and 778 matched controls were genotyped to detect these four SNPs. Statistically significant association was found between the IVS1+11C > T SNP and increased CRC risk in the Japanese population. A haplotye-based association study was also carried out, and a statistically significant association was found between the IVS1+11T – IVS6+35G – IVS10–2A – 972C (TGAC) haplotype containing the IVS1+11T allele and CRC risk. In the subsite-specific analysis, the IVS1+11C > T SNP was detected to be nearly statistically significantly associated and the TGAC haplotype was found to be statistically significantly associated with an increased risk of distal colon, but not proximal colon or rectal cancer. We also found that a novel –280G > A SNP in the 5′ flanking region of *MUTYH* and a previously reported 1389G > C (Thr463Thr) SNP were both in complete linkage disequilibrium with the IVS1+11C > T. Our results suggest that the –280A/IVS1+11T/1389C or the TGAC haplotype of *MUTYH* may be novel CRC susceptibility alleles.

Materials and Methods

Specimens. Blood specimens from 685 CRC cases and 778 controls were collected in a previous study. DNA was extracted from these specimens and written informed consent was obtained

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[14]Hong Tao is a scholarship student (2006–08) of the Rotary Yoneyama Scholarship endowed by Rotary International, Japan.

doi: 10.1111/j.1349-7006.2007.00694.x

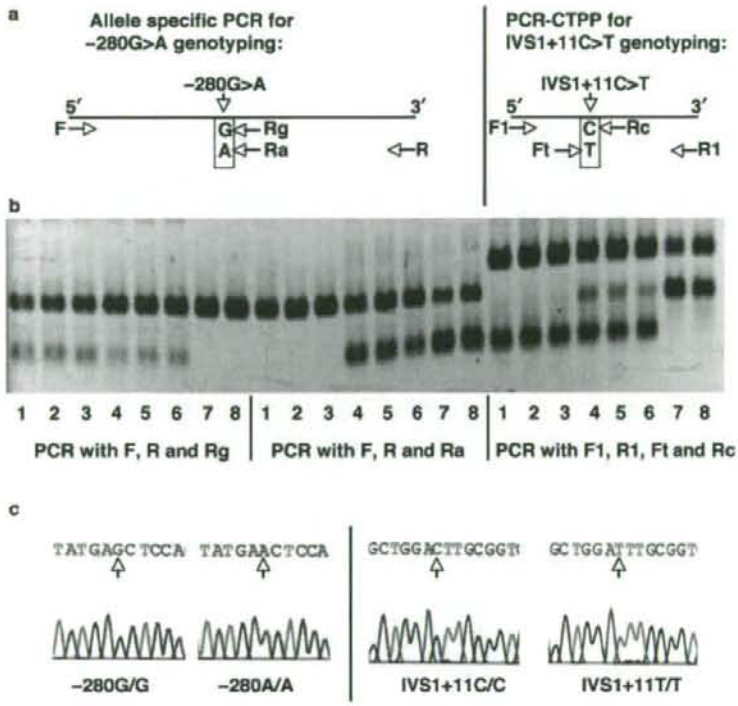

Fig. 1. Genotyping of the –280G > A and IVS1+11C > T single nucleotide polymorphisms (SNPs) of the *MUTYH* gene. (a) The schematic diagrams of the allele-specific polymerase chain reaction (PCR) used to genotype the –280G > A SNP (left) and the PCR with confronting two-pair primers (PCR-CTPP) used to genotype the IVS1+11C > T SNP (right). PCR primers are indicated by the horizontal arrows, and F and R mean forward primer and reverse primer, respectively. The location of each SNP is indicated by a vertical arrow. (b) Agarose gel electrophoresis of the PCR products. Eight samples, three from homozygous carriers of the wild-type allele (No. 1–3), three from heterozygous (No. 4–6) and two from homozygous (No. 7 and 8) carriers of the variation, were genotyped for –280G > A (left and middle) and IVS1+11C > T (right). (c) Sequence electropherograms of the region containing the –280G/G and A/A (left two) and IVS1+11C/C and T/T (right two). The positions of the SNPs are indicated by vertical arrows.

from each individual patient.[25] The characteristics of the cases and controls have been described previously.[25–28] In brief, the cases were composed of a consecutive series of patients with histologically-confirmed incident colorectal adenocarcinomas, and controls were composed of individuals that had no diagnosis of CRC. Other eligibility criteria were as follows: age 20–74 years at the time of diagnosis for the cases or at the time of selection for the controls, residents of the study area (Fukuoka City and three adjacent areas), no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease, and mental competence to give informed consent and participate in the interview. The number of control candidates by gender and 10-year age class was determined in accordance to the expected sex- and age-specific number of incident cases of colorectal cancer. For the reverse transcriptase-polymerase chain reaction (RT-PCR) experiment, total RNA was extracted from the non-cancerous colorectal mucosa of six CRC patients and converted to cDNA, as described previously.[19] This study was approved by the Institutional Review Board (IRB) of Hamamatsu University School of Medicine (12–14, 18-4).

Target SNPs and genotyping. The six SNPs genotyped in this study were as follows: IVS1+11C > T (rs2275602), IVS6+35G > A (rs3219487), IVS10–2A > G (5′-flanking sequence: 5′-CAC TCA ACC CTG TGC CTC TC-3′; 3′-flanking sequence: 5′-GGT GGA GCA GGA ACA GCT CT-3′), 972G > C (Gln324His) (rs3219489), G382D (rs36053993), and –280G > A (5′-flanking sequence: 5′-ATT ACT ACT AAC CGT TAT GA-3′; 3′-flanking sequence: 5′-CTC CAG ACT ACA TCT CCC GC-3′). The IVS10–2A > G and –280G > A had not been presented in the SNP database (dbSNP) of the National Center for Biotechnology Information (NCBI) Entrez system. Genotyping of the four target SNPs, namely, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G and 972G > C (Gln324His), was carried out by PCR with confronting two-pair primers (PCR-CTPP), as described previously (Fig. 1a,b),[19] and genotyping of the G382D SNP was carried out by PCR-restriction fragment length polymorphism (PCR-RFLP). Genotyping of the –280G > A SNP was carried out by two independent allelic-specific PCRs (Fig. 1a,b). The PCR primers used were: IVS1+11C > T SNP: F1 (5′-AAC TAT GAG CCC GAG GCC TTC C-3′), R1 (5′-CAG CAG AAC ACG GAG GCC C-3′), F2 (5′-AGT CGT CTG TGG GTA CGC TGG AT-3′), and R2 (5′-CCA GGA GAC GGA CCG CAA G-3′); IVS6+35G > A: F1 (5′-CCA GTG TGG GTC TCA GAG G-3′), R1 (5′-CCC TAG CTC CTC TAC CAC CTG-3′), F2 (5′-CTA GGG TAG GGG AAA TAG GAA CA-3′), and R2 (5′-CAC CCG TCA GTC CCT CTA TC-3′); IVS10–2A > G SNP, those described previously,[19] 972G > C (Gln324His) SNP: F1 (5′-CCT GTC GGG CAG TCC TGA CG-3′), R1 (5′-CGC TGA AGC TGC TCT GAG GGC-3′), F2 (5′-CCC AGC TCC CAA CAC TGG ACA C-3′), and R2 (5′-GAG GCA GGC ACA GGT GGC AC-3′); G382D SNP: F (5′-GCC CAA ATT CTG CTG GTG C-3′) and R (5′-GCC CAA CGC TGT AGT TCC TG-3′); –280G > A SNP, F (5′-TAC TGT TCT CAT GGT GCC CC-3′), R (5′-GCC TCG GGC TCA TAG TTC TAG-3′), Ra (5′-GCG GGA GAT GTA GTC TGG AGT-3′), and Rg (5′-CGG GAG ATG TAG TCT GGA GC-3′). PCR products were fractionated by electrophoresis on a 2.0% agarose gel and stained with ethidium bromide. All the cases and controls were genotyped for all of the above SNPs.

Statistical analysis. χ^2 tests were used for deviation from the Hardy–Weinberg equilibrium (HWE) among the controls, and the significance level was set at 0.05. Associations between *MUTYH* genotypes or haplotypes and risk of CRC were assessed by calculating the odds ratio (OR) and 95% confidence interval (CI). SAS version 8.2 software (SAS institute, Inc., Cary, NC, USA) was used to carry out the statistical analysis. A *P*-value less than 0.05 was accepted as statistically significant in all cases. Adjustment for multiple testing was performed using false discovery rate (FDR) principle.[29] Haplotypes were inferred by

doi: 10.1111/j.1349-7006.2007.00694.x

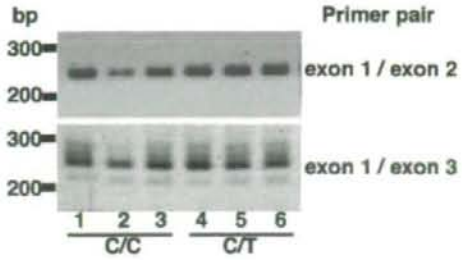

Fig. 2. Reverse transcription-polymerase chain reaction (RT-PCR) analysis. RT-PCR was carried out with a set of primers located at exon 1 and exon 2 (upper panel) and a set of primers located at exon 1 and exon 3 (lower panel). cDNAs from three homozygous carriers of the wild-type allele (lanes 1–3) and three heterozygous carriers of the variation (lanes 4–6) were used as the templates.

the expectation maximization algorithm with the SNPAlyze Version 5.0 software (DYNACOM, Yokohama, Japan). Five haplotypes with a frequency of greater than 1% were selected for further statistical analysis. The linkage disequilibrium analysis of the haplotypes was carried out using the SNPAlyze Version 5.0 software (DYNACOM).

RT-PCR analysis. Reverse transcription-polymerase chain reaction was carried out for the IVS1+11C/C and C/T genotype, respectively, with 1 μL of each cDNA prepared from the non-cancerous colorectal mucosa of six CRC patients. The two primer pairs shown in Fig. 2 were used: one pair was composed of a forward primer in exon 1 and a reverse primer in exon 2, and the other pair was composed of the same forward primer in exon 1 and a reverse primer in exon 3. The sequences of the primer sets are available on request.

Results

Target SNP selection. Among the variants registered in the dbSNPs of the NCBI Entrez system, there were six *MUTYH* SNPs, namely, IVS1+11C > T (rs2275602), IVS1+1841G > A (rs3219472), IVS1+3221T > G (rs3219476), IVS6+35G > A (rs3219487), IVS14–40G > C (rs3219493) and 972G > C (Gln324His) (rs3219489), that have been detected in the Japanese population. Among the SNPs reported in previous publications,[19,20] five *MUTYH* SNPs, namely, IVS1+11C > T (rs2275602), IVS6+35G > A (rs3219487), IVS10–2A > G (5′-flanking sequence: 5′-CAC TCA ACC CTG TGC CTC TC-3′; 3′-flanking sequence: 5′-GGT GGA GCA GGA ACA GCT CT-3′), 972G > C (Gln324His) (rs3219489), and 1389G > C (Thr463Thr) (5′-flanking sequence: 5′-CCA GGT GCT CGC TGG CTG AC-3′; 3′-flanking sequence: 5′-CAG GAG GAA TTT CAC ACC GC-3′) have been reported in the Japanese population. However, since the IVS6+35G > A and IVS1+11C > T had been found to be in complete linkage disequilibrium with IVS14–40G > C and 1389G > C (Thr463Thr), respectively. The remaining six SNPs were initially selected as candidates. For the haplotype association analysis, a pilot study was carried out by genotyping the six SNPs in 30 healthy Japanese individuals. Analysis with the SNPAlyze Version 5.0 software revealed the five haplotypes with a frequency of more than 1%, and they comprised all of the total predicted haplotype variation. As we were able to distinguish these five haplotypes with four SNPs, namely, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G, and 972G > C (Gln324His), the 4 SNPs were ultimately chosen as the haplotype-tagging SNPs.

Association between the IVS1+11C > T SNP and increased risk of CRC. The 685 cases and 778 controls were genotyped for the *MUTYH* SNPs by PCR-CTPP, and the accuracy of the genotyping was verified by sequencing five specimens for each genotype of each SNP. The concordance rate was 100% (data not shown). The frequencies of each SNP are summarized in Table 1. The genotypic distributions of all the SNPs detected were in HWE. The IVS1+11C > T SNP, whose functional role has never been investigated, was shown to be statistically significantly associated with increased CRC risk. The crude OR was 1.43 (95% CI, 1.012–2.030; $P = 0.042$). After adjustments for gender, age and place of residence, the OR was estimated to be 1.46 (95% CI, 1.024–2.069; $P = 0.036$) (Table 1). The *P*-value remained less than 0.05 after FDR adjustment (Table 1). No statistically significant differences in the frequency of any of the other three SNPs, IVS6+35G > A, IVS10–2A > G, and 972G > C, were observed between the cases and controls (Table 1). Furthermore, the association between the SNPs of *MUTYH* and the risk of CRC was examined by the anatomic subsite of the CRC. It showed that the IVS1+11 A/T + T/T genotypes were nearly statistically significantly associated with an increased risk of distal colon cancer risk (OR, 1.58; 95% CI, 0.984–2.544; $P = 0.058$) (Table 2). Since monoallelic mutation of G382D has recently been shown to be associated with CRC risk in Caucasians,[16] the 685 cases and 778 control subjects were also examined for G382D, but no homozygotes or heterozygotes for this mutation were detected (data not shown). Because the complete linkage disequilibrium between IVS1+11C > T and 1389G > C had already been reported,[19] the results suggested that the IVS1+11C > T and 1389G > C variants of *MUTYH* may confer susceptibility to CRC in the Japanese population.

Table 1. Genotypes of the four *MUTYH* single nucleotide polymorphisms (SNPs) and risk of colorectal cancer

Variation[†]	Genotype	No. of controls (%)/cases (%)	Not adjusted		Adjusted[‡]		
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	FDR adjusted *P*-value[§]
IVS1+11C > T	C/C	714 (91.8)/607 (88.6)	1.00 (reference)	–	1.00 (reference)	–	–
	C/T + T/T	64 (8.2)/78 (11.4)	1.43 (1.012–2.030)	0.042	1.46 (1.024–2.069)	0.036	0.036
IVS6+35G > A	G/G	628 (80.7)/539 (78.7)	1.00 (reference)	–	1.00 (reference)	–	–
	G/A	143 (18.4)/140 (20.4)	1.14 (0.880–1.480)	0.321	1.14 (0.878–1.485)	0.321	0.963
	A/A	7 (0.9)/6 (0.9)	1.00 (0.334–2.990)	0.998	0.97 (0.320–2.926)	0.953	>1.0
IVS10–2A > G	A/A	741 (95.2)/662 (96.6)	1.00 (reference)	–	1.00 (reference)	–	–
	A/G + G/G	37 (4.8)/23 (3.4)	0.70 (0.409–1.183)	0.178	0.67 (0.390–1.139)	0.138	0.276
972G > C (Gln324His)	G/G	215 (27.6)/194 (28.3)	1.00 (reference)	–	1.00 (reference)	–	–
	G/C	395 (50.8)/350 (51.1)	0.98 (0.771–1.250)	0.883	0.96 (0.751–1.223)	0.733	>1.0
	C/C	168 (21.6)/141 (20.6)	0.93 (0.692–1.251)	0.632	0.90 (0.670–1.220)	0.511	>1.0

[†]Nucleotide +1 is the A of the ATG-translation initiation codon. [‡]Adjustment was made for gender, 5-year age class, and residential area. [§]False discovery rate (FDR) adjusted *P*-value. CI, confidence interval; OR, odds ratio.

Table 2. ***MUTYH*** **genotypes and the risk of colorectal cancer (CRC) stratified by anatomic subsite**

Variation†	Genotype	Proximal colon‡ ($n = 150$)			Distal colon‡ ($n = 232$)			Rectum‡ ($n = 290$)		
		No.	OR (95% CI)	*P*-value	No.	OR (95% CI)	*P*-value	No.	OR (95% CI)	*P*-value
IVS1+11C > T	C/C	133	1.00 (reference)	–	203	1.00 (reference)	–	259	1.00 (reference)	–
	C/T + T/T	17	1.50 (0.843–2.664)	0.169	29	1.58 (0.984–2.544)	0.058	31	1.36 (0.861–2.154)	0.187
IVS6+35G > A	G/G	122	1.00 (reference)	–	178	1.00 (reference)	–	229	1.00 (reference)	–
	G/A	28	1.03 (0.655–1.629)	0.887	51	1.27 (0.884–1.835)	0.195	58	1.07 (0.757–1.510)	0.703
	A/A	0	–	0.984	3	1.52 (0.383–6.056)	0.551	3	1.15 (0.286–4.581)	0.848
IVS10–2A > G	A/A	143	1.00 (reference)	–	226	1.00 (reference)	–	281	1.00 (reference)	–
	A/G + G/G	7	0.98 (0.422–2.268)	0.959	6	0.48 (0.197–1.156)	0.101	9	0.62 (0.295–1.319)	0.216
972G > C (Gln324His)	G/G	34	1.00 (reference)	–	72	1.00 (reference)	–	83	1.00 (reference)	–
	G/C	95	1.57 (1.020–2.426)	0.040	105	0.78 (0.553–1.108)	0.167	144	0.90 (0.655–1.247)	0.538
	C/C	21	0.78 (0.434–1.405)	0.409	55	0.99 (0.654–1.484)	0.943	63	0.94 (0.639–1.392)	0.768

†Nucleotide +1 is the A of the ATG-translation initiation codon. ‡Adjustment was made for gender, 5-year age class, and residential area. CI, confidence interval; OR, odds ratio.

Table 3. Haplotype frequency based on the four ***MUTYH*** **single nucleotide polymorphisms (SNPs) and risk of colorectal cancer**

Haplotype†				Frequency (%)‡		Not adjusted		Adjusted§		
IVS1+11C > T	IVS6+35G > A	IVS10–2A > G	972G > C	Control	Case	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	FDR adjusted *P*-value¶
C	G	A	C	42.9	40.4	1.00 (reference)	–	1.00 (reference)	–	–
C	G	A	G	40.9	41.5	0.93 (0.791–1.089)	0.360	1.10 (0.936–1.293)	0.248	0.992
C	A	A	G	9.8	10.7	1.08 (0.840–1.397)	0.537	1.18 (0.910–1.519)	0.215	0.645
T	G	A	C	4.0	5.8	1.43 (1.005–2.029)	0.046	1.56 (1.098–2.228)	0.013	0.013
C	G	G	G	2.5	1.6	0.63 (0.370–1.079)	0.090	0.66 (0.387–1.136)	0.135	0.270

†Nucleotide +1 is the A of the ATG-translation initiation codon. ‡Inferred common haplotypes with frequency >1% are listed. §Adjustment was made for gender, 5-year age class, and residential area. ¶False discovery rate (FDR) adjusted *P*-values. CI, confidence interval; OR, odds ratio.

Table 4. ***MUTYH*** **haplotypes and the risk of colorectal cancer (CRC) stratified by anatomic subsite**

Haplotype†				Proximal colon‡		Distal colon‡		Rectum‡	
IVS1+11C > T	IVS6+35G > A	IVS10–2A > G	972G > C	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
C	G	A	C	1.00 (reference)	–	1.00 (reference)	–	1.00 (reference)	–
C	G	A	G	1.13 (0.855–1.484)	0.397	1.10 (0.871–1.389)	0.424	1.06 (0.856–1.311)	0.595
C	A	A	G	1.00 (0.634–1.587)	0.990	1.27 (0.885–1.816)	0.196	1.15 (0.822–1.594)	0.424
T	G	A	C	1.61 (0.904–2.879)	0.106	1.81 (1.131–2.884)	0.013	1.36 (0.854–2.166)	0.195
C	G	G	G	0.99 (0.430–2.296)	0.988	0.43 (0.166–1.116)	0.083	0.62 (0.296–1.316)	0.216

†Nucleotide +1 is the A of the ATG-translation initiation codon. ‡Adjustment was made for gender, 5-year age class, and residential area. CI, confidence interval; OR, odds ratio.

Association between the TGAC haplotype containing the IVS1+11T and increased risk of CRC. Haplotype-based association studies are known to have greater power than individual SNP-based association studies.[30] Haplotype analyses were carried out based on the genotyping data of the four SNPs of *MUTYH*, namely, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G, and 972G > C. There were five haplotypes with a frequency greater than 1%, CGAC, CGAG, CAAG, TGAC, and CGGG (Table 3), and since the CGAC haplotype was detected in 42.7% of the controls (Table 3), the highest percentage, the CGAC haplotype was used as the reference haplotype, and the following statistical analysis was carried out using the SAS system. Consistent with the results for each SNP, the TGAC haplotype containing the IVS1+11T allele was statistically significantly associated with increased CRC risk. The crude OR was 1.43 (95% CI, 1.005–2.029; $P = 0.046$) and after adjustment for gender, age and place of residence, the OR was 1.56 (95% CI, 1.098–2.228; $P = 0.013$) (Table 3). The *P*-value remained less than 0.05 after FDR adjustment (Table 3). The results of subsite-specific analysis revealed a significant association between the TGAC haplotype and increased risk of distal colon cancer (OR, 1.81; 95% CI, 1.131–2.884; $P = 0.013$) (Table 4). These results suggested that the TGAC haplotype containing the c.36+11T SNP confers susceptibility to CRC, especially to distal colon cancer, in the Japanese population.

RT-PCR analysis and detection of a novel SNP –280G > A linked with IVS1+11C > T. The association between the IVS1+11C > T SNP of *MUTYH* and CRC risk suggested a functional difference between IVS1+11C and IVS1+11T. The IVS1+11C > T SNP is located in the boundary region between *MUTYH* exon 1 and intron 1, and many reports have suggested that gene variants in the neighborhood of the junction are often accompanied by abnormal splicing.[31–33] In order to investigate whether the IVS1+11C > T SNP affects the splicing of *MUTYH*, an RT-PCR analysis was carried out by using cDNAs from carriers of the IVS1+11C/T and C/C genotype. However, no splicing abnormalities were detected in the cases carrying the IVS1+11C/T genotype (Fig. 2). On the other hand, during our checking of the sequences around the first exon of *MUTYH* to exclude variation at the splice site, a novel SNP of –280G > A was detected in the sample carrying the IVS1+11C > T SNP (Fig. 1b,c). Further genotyping was carried out in all subjects,

doi: 10.1111/j.1349-7006.2007.00694.x

and –280G > A was found to be 100% linked with IVS1+11C > T. The –280G > A SNP was demonstrated to be in complete linkage disequilibrium with the IVS1+11C > T SNP ($r^2 = 1$) by the SNPAlyze Version 5.0 software. Since the –280G > A SNP and 1389G > C (Thr463Thr) SNP were both in complete linkage disequilibrium with the IVS1+11C > T SNP, the results suggested that the Japanese individuals with the –280 A/IVS1+11T/1389C alleles or the –280A – IVS1+11T – IVS6+35G – IVS10-2A – 972C – 1389C haplotype were significantly associated with increased CRC risk.

Discussion

In this Japanese population-based case-control study four *MUTYH* SNPs, namely, IVS1+11C > T, IVS6+35G > A, IVS10–2A > G, and 972G > C, were genotyped in 685 CRC cases and 778 controls. The frequency distribution of IVS1+11T and the IVS1+11T – IVS6+35G – IVS10-2A – 972C (TGAC) haplotype were significantly associated with increased CRC risk. Subsite-specific analysis showed that the IVS1+11C > T SNP was nearly statistically significantly associated ($P = 0.058$) and the TGAC haplotype were statistically significantly associated ($P = 0.013$) with an increased risk of distal colon, but not proximal colon or rectal cancer. Next, we found that the IVS1+11C > T SNP was in complete linkage disequilibrium with –280G > A. No aberrant splicing induced by IVS1+11T allele was detected by RT-PCR. Together with the previously detected 1389G > C (Thr463Thr), a SNP in complete linkage disequilibrium with IVS1+11C > T, our results suggested that individuals who have the *MUTYH* – 280 A/IVS1+11T/1389C alleles or the TGAC haplotype are more susceptible to CRC in the Japanese population.

The present study is the first Japanese population-based CRC case-control study to evaluate the association between the SNPs of *MUTYH* and the risk of CRC by the anatomic subsite of the colorectal cancer. The results demonstrate that the IVS1+11C > T SNP and TGAC haplotype confer susceptibility to distal colon cancer in the Japanese population. Since this study investigated the association between four SNPs and five haplotypes of *MUTYH* and the risk of CRC on the same set of samples, a method for multiple testing is applicable to this study. Therefore, the method of FDR was used for all of the results, and the statistical significance of the associations was found to remain essentially unchanged (Tables 1 and 3). These results coincide with the fact that tumors arising from different subsites of the colorectum differ in their population distribution, clinical features as well as genetic pathways.[34–37] It was suggested from our results that the IVS1+11 > T SNPs and TGAC haplotype of *MUTYH* may be involved in distal colon carcinogenesis and that the risk of cancer arising from each anatomic subsite of the colorectum may be modified by different genetic pathways. Further studies need to be conducted to elucidate the underlying mechanisms.

The development of CRC is a multistep, multifactor process.[38] Some studies have demonstrated that environmental factors and physical conditions may modify the genetic risk of CRC associated with SNPs.[39,40] This may also hold true for the CRC risk associated with *MUTYH* SNPs. In the present study, adjustment was made for gender, age and place of residence to evaluate the association between the *MUTYH* SNPs and CRC risk. The adjusted OR and 95% CI remained essentially unchanged after the adjustments as compared with the values obtained without the adjustments (Tables 1 and 3). Furthermore, when the body mass index, disease history, physical activity, dietary factors, smoking and alcohol consumption status were taken into consideration, the *P*-value for IVS1+11C > T was 0.058, a nearly significant value, and the *P*-value for the TGAC haplotype remained less than 0.05 (data not shown). This result suggested that the association between the *MUTYH* SNPs and the risk of CRC was not significantly modified by environmental factors or the physical condition.

In the present study, the 972G/C genotype was statistically significantly associated with increased risk of proximal colon, but not distal colon or rectal cancer (Table 2). The functional analysis revealed no difference between the C/C type and G/G type,[4] and the 972C allele is more frequently detected in Japanese and Chinese than in European populations as shown in the dbSNPs of the NCBI Entrez system. Taking this into consideration with our result, it could be suggested that the 972C allele may be inversely associated with the development of at least proximal colon cancer in the Japanese population. Alternatively, this inverse association of the 972C allele with the risk of proximal colon cancer in the Japanese population may arise from its interaction with other allele(s). The IVS10–2A > G SNP had been demonstrated to generate a protein without nuclear expression and the IVS10–2G allele was suggested to be associated with a low BER function in the cell nuclei and thereby, act as a risk allele for cancer. However, the results of analyses in this study revealed an OR of less than 0.7 (except for cancer of the proximal colon) for the IVS10–2G allele and the CGGG haplotype, which contains the IVS10–2G allele, although the *P*-value did not reach statistical significance (Tables 1–4). These results remained essentially unchanged even after adjustments for environmental factors and physical conditions (as described above). Investigation of some other additional clinical factors, such as pathological stage, recurrence or survival, might yield some association. On the other hand, some studies have suggested that SNPs of repair genes may be associated with reduced cancer risk or fewer recurrences, and that effective host DNA repair capacity may be associated with poorer survival.[41–43] These observations suggest that mutations in the repair genes may also be inversely associated with malignant alterations, in addition to their more widely recognized association with increased cancer risk. The inverse association of the IVS10–2A > G SNP detected in this study with colorectal cancer risk might be explained by the contention that individuals with the A allele may be more resistant to ROS or other stresses than individuals with the G allele, and that the A allele has a protective effect on cells with mutations, similar to the situation suggested by Wang *et al.* for the XRCC1 Arg194Trp variant, a SNP associated with a reduced risk for various types of cancers.[44] Research on the effect of *MUTYHβ* isoforms on the cellular responses to various mutagens are expected help in clarifying this issue.

Besides the RT-PCR experiment, reporter assay was also carried out to investigate whether the –280G > A and IVS1+11C > T SNPs may affect the promoter activity of *MUTYH*. The dual-luciferase reporter assay experiments detected high transcriptional activity of the region (–411~+356) of *MUTYH* (data not shown). This information will be of use for future analyses. The reporter plasmids containing the wild-type and mutant sequence for the responses to oxidative stress were also investigated using the colon cancer cell line HCT116. ROS was induced by glucose oxidase, menadione or H_2O_2 at appropriate concentrations and treatment durations. However, the two linked SNPs, –280G > A and IVS1 +11C > T, did not affect the promoter activity in our setting (data not shown). This study did not detect any functional differences in the –280G > A/IVS1+11C > T/1389G > C SNPs, and there remains the possibility that the three SNPs might be linked with other SNPs and these SNPs might affect the susceptibility to CRC.

Acknowledgments

This work was supported in part by Grants-in-Aid for Young Scientists Category B (17790258) from the Japan Society for the Promotion of Science, Scientific Research in a Priority Area (18014009) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Ministry of Health, Labour and Welfare (15-2, 15-22, 19-19) of Japan and the 21st century COE program 'Medical Photonics', from the Smoking Research Foundation, and from the Foundation for Promotion of Cancer Research.

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doi: 10.1111/j.1349-7006.2007.00694.x

Life Sciences 83 (2008) 301–304

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

MDR1 C3435T polymorphism has no influence on developing *Helicobacter pylori* infection-related gastric cancer and peptic ulcer in Japanese

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ARTICLE INFO

Article history:
Received 29 April 2008
Accepted 25 June 2008

Keywords:
MDR1
Helicobacter pylori
Gastric cancer
Peptic ulcer
MDR1-C3435T polymorphism

ABSTRACT

Aims: P-glycoprotein, the gene product of *multidrug-resistant transporter-1* (*MDR1*), confers multidrug resistance against antineoplastic agents but also affects the kinetic disposition of some drugs and carcinogens. *MDR1* C3435T polymorphism influences the development of colon cancer and adult acute myeloid leukemia by the association with transporting carcinogen. The aim of this study was to clarify the association of *MDR1* C3435T polymorphism with susceptibility to gastric cancer and peptic ulcers in patients with Japanese *H. pylori* infection.

Main methods: We assessed the *MDR1* C3435T polymorphism in *H. pylori*-positive gastritis alone patients ($n=150$), gastric cancer ($n=292$), gastric ulcer ($n=215$), and duodenal ulcer ($n=163$) and *H. pylori*-negative subjects ($n=168$) as control by a PCR-based method.

Key findings: No significant difference existed in frequencies of *MDR1* C3435T polymorphisms between *H. pylori*-negative controls and *H. pylori*-positive gastritis alone patients. Moreover, *MDR1*-3435 T allele carriage didn't affect the risk of gastric cancer or peptic ulcer development. The age- and sex-adjusted odds ratios (ORs) of *MDR1* 3435 T allele carriers relative to the C/C genotype group for gastric cancer, gastric ulcer and duodenal ulcer risk were 0.96 (95%CI: 0.56–1.66), 1.16 (95%CI: 0.72–1.84) and 1.00 (95%CI: 0.61–1.62), respectively.

Significance: In this preliminary data, the association with *MDR1* C3435T polymorphism and risk for developing *H. pylori*-related gastric cancer and peptic ulcer in Japanese was low. P-glycoprotein might not be involved in the carcinogenesis of *H. pylori*-related gastric cancer.

Introduction

In 1994, WHO/IARC designated *Helicobacter pylori* (*H. pylori*) as a definite biological group 1 carcinogen of gastric cancer. Around 50% of the Japanese population are infected with *H. pylori*, and have a five-times higher risk of gastric cancer development in comparison with those without infection (Hamajima et al., 2004). Of gastric carcinogenesis-related host genetic factors, the polymorphism of not only inflammation-related cytokines (El-Omar et al., 2000; El-Omar et al., 2003; Sugimoto et al., 2007a; Sugimoto et al., 2007b), but also drug metabolism-related enzymes, such as cytochrome P450 (CYP) 2A6, CYP2E1, CYP2C19, glutathione *S*-transferase and *N*-acetyltransferase have intensively been studied (Yokose et al., 1998; Cai et al., 2001; Gao et al., 2002; Tsukino et al., 2002; Park et al., 2003; Suzuki et al., 2004; Sugimoto et al., 2005).

In the gastrointestinal tract, P-glycoprotein (P-gp), an ATP-binding cassette (ABC) transporter, is expressed in a high concentration on the apical surfaces of superficial columnar epithelial cells of the colon and distal small bowel (Fojo et al., 1987; Thiebaut et al., 1987). P-gp, the gene product of multidrug-resistant transporter-1 (*MDR1*), confers multidrug resistance against antineoplastic agents but also plays an important role in affecting the kinetics disposition of common drugs and carcinogens (Cascorbi et al., 2001; Pauli-Magnus et al., 2001).

MDR1 is located in chromosome 7q21.1, composed of 28 exons and 209 kilobases in length. More than 40 single nucleotide polymorphisms have been discovered in *MDR1* (Hoffmeyer et al., 2000; Cascorbi et al., 2001; Kroetz et al., 2003). The *MDR1* C3435T polymorphism in exon 26 was most extensively investigated and was first shown to correlate with the activity of P-gp in the duodenum (Hoffmeyer et al., 2000). Individuals with the *MDR1* 3435 T/T genotype have significantly lower duodenal P-gp expression and higher serum digoxin

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doi:10.1016/j.lfs.2008.06.022

Table 1
Demographic characteristics and frequencies of *MDR1* C3435T genotypes

		H. pylori-negative control (n=168)	Gastritis alone (n=292)	Gastric ulcer (n=215)	Duodenal ulcer (n=163)	Gastric cancer (n=150)	*P* value
Age (mean, yr ± SD)		46.0±0.7	52.6±0.7	52.6±0.9	50.7±1.1	68.7±0.8	<***0.01***
Sex (male/female, n/n)		105/63	203/89	177/38	135/28	115/35	<***0.01***
Histology	Intestinal type					111	
	Diffuse type					39	
Clinical stage	Stages 1–2					114	
	Stages 3–4					36	
MDR1 C3435T polymorphism	C/C genotype	49 (29.2%)	109 (37.2%)	56 (26.1%)	46 (28.2%)	49 (32.7%)	0.09
	C/T genotype	96 (57.1%)	141 (48.1%)	117 (54.4%)	81 (49.7%)	73 (48.6%)	
	T/T genotype	23 (13.7%)	43 (14.7%)	42 (19.5%)	36 (22.1%)	28 (18.7%)	

Abbreviations are; MDR1; multidrug-resistant transporter-1.

levels after orally dosed in comparison with those with C/C genotype in Caucasians (Hoffmeyer et al., 2000). However, the effect of *MDR1* C3435T polymorphism on the P-gp expression is controversial and appears to be conflicting by several ethnic and/or geographic factors (Nakamura et al., 2002). Nakamura et al. (Nakamura et al., 2002) reported that plasma levels of orally dosed digoxin were higher in Japanese with the *MDR1* 3435 C/C genotype in comparison with those with the T/T genotype and that mRNA levels of *MDR1* in the duodenum were also lower in the group with the *MDR1* 3435 C/C genotype. Kimchi-Sarfaty et al. (2007) have recently reported that *MDR1* C3435T polymorphism affects the timing of translational folding and insertion of P-gp into the membrane, thereby altering the structure of substance.

Interestingly, *H. pylori* infection upregulates the gastric mucosal P-gp levels with upregulations of COX-2 and prostaglandin in gastric mucosa (Nardone et al., 2004). P-gp is also reportedly associated with the development of neoplastic diseases (Siegsmund et al., 2002; Tafuri et al., 2002; Humeny et al., 2003; Jamroziak et al., 2004; Kurzawski et al., 2005; Koyama et al., 2006; Osswald et al., 2007; Tahara et al., 2007). Although Tahara et al. (2007) reported that the carrier of *MDR1* 3435 T/T genotype had significantly prevented effects in gastric cancer developments, the relationship between the *MDR1* C3435T polymorphism and gastric cancer risks remains totally obscure. Then, we intended to examine whether *MDR1* C3435T polymorphism is associated with gastric cancer risks in Japanese patients with *H. pylori* infection.

Materials and methods

Subjects

A total of 820 Japanese patients infected with *H. pylori* infection on the basis of rapid urease test (RUT) (Helico Check, Otsuka Co., Tokushima, Japan) and 168 *H. pylori*-negative subjects were enrolled in this study at the University Hospital of Hamamatsu University School of Medicine from January 2001 to December 2007. When a result of RUT was obtained from the biopsied specimens under gastroduodenoscopy, they were invited to participate in the study. After their written informed consents were obtained, patients were enrolled in the study. They consisted of the gastric cancer (n=150), gastric ulcer (n=215), duodenal ulcer (n=163), or gastritis alone (n=292) patients and *H. pylori*-negative subjects as control group (n=168). These *H. pylori*-positive patients had endoscopically and histologically proven active chronic gastritis, peptic ulcer or gastric cancer. The gastric cancer group was further pathologically classified into the two subgroups, the intestinal-type group and the diffuse-type group, in according to the Lauren (1965) classification (Lauren, 1965). Gastritis alone group was defined as endoscopical and histological gastritis with no peptic ulcers, gastric cancer, or any esophageal diseases. Demographic clinical characteristics of patients enrolled in the study are summarized in Table 1. The protocol was approved in advance by the Human Institutional Review Board of Hamamatsu University School of Medicine.

Genotyping of MDR1 C3435T polymorphism

DNA was extracted from peripheral blood leukocytes of each patient, using a commercially available kit (IsoQuick, ORCA Research Inc., Bothell, WA). *MDR1* C3435T polymorphism was determined by a PCR-RFLP method as described by Cascorbi et al. (2001). Amplification primers for the 197 bp fragment were 5′-TGT TTT CAG CTG CTT GAT GG-3′ [205,700–205,719 of ABCB1 gene (AY910577)] and 5′-AAG GCA TGT ATG TTG GCC TC-3′ [205,896–205,877 of *ABCB1* (AY910577)]. The PCR products were digested with *Sau*3AI (Takara Bio Inc., Shiga, Japan) at 37 °C for 1 h. Fragments were separated by electrophoresis on 3% agarose gels and stained with ethidium bromide. The result of PCR-RFLP was identified only by agarose gel electrophoresis.

Data analysis

Hardy–Weinberg equilibrium of allele frequencies at individual loci was assessed by comparing the observed and expected genotype frequencies using the chi-squared test. Statistical differences in mean age, male/female and *MDR1*-C3435T genotype/allele frequencies among the different disease groups were determined by one-way ANOVA or the chi-square test. The effects of genotypes/alleles of *MDR1* C3435T polymorphism on the risk of gastric cancer and

Table 2
Influences of *MDR1* C3435T genotypes on gastric cancer, gastric ulcer and duodenal ulcer risks

Disease	Genotype/allele carriage	n (%)	Adjusted OR	95% CI	*P* value
Gastric cancer	C/C	49 (32.7%)	1.000 (ref)		
	C/T	73 (48.6%)	0.97	0.55–1.71	0.90
	T/T	28 (17.7%)	1.02	0.47–2.21	0.96
	T allele carriage	101 (67.3%)	0.96	0.56–1.66	0.89
Gastric ulcer	C/C	56 (26.1%)	1.000 (ref)		
	C/T	117 (54.4%)	1.07	0.66–1.74	0.78
	T/T	42 (19.5%)	1.51	0.78–2.89	0.22
	T allele carriage	159 (73.9%)	1.16	0.72–1.84	0.55
Duodenal ulcer	C/C	46 (28.2%)	1.000 (ref)		
	C/T	81 (49.7%)	0.86	0.51–1.44	0.57
	T/T	36 (22.1%)	1.54	0.78–3.02	0.21
	T allele carriage	117 (71.8%)	1.00	0.61–1.62	0.99

Odds ratios (ORs) were adjusted by age and sex. Abbreviations are: *MDR1* = *multidrug-resistant transporter*-1, and CI = confidence interval.

Table 3
Influences of the *MDR1* C3435T genotype status on the development of two different histological types of gastric cancer

Histological type	Genotype/allele carriage		Adjusted OR	95% CI	*P* value
Intestinal type	C/C	38 (34.2%)	1.000		
(n=111)	C/T	51 (46.0%)	0.86	0.46–1.60	0.63
	T/T	22 (19.8%)	0.94	0.41–2.14	0.89
	T allele carriage	73 (65.8%)	0.86	0.48–1.55	0.62
Diffuse type	C/C	11 (28.2%)	1.000		
(n=39)	C/T	22 (56.4%)	1.31	0.56–3.06	0.53
	T/T	6 (15.4%)	1.07	0.33–3.42	0.91
	T allele carriage	28 (71.8%)	1.23	0.55–2.77	0.62

Odds ratios (ORs) were adjusted by age and sex. Abbreviations are: *MDR1* = *multidrug-resistant transporter-1*, and CI = confidence interval.

peptic ulcer development were expressed as odds ratios (ORs) with 95% confidence intervals (CIs) adjusted by age and sex with reference to gastritis alone subjects. All *P* values were 2-sided, and *P* values less than 0.05 were considered statistically significant.

The sample size was calculated on the basis of the frequencies of *MDR1*-3435 genotypes in the healthy Japanese *H. pylori*-negative population reported by Tahara et al. (2007): 40.8% for *MDR1*-3435 T/T genotypes. We chose to perform an unmatched case-control study (assuming 1.00 controls per gastric cancer patient). When the desired power of our study (1-β) was set at 80% with a significance level of 0.05 in a two-sided test, at least 66 gastric cancer patients and 66 *H. pylori*-negative controls were required. Therefore, our sample size was considered sufficient to study *MDR1*-3435 polymorphisms in relation to gastric cancer risks.

Results

Patient characteristics

The mean age of subjects with gastric cancer was significantly higher than those of any other groups ($p<0.01$, Table 1) and the frequency of gastric cancer patients was significantly greater in men than in women ($p<0.01$, Table 1). Then, the ORs of development of gastric cancers and peptic ulcers were adjusted by age and sex.

Effects of MDR1 C3435T polymorphism on developing gastric cancer and peptic ulcer

The genotype frequencies in the control group did not deviate significantly from those expected under the Hardy–Weinberg equilibrium (Table 1). No significant difference existed in frequencies of *MDR1* C3435 T polymorphism between *H. pylori*-negative controls and *H. pylori*-positive gastritis alone patients ($p>0.05$).

The frequencies of the *MDR1* 3435 C/C, C/T and T/T genotypes were 29.2% (n=29), 57.1% (n=96) and 13.7% (n=23) in the control group without *H. pylori* infection, whereas those in the gastric cancer group were 32.7% (n=49), 48.6% (n=73) and 18.7% (n=28), respectively (Table 1). The age- and sex-adjusted ORs of *MDR1* 3435 T allele carriers relative to the C/C genotype group for gastric cancer was 0.96 [95%CI: 0.56–1.66, n=101 (67.3%)]; there was no association with gastric cancer development and *MDR1* C3435T polymorphism (Table 2). When the gastric cancer patients were classified into the intestinal-type and diffuse-type subgroups, the adjusted ORs of T allele carriers for the respective subgroups were 0.86 [95%CI: 0.48–1.55, n=73 (65.8%)] and 1.23 [95%CI: 0.55–2.77, n=28 (71.8%)] (Table 3).

The frequencies of the *MDR1* 3435 C/C, C/T and T/T genotype in the gastric ulcer and duodenal ulcer group were 26.1%, 54.4% and 19.5%, and 28.2%, 49.7% and 22.1%, respectively (Table 1). Although patients with *MDR1*-3435 T/T genotype had tendency with higher risk of peptic ulcer development, *MDR1* C3435T polymorphism was not significantly associated with the risks of the peptic ulcer development (Table 2).

Discussion

P-gp as a physiological barrier plays an important role in the detoxification system of normal tissues, such as the gastrointestinal and renal epithelium (Kankesan et al., 2004). P-gp is also associated with the regulation of cellular apoptosis, differentiation and proliferation (Fantappie et al., 2007). Recent studies have demonstrated the local over-expression or down-expression of P-gp in various cancer cells and tissues, suggesting that local expression level of P-gp would be associated with carcinogenesis. The theoretical possibility that the lower P-gp activity may result in higher intracellular concentrations of mutagens, which can induce more DNA damage in cells, thereby eventually resulting in the transformation of normal cells to mutated cells. Indeed, in susceptibility to cancer of gastrointestinal tract, the *MDR1* 3435 T allele carrier were found more frequently in patients of colon cancer, and the carriage of *MDR1* 3435 T allele and TT genotype is a risk factor for colon cancer development (Humeny et al., 2003; Kurzawski et al., 2005). Moreover, Osswald et al. (2007) and Koyama et al. (2006) reported that patients who carried the C allele of *MDR1* T-129C, but not G2677A, T and C3435T was associated with the lower expression of P-gp and developed more frequently colorectal cancer. However, some reports indicated that no significant difference was observed in frequencies of *MDR1* C3435T genotype between controls and patients with colorectal cancer or esophageal cancer (Potocnik et al., 2002; Bae et al., 2006; Komoto et al., 2006; Lee et al., 2006). As stated above, there have been controversial reports about the association of *MDR1* C3435T polymorphism with susceptibility to cancers.

On the other hand, the polymorphic effects of *MDR1* C3435T on the development of gastric cancer have been reported only one paper, Tahara et al. (2007) reported that the carrier of *MDR1* 3435 TT genotype had significantly prevented effects in gastric cancer development (OR: 0.43; 95%CI: 0.23–0.79). Of *H. pylori* infection-related disorders, the positivities and expressions of P-gp are significantly higher in *H. pylori*-associated gastritis and gastric cancer than in the normal gastric mucosa without *H. pylori* infection (Nardone et al., 2004; Babic et al., 2005). P-gp is generally undetectable in normal gastric mucosa, whereas the expression of P-gp is correlated with the grade of *H. pylori* infection, atrophy and acute and chronic infiltration (Nardone et al., 2004). Therefore, P-gp is considered to be associated with the pathogenesis of *H. pylori*-related disorders including gastric cancer. However, our results indicated that *MDR1* C3435T polymorphism had no association with gastric cancer development. Previous Japanese data of frequency of *MDR1* C3435T polymorphism was 35% in *MDR1*-3435 C/C genotype, 53% in C/T genotype and 12% in T/T genotype (Horinouchi et al., 2002), as observed in this study, whereas the frequency in control patients reported by Tahara et al. (2007) was 26.9%, 41.4% and 31.7%, which had higher frequency of T allele carrier. The difference of frequency of *MDR1* C3435T genotypes might cause the different result. We are tempted to assume that the influence of grade of gastritis induced by *H. pylori* infection on the gastric mucosal P-gp level might be much stronger than that of *MDR1* polymorphism and/or that carcinogen(s) for gastric cancer might not be the candidate substrate(s) for P-gp. In this respect, the candidate carcinogens for gastric cancer might differ from those for colonic cancer.

Conclusion

We demonstrated that the *MDR1* C3435T polymorphism was not associated with an increased risk for the developing of gastric cancer. Although the possible association of *MDR1* C3435T polymorphism in this process was reported for other neoplastic diseases, the role of P-gp in gastric carcinogenesis appears to be of a limited importance

based upon the present study findings. Further studies are required to determine the exact role of *MDR1* C3435T polymorphism in the gastric cancer development in relation to other polymorphisms already known to be associated with gastric carcinogenesis.

Acknowledgments

This work was supported in part by a Grant-in-Aid from the YOKOYAMA Foundation for Clinical Pharmacology, from the 21st century Center of Excellence (COE) program Medical Photonics (Hamamatsu University School of Medicine), and from the Ministry of Education, Culture, Sports, Science and Technology of Japan (19790479).

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