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Association between insulin-like growth factor-1 polymorphisms and stomach cancer risk in a Japanese population

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The insulin-like growth factor (IGF) signaling system plays a central role in cellular growth, differentiation and proliferation. Although the association between *IGF1* gene polymorphisms and cancer risk has been evaluated for several carcinomas, this association has not yet been examined for stomach cancer. We investigated the association between *IGF1* polymorphisms and the risk of stomach cancer in a Japanese population. A total of 703 patients with stomach cancer and 1462 non-cancer control subjects were enrolled in this case–control study. Associations between polymorphisms of 10 *IGF1* loci and the risk of stomach cancer were evaluated using odds ratios (OR) and 95% confidence intervals (CI) in multiple logistic regression models. We observed that the C allele in rs1520220 and the G allele in rs4764887 were significantly associated with stomach cancer risk in the per-allele model after adjusting for other risk factors (OR: 1.14 [95% CI: 1.00–1.30] and OR: 1.18 [95% CI: 1.02-1.36], respectively). We also observed a positive and dose-dependent association between the number of risk alleles and stomach cancer risk (*P*-trend: 0.019) when examining the two loci in the same model. These associations were still seen after adjusting for potential confounders, including sex, age, smoking status, history of diabetes and family history of stomach cancer. We did not find any significant interaction between these factors and the number of risk alleles. In conclusion, we observed a significant association between *IGF1* polymorphisms and stomach cancer risk among a Japanese population. Examination of the biological significance of *IGF1* is warranted. (*Cancer Sci* 2011; 102: 2231–2235)

Stomach cancer remains one of the most common cancers in Japan, as well as worldwide.[1] The known and suspected risk factors for stomach cancer include smoking, diabetes, family history of stomach cancer, green tea intake and *Helicobacter pylori* infection. Recent studies have focused on the detection of single nucleotide polymorphisms (SNP) that have an incident and prognostic impact in cancer, including stomach cancer. Specifically, SNP in the gene encoding insulin-like growth factor (IGF) have been studied in the context of several cancers, but to our knowledge, their influence in the development of stomach cancer has not yet been investigated.

The IGF signaling system plays a central role in cellular growth, differentiation and proliferation.[2] IGF1 is reported to be a potential proliferative molecule, affecting almost every cell type via the RAS-mitogen-activated protein kinase signaling pathway.[3,4] It is also reported to be a powerful antiapoptotic molecule, activating the phosphatidylinositol-3 kinase–AKT pathway, which ultimately activates downstream transcription factors that regulate gene expression of proliferative, differentiation and antiapoptotic factors.[3,4] Given this role of IGF1 in stimulating cellular growth and inhibiting apoptosis, abnormalities in IGF1 levels might contribute to the development of cancer. IGF1 polymorphisms have been found to be associated with several cancer risks, such as prostate cancer,[5–7] breast cancer,[8] ovarian cancer[9,10] and colorectal cancer.[11,12] However the association between this SNP and stomach cancer risk was not reported.

Treatments targeting the IGF1 protein function are in fact currently under investigation, including the use of monoclonal antibodies targeting the IGF receptor in several cancers.[13] Although these treatments appear promising, the oncogenic potential of IGF1 in the context of stomach cancer must be verified before the clinical use of any such treatments in patients with stomach cancer. We have reported polymorphisms in *IGF1* are associated with the prognosis of stomach cancer,[14] warranting potential importance of *IGF1* loci on stomach cancer risk.

Here, we conducted a case–control study to investigate the association between stomach cancer risk and common genetic variations in *IGF1* in a Japanese population.

Methods

Subjects. The subjects were selected from the database of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), developed by the Aichi Cancer Center Hospital (ACCH) in Nagoya, Japan.[15,16] The framework of this program has been detailed elsewhere.[15,16] Briefly, 23 408 HERPACC-enrolled, first-visit outpatients treated between January 2001 and November 2005 at ACCH were asked to provide information on lifestyle factors in addition to blood samples. Approximately 60% of patients provided blood samples, and 22 727 (97.1%) patients completed the self-administered questionnaire on lifestyle factors. All questionnaires were then checked by trained interviewers. This study was approved by the Institutional Ethics Review Board of ACCH, and written informed consent was obtained from all participants.

In the present study, the case subjects were 703 patients with incident, histologically confirmed stomach cancer, and the unmatched controls were 1462 patients without a present diagnosis or previous history of cancer, according to the cancer registry and medical records. Both groups participated in HERPACC during the same time period and provided blood samples.

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Selection and genotyping of IGF1 polymorphisms. For each subject, DNA was extracted from the buffy coat fraction with the DNA Blood Mini Kit (Qiagen, Tokyo, Japan). Based on the HapMap database for Japanese population in Tokyo,[17,18] tag SNP for *IGF1* were selected if they fit the following criteria: a minor allele frequency >30% and a haplotype R^2 value >0.95. Ten loci were selected, namely rs574214, rs6214, rs1520220, rs6539035, rs4764887, rs2288378, rs2195239, rs12423791, rs2162679 and rs5742612. Genotyping was performed using the TaqMan method with probes from Applied Biosystems (Foster City, CA, USA) and Fluidigm EP1 SNP Genotyping 96.96 Dynamic Array (Fluidigm Corp., South San Francisco, CA, USA). Approximately 10% of subjects were examined in duplicate to confirm consistency in genotyping.

Assessment of exposure. All lifestyle and background characteristics were reported through a self-administered questionnaire. Cumulative smoking dose was measured in pack-years (PY), the product of the average number of packs consumed per day and the number of years of smoking. Subjects were classified as non-smokers, former smokers and current smokers. Non-smokers were defined as subjects with PY = 0, and former smokers as those who had quit smoking at least 1 year prior to the survey. Current smokers were further categorized as those with $0 < PY \leq 20$, $20 < PY \leq 40$ or $PY > 40$. Green tea consumption was measured as the average number of cups consumed per day. History of diabetes and family history of stomach cancer among parents and siblings were also self-reported. Serum IgG levels for *Helicobacter pylori* (HP) were measured using a commercially available direct enzyme-linked immunosorbent assay (ELISA) kit (''E Plate 'Eiken' HP Antibody''; Eiken Kagaku, Tokyo, Japan). This ELISA kit was developed in Japan using the antigen extracted from the domestic strain of HP in Japan and is commonly used in medical studies.[19,20] Positivity for HP infection was defined as an anti-HP IgG antibody level >10 U/mL in serum.

Statistical analysis. Associations between polymorphisms and stomach cancer were assessed by odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression models. The OR and 95% CI were estimated for per-allele, dominant and recessive models. The association between genotype and risk of stomach cancer was adjusted for potential confounders, including sex, smoking status, history of diabetes and family history of stomach cancer in a first-degree relative. The *P*-values for heterogeneity were assessed by adding interaction terms between the number of risk alleles and confounders in the models. Discrepancies between the expected and observed genotype and allele frequencies in the cases and controls were assessed according to the Hardy–Weinberg equilibrium (HWE) using the Chi-squared test. Linkage disequilibrium (LD) was measured using *D*'.

All statistical analyses were performed using Stata version 10.0 (StataCorp., College Station, TX, USA). A *P*-value of <0.05 was considered statistically significant. The LD estimates were calculated using Haploview (Broad Institute, MA, USA).

Results

The characteristics of cases and controls are shown in Table 1. Histological information was available for 98.2% of cases (diffuse type in 380 cases and intestinal type in 310 cases). Both heavy smoking and family history of stomach cancer in a first-degree relative were more prevalent in cases than controls.

The genotype distributions for the 10 *IGF1* loci and their OR and 95% CI for stomach cancer are shown in Table 2. Distributions of six of the 10 loci, namely rs5742714, rs1520220, rs2195239, rs4764887, rs2288378 and rs5742612, were in accordance with the HWE, while those of the other four were not (Supporting Information Table S1). These four loci were therefore excluded from further analyses. Among the remaining six candidate loci, rs1520220 and rs4764887 were significantly associated with stomach cancer risk after adjusting for sex, age, smoking and drinking habits, history of diabetes and family history of stomach cancer (OR: 1.14 [95% CI: 1.00–1.30] and OR: 1.18 [95% CI: 1.02–1.36], respectively). rs4764887 was also significantly associated with stomach cancer risk in the dominant model (OR: 1.45 [95% CI: 1.02–2.06]).

Table 1. Characteristics of cases and controls

Variables	Cases (%)	Controls (%)	*P*-value
Total	703	1462	
Sex			
Male	527 (75.00)	1094 (74.80)	0.95
Female	176 (25.00)	368 (25.20)	
Age (years)			
<40	34 (4.90)	75 (5.10)	0.77
40–49	72 (10.20)	147 (10.00)	
50–59	246 (35.00)	478 (32.70)	
60–69	214 (30.40)	482 (33.00)	
>70	137 (19.50)	280 (19.20)	
Smoking status			
PY = 0	223 (31.70)	577 (39.50)	<0.001
0 < PY ≤ 20	104 (14.80)	273 (18.70)	
20 < PY ≤ 40	162 (23.00)	313 (21.40)	
PY > 40	207 (29.50)	290 (19.80)	
Unknown	7 (1.00)	9 (0.60)	
Green tea intake†			
0–1/day	254 (36.10)	531 (36.30)	0.93
>1/day	449 (63.90)	931 (63.70)	
History of diabetes			
Yes	56 (8.00)	126 (8.60)	0.6
No	647 (92.00)	1336 (91.40)	
Family history of stomach cancer‡			
Yes	155 (22.00)	270 (18.50)	0.05
No	548 (78.00)	1192 (81.50)	
Histology			
Diffuse type	380 (54.10)	–	–
Intestinal type	310 (44.10)	–	
Unknown	13 (1.80)	–	

†Green tea intake measured in average number of cups per day.
‡Family history of stomach cancer in first-degree relatives.
PY, pack-year.

H. pylori infection was tested in 1274 (87.1%) of 1462 control patients. There was no significant association between *H. pylori* infection and these two SNP, rs1520220 and rs4764887 (OR: 1.09 [95% CI: 0.93–1.28] and OR: 0.99 [95% CI: 0.83–1.17], respectively).

rs1520220 and rs4764887 showed LD ($D' = 0.94$ and $R^2 = 0.35$). The number of risk alleles, namely the C allele in rs1520220 and the G allele in rs4764887, was found to have a positive association with stomach cancer risk (OR: 1.10 [95% CI: 1.02–1.18], *P*-trend: 0.019; Table 3). Haplotype analysis also showed that the combination of C allele in rs1520220 and G allele in rs4764887 had the strongest association with stomach cancer risk (Table 4). We performed stratified analysis according to sex, smoking, history of diabetes, family history of stomach cancer and histological subtype (Table 5) to explore potential effect modification between potentially confounding factors and the total number of risk alleles in these two loci, but no significant interactions were found.

Discussion

In this case–control study assessing the association of six genetic variants in *IGF1* on stomach cancer risk, we found that

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Table 2. Genotype distributions of *IGF1* polymorphisms and their odds ratios (OR) for stomach cancer risk

				Per-allele model						Dominant model			Recessive model		
				Model 1†			Model 2‡								
				OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
rs5742714: risk allele (C) frequency in control subjects = 0.219 (HWE: *P* = NS)															
Case/control	CC	CG	GG	1.02	0.88–1.19	0.76	1.02	0.87–1.19	0.82	1.04	0.87–1.25	0.67	0.97	0.66–1.45	0.90
	38/81	238/479	427/902												
rs1520220: risk allele (C) frequency in control subjects = 0.504 (*P* = NS)															
Case/control	GG	GC	CC	1.13	0.99–1.29	0.052	1.14	1.00–1.30	0.050	1.23	0.99–1.53	0.06	1.14	0.94–1.40	0.19
	148/361	357/728	198/373												
rs4764887: risk allele (G) frequency in control subjects = 0.717 (*P* = NS)															
Case/control	AA	AG	GG	1.18	1.03–1.36	0.021	1.18	1.02–1.36	0.027	1.45	1.02–2.06	0.038	1.18	0.99–1.42	0.070
	45/132	260/563	398/767												
rs2288378: risk allele (A) frequency in control subjects = 0.215 (*P* = NS)															
Case/control	AA	AG	GG	1.02	0.88–1.19	0.77	1.02	0.87–1.19	0.83	1.05	0.87–1.26	0.61	0.93	0.62–1.40	0.73
	36/80	236/469	431/913												
rs219523: risk allele (G) frequency in control subjects = 0.546 (*P* = NS)															
Case/control	CC	CG	GG	1.11	0.98–1.27	0.096	1.11	0.98–1.27	0.11	1.23	0.98–1.55	0.08	1.10	0.91–1.34	0.314
	127/312	346/703	230/447												
rs5742612: risk allele (T) frequency in control subjects = 0.703 (*P* = NS)															
Case/control	CC	CT	TT	1.07	0.96–1.23	0.38	1.06	0.92–1.22	0.82	1.17	0.85–1.64	0.32	1.06	0.88–1.26	0.56
	55/133	289/602	359/727												

†Crude model. ‡Adjusted for sex, age, smoking status, history of diabetes and family history of gastric cancer. CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

Table 3. Association between the number of risk alleles of rs1520220 and rs4764887 and the risk of stomach cancer

Number of risk alleles†	0	1	2	3	4	Trend
Case	42	69	229	170	193	
Control	124	164	476	337	361	
Univariate analysis (OR)	1.0	1.24	1.42	1.49	1.58	1.10
95% CI	Ref	0.79–1.95	0.97–2.08	1.00–2.21	1.07–2.33	1.02–1.18
P-value	–	0.34	0.073	0.048	0.022	0.018
Multivariate analysis (OR)‡	1.0	1.22	1.36	1.44	1.55	1.10
95% CI	Ref	0.78–1.92	0.92–2.01	0.97–2.15	1.05–2.31	1.02–1.18
P-value	–	0.39	0.12	0.070	0.029	0.019

†Risk alleles are C in rs1520220 and G in rs4764887, and number of risk alleles indicates the total number of risk alleles in two loci. ‡Adjusted for sex, age, smoking status, history of diabetes and family history of gastric cancer. CI, confidence interval; OR, odds ratio; Ref, reference.

Table 4. Association between haplotype of rs1520220 and rs4764887 and the risk of stomach cancer

Haplotype	A-G	A-C	G-G	G-C
Case (frequencies)	0.241525	0.007408	0.222913	0.521854
Control (frequencies)	0.27409	0.008742	0.221806	0.495362
Univariate analysis (OR)	1	0.96	1.14	1.21
95% CI	Ref	0.42–2.22	0.95–1.37	1.04–1.41
P-value	–	0.927	0.168	0.016
Multivariate analysis (OR)†	1	0.96	1.14	1.21
95% CI	Ref	0.420–2.22	0.95–1.37	1.03–1.41
P-value	–	0.927	0.168	0.016

†Adjusted for sex, age, smoking status, history of diabetes and family history of gastric cancer. CI, confidence interval; OR, odds ratio.

the C allele of rs1520220 and G allele of rs4764887 in the *IGF1* gene, two SNP we noted to be in close LD, showed a significant and dose-dependent association with increased stomach cancer risk. This association was consistent regardless of age, sex, smoking habit, history of diabetes and family history of stomach cancer, suggesting that these *IGF1* SNP affect cancer risk independently of potential risk factors. To our knowledge, this is the first study to identify an association between *IGF1* genetic variants and stomach cancer risk.

The functional consequences of SNP at rs1520220 and rs4764887 are still unclear. rs1520220 is located in intron 3 of the *IGF1* gene. A variant allele at an intron boundary might lead to alternative splicing and a subsequent change in protein function. This is supported by previous reports, which showed that genetic variants of rs1520220 were associated with increased levels of circulating IGF1 and risk of several types of cancer.[20,21] Although rs4764887 is also located in intron 3 of *IGF1*, there are no reports evaluating the impact of the SNP at rs4764887 on IGF1 function and cancer risk. Our finding that rs4764887 is in close LD with rs1520220 might partly explain the increase in stomach cancer risk associated with rs4764887. Replication of this association between these loci and stomach cancer in other populations and biological examination of these loci are warranted. Notably, our recent study showed that rs1520220 is associated with a prognosis of stomach cancer,[14] indicating potential biological importance of this locus in stomach cancer proliferation.

In the present study we genotyped several *IGF1* SNP, which have previously been found to be associated with an increased

Table 5. Stratified analysis according to potential confounding factors for the number of risk alleles in *IGF1* rs1520220 and rs4764887

Number of risk alleles†	0	1	2	3	4	Allele model‡			*P*-heterogeneity
Exposure	Case/control	Case/control	Case/control	Case/control	Case/control	OR	95% CI	*P*-value	
Sex									
Male	31/101	55/120	178/347	125/266	138/260	1.08	0.99–1.18	0.077	0.652
Female	11/23	14/44	51/129	45/71	55/101	1.13	0.97–1.32	0.12	
Smoking status									
PY = 0	17/40	21/69	66/182	51/128	68/158	1.05	0.92–1.20	0.44	0.442
0 < PY ≤ 20	4/34	12/32	33/87	27/55	28/65	1.22	1.01–1.47	0.038	
20 < PY ≤ 40	10/26	13/33	56/109	41/82	42/63	1.13	0.96–1.34	0.14	
PY > 40	11/24	23/29	69/95	50/70	54/72	1.06	0.91–1.23	0.47	
Unknown	0/0	0/1	5/3	1/2	1/3	–	–	–	
Family history									
No	32/102	52/134	179/387	132/273	153/296	1.11	1.02–1.21	0.020	0.576
Yes	10/22	17/30	50/89	38/64	40/65	1.07	0.90–1.26	0.44	
DM history									
No	39/115	65/145	208/441	160/307	175/328	1.10	1.01–1.19	0.025	0.835
Yes	3/9	4/19	21/33	10/30	18/33	1.15	0.87–1.52	0.32	
Histology									
Diffuse	22/20	33/38	110/97	90/80	107/86	1.05	0.92–1.19	0.47	
Intestinal	20/22	35/34	96/133	75/95	84/109	0.96	0.84–1.09	0.53	

†Risk alleles are C in rs1520220 and G in rs4764887 and number of risk alleles indicates the total number of risk alleles in two loci. ‡Adjusted for sex, age, smoking status, history of diabetes and family history of gastric cancer. CI, confidence interval; OR, odds ratio; PY, pack-year.

risk of several other types of cancer. The present study identified two *IGF1* SNP associated with stomach cancer risk, of which rs1520220 has been shown to be associated with increased risk of breast cancer in one study,[20] but not in another study.[21] Another study also showed there was no association between rs1520220 polymorphism and ovarian cancer risk.[22] In contrast, no studies have examined the relationship between the *IGF1* SNP rs4764887 and serum IGF1 levels and cancer risk. Although the present study eliminated *IGF* SNP rs6214 because its genotype frequency was not in HWE, this SNP has been shown to be associated with colorectal cancer risk.[12]

Additionally, the present study did not examine several *IGF1* SNP, which were previously studied in relation to other cancers. rs6220 has been shown to be associated with higher levels of IGF1 and increased risk of breast cancer[21–23] and prostate cancer,[24] but not colorectal cancer.[12] rs7136446 has also been reported to be associated with increased IGF1 levels and prostate cancer risk.[24] Neither loci satisfied the inclusion criteria on tag SNP selection, and were therefore not examined in the present study.

The strengths of the present study include its large sample size and comprehensive characterization of variations in *IGF1* loci. Given that our allele frequencies were comparable with those previously reported in a public database such as HapMap JPT, bias in the distribution of selected polymorphisms was negligible. Several potential limitations of the present study also warrant mention. First, hospital-based, non-cancer patients were selected as controls. Nonetheless, because cases and controls were selected from the same hospital and lived mostly in the Tokai area of central Japan, the internal validity of this case–control study was likely to be acceptable. Second, information on lifestyle factors considered to be potential confounders was self-reported, and might therefore have been inaccurate. However, any potential misclassification was likely to have been non-differential and to have underestimated the causal association. Third, information on the presence of *H. pylori* infection was unavailable for the majority of patients. However, among the 1274 controls known to have *H. pylori* infection, no significant association between *H. pylori* infection and the *IGF1* genotype was found, further suggesting that *IGF1* SNP affect cancer risk independently of *H. pylori*. Last, four of 10 loci were not in accordance with HWE. This might limit coverage of *IGF1* by tagSNP.

In conclusion, we found that *IGF1* genetic variations at rs1520220 and rs4764887 were significantly associated with stomach cancer risk among a Japanese population. Further studies are warranted, including analysis of circulating IGF1.

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Disclosure Statement

None of the authors have any financial or other interests that could be construed as a conflict of interest with regard to the submitted manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Genotype distributions of four *IGF1* loci excluded from analysis because of violation of the Hardy–Weinberg equilibrium.

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