

## Original Article



# Association between the *MUC1* rs4072037 Polymorphism and Risk of Gastric Cancer and Clinical Outcomes

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## ABSTRACT

**Purpose:** *Mucin 1 (MUC1)* was identified as a gastric cancer (GC) susceptibility gene by genome-wide association studies in Asians and candidate gene studies in Europeans. This study aimed to investigate the association between the *MUC1* rs4072037 polymorphism and GC in terms of the Lauren classification and long-term clinical outcomes.

**Materials and Methods:** A total of 803 patients with GC and 816 unrelated healthy controls were enrolled in the study. The association between the *MUC1* rs4072037 variant and GC histological types and clinical outcomes, including tumor recurrence and prognosis was investigated.

**Results:** The major A allele of rs4072037 was associated with increased GC risk ( $P < 0.05$ ). In subtype analysis, the association was most significant for diffuse-type GC ( $P < 0.05$ ) and in a dominant model ( $P < 0.05$ ), whereas there was no association with intestinal-type GC ( $P > 0.05$ ). Cox proportional hazards analysis revealed the heterozygote AG rs4072037 allele as an independent risk factor influencing tumor recurrence and disease-related death in diffuse-type GC ( $P < 0.05$ ), but not in intestinal-type GC ( $P > 0.05$ ).

**Conclusions:** The exonic single nucleotide polymorphism rs4072037 in *MUC1* was associated with diffuse-type GC and was an independent risk factor influencing tumor recurrence and disease-related death in diffuse-type GC.

**Keywords:** Single nucleotide polymorphism; Gastric cancer; Histologic type

## INTRODUCTION

According to the World Health Organization (WHO) report, gastric cancer (GC) is the third leading cause of death after lung and liver cancer [1]. Many different classification systems have been proposed for GC. However, the most useful and widely used system remains the one proposed by Lauren in 1965 [2]. More than 90% of GCs are adenocarcinomas, which are further classified into diffuse- and intestinal-type [3]. Typically, the intestinal-type GC arises from pathological changes in the gastric epithelial resulting from chronic gastritis mainly due to *Helicobacter pylori* infection, atrophic gastritis, intestinal metaplasia, and dysplasia [4]. On the other hand, the origin of the diffuse-type is considered to be the gastric epithelial stem cells or precursors present in the isthmus region of the middle portion of the epithelium. Genetic and epigenetic events acting on the stem or precursor cells may cause

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#### Author Contributions

Conceptualization: K.B.S., S.K.Y.; Data curation: K.B.S., L.J.C., Y.J.H.; Formal analysis: K.B.S.; Funding acquisition: S.K.Y.; Investigation: L.J.C.; Methodology: Y.J.H.; Writing - original draft: K.B.S.; Writing - review & editing: S.K.Y.

#### Conflict of Interest

No potential conflict of interest relevant to this article was reported.

them to deviate from their normal differentiation program and lead to the development of a diffuse-type GC [5], although the details of this are yet to be elucidated. In contrast to the steady decline in the incidence of intestinal-type GC (mainly due to the reduced prevalence of *H. pylori* infection), the incidence of the diffuse-type GC appears to be increasing [6]. Moreover, some diffuse-type GCs develop into a highly malignant form, linitis plastica [7]. Identification of genetic predisposing factors and molecular pathways for diffuse-type GC development will provide new insights for effective prevention, early diagnosis, and therapeutic strategies.

*Mucin 1 (MUC1)* is a highly polymorphic membrane-associated mucin that is often aberrantly expressed in cancers [8]. The observation that *MUC1* plays a role in the progression of GC highlights the importance of understanding all aspects of the variations of this gene [9]. Additionally, the single nucleotide polymorphism (SNP), rs4072037, is known to determine a splicing acceptor site in the second exon of *MUC1* [10]. Therefore, *MUC1* rs4072037 polymorphism plays a key role in GC.

The Japanese genomic-wide association study (GWAS) on diffuse-type GC and subsequent fine mapping and functional studies identified *MUC1* at chromosome 1q22 as a susceptibility gene for diffuse-type GC [10,11]. *MUC1* is the second major diffuse-type GC susceptibility gene to be identified, and the minor allele of the exonic SNP rs4072037 in *MUC1* is inversely associated with the risk of diffuse-type GC [10]. Functional studies have shown that rs4072037 regulates alternative splicing of the second exon and modifies the transcriptional activity of the *MUC1* promoter [9,10]. However, the mechanism by which rs4072037 contributes to GC development is yet to be elucidated.

Although several studies have confirmed the association between rs4072037 and GC in different ethnic populations [2,12-17], there are limited reports on diffuse- versus intestinal-type GC or the association with long-term clinical outcomes. Given that the intestinal and diffuse-type GCs develop through distinct mechanisms, this study aimed to investigate the association between *MUC1* rs4072037 polymorphism, risk of diffuse- or intestinal-type GC, and various clinical outcomes, including tumor recurrence and prognosis.

## MATERIALS AND METHODS

### Patients

A total of 803 patients with GC and 816 unrelated healthy controls were enrolled in the study. All the GC patients were recruited from the Asan Medical Center between 2001 and 2002. Healthy individuals were volunteers recruited from the University of Ulsan College of Medicine. This study was approved by the Institutional Review Board of the Asan Medical Center (2011-0074). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent (or a substitute for it) was obtained from all patients included in the study. We investigated the association between the *MUC1* rs4072037 polymorphism and histological types in GC. We also investigated the association between rs4072037 polymorphism and clinical outcomes, including tumor recurrence and prognosis. TNM stage was described according to the seventh American Joint Committee on Cancer (AJCC) guidelines.

### Genotyping

Genomic DNA was purified from buffy coat of blood from the patients using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. DNA purity was assessed using the ratio of absorbance at 260 and 280 nm ( $A_{260}/A_{280}$ ). DNA samples with  $A_{260}/A_{280}$  ratios greater than 1.8 were used. Samples were genotyped using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based system (Sequenom, San Diego, CA, USA) at Analytical Genetics Technology Center, Princess Margaret Hospital/University Health Network in Toronto, Canada.

### Statistical analyses

A goodness-of-fit  $\chi^2$  test was used to test for deviation from Hardy–Weinberg equilibrium for rs4072037 in the controls. Association analysis and evaluation of odds ratios (ORs) with 95% confidence intervals (CIs) were computed by logistic regression analysis using PLINK software (S. Purcell, 2009, version 1.07) downloaded from <http://pngu.mgh.harvard.edu/purcell/plink/>. Statistical analysis of the clinical data was performed using SPSS, Version 19.0 for Windows (IBM Corp., Armonk, NY, USA). Student's t-test was used for comparison of the means. The  $\chi^2$  test was used for cross-tabulation analysis. The Kaplan–Meier method using log-rank tests was used to analyze univariate survival and recurrence rates, and Cox proportional hazard modeling was used to analyze multivariate factors. P-value <0.05 was considered to indicate statistical significance.

## RESULTS

The clinical characteristics of the 803 GC cases and 816 healthy controls are summarized in **Table 1**. There was no significant difference in age or sex between the GC cases and control group ( $P>0.05$ ). Most patients ( $n=735$ , 91.5%) had non-cardia cancer, and only 68 patients (8.5%) had cardia cancer in the case group. About half ( $n=423$ , 52.7%) of the case group had diffuse-type carcinoma, 345 (43%) had intestinal-type carcinoma, and 35 (4.4%) had mixed-type carcinoma. Early GC (T1 cancer) was present in 359 patients (44.7%), and advanced GC was present in 427 (53.1%) patients. The clinical characteristics of the patients based on the Lauren classification are summarized in **Table 2**.

### Association between rs4072037 and GC

The observed genotypic frequencies of rs4072037 were in agreement with Hardy–Weinberg equilibrium in the control group. The genotypic distribution and its association with GC are shown in **Table 3**. Allelic testing revealed that the minor G allele of rs4072037 was inversely associated with a risk of GC (OR, 0.74; 95% CI, 0.60–0.91;  $P=0.004$ ). In subtype analysis, the association was the most significant for diffuse-type GC (allelic OR, 0.63; 95% CI, 0.48–0.82;  $P=6.14\times 10^{-4}$ ) and in a dominant model (OR, 0.61; 95% CI, 0.45–0.82;  $P=8.84\times 10^{-4}$ ), whereas there was no association with intestinal-type GC.

### Analysis of clinical follow-up characteristics according to rs4072037 polymorphism in the case group

**Table 4** shows the analysis of clinical follow-up characteristics based on rs4072037 polymorphism among the cases. Patients with the GG (minor allele homozygote) rs4072037 genotype had smaller tumors than the heterozygotes and major-allele homozygotes ( $P<0.05$ ). Intestinal-type GC was predominant among patients with the AG genotype and rs4072037 polymorphism ( $P<0.05$ ). Also, depth of invasion was deeper among AG patients.

**Table 1.** Clinical characteristics of case and control groups

Characteristics	Case group (n=803)	Control group (n=816)
<b>Sex</b>		
Male	541 (67.4)	550 (67.6)
Female	262 (32.6)	264 (32.4)
Age (yrs)	50.7±33.4	50.7±12.2
Follow-up (mo)	62.6±40.8	
<b>Location of tumor</b>		
Non-cardia	735 (91.5)	
Cardia	68 (8.5)	
Tumor size (mm)	50.7±12.1	
<b>Histologic types</b>		
Diffuse	423 (52.7)	
Intestinal	345 (43.0)	
Mixed	35 (4.4)	
<b>Gastrectomy</b>		
Subtotal	552 (68.7)	
Total	223 (27.8)	
Others	28 (3.5)	
<b>Depth of invasion</b>		
Mucosa/Submucosa	359 (44.7)	
Muscularis propria	85 (10.6)	
Subserosa	158 (19.7)	
Serosa/adjacent organs	184 (22.9)	
Unknown	17 (2.1)	
<b>Nodal stages</b>		
N0	457 (56.9)	
N1	94 (11.7)	
N2	83 (10.3)	
N3	145 (18.1)	
Nx	24 (3.0)	
<b>TNM stages</b>		
Stage I	393 (48.9)	
Stage II	152 (18.9)	
Stage III	234 (29.1)	
Stage IV	24 (3.0)	
<b>Neoadjuvant chemotherapy</b>		
No	453 (56.5)	
Yes	348 (43.5)	

Values are presented as number of patients (% or mean±standard deviation).  
TNM = tumor, node, metastasis.

Additionally, lymph node metastasis was less frequent among patients with GG genotype than among patients with the other two genotypes ( $P < 0.05$ ). However, there were no significant differences among gender, age, and tumor location.

There were no statistically significant differences between all categories of diffuse-type GC. However, GG homozygotes with intestinal-type GC had smaller tumors than the other two genotypic groups ( $P < 0.05$ ), and lymph node metastasis was less frequent among GG patients with the rs4072037 polymorphism than among the other 2 groups ( $P < 0.05$ ). There were no statistically significant differences in gender, age, tumor location, and depth of invasion among patients with intestinal-type GC.

### Tumor recurrence according to rs4072037 polymorphism

**Fig. 1** summarizes the univariate analysis of the recurrence rate of diffuse-type GC using Kaplan-Meier analysis with the log-rank test. Recurrence rate was higher among AG rs4072037 heterozygotes with diffuse-type GC. **Fig. 2** shows the univariate analysis of the

**Table 2.** Clinical outcomes according to Lauren classification in gastric cancer patients

Characteristics	Diffuse type (n=423)	Intestinal type (n=345)	P-value
Sex			<0.001
Male	241	274	
Female	182	71	
Age (years)	46.7±11.4	55.7±11.1	<0.001
Follow-up (mo)	62.2±42.5	63.7±39.9	0.631
Location of tumor			0.755
Non-cardia	386	317	
Cardia	37	28	
Tumor size (mm)	56.4±36.1	43.2±28.3	<0.001
Gastrectomy			<0.001
Subtotal	253	273	
Total	151	64	
Others	19	8	
Depth of invasion			<0.001
Mucosa/Submucosa	169	175	
Muscularis propria	33	47	
Subserosa	81	70	
Serosa/adjacent organs	125	51	
Unknown	15	2	
Nodal stages			0.001
N0	217	221	
N1	45	44	
N2	47	31	
N3	95	44	
Nx	19	5	
TNM stages			<0.001
Stage I	177	200	
Stage II	80	61	
Stage III	147	79	
Stage IV	19	5	
Adjuvant chemotherapy			0.005
No	215	215	
Yes	208	130	

TNM = tumor, node, metastasis.

tumor recurrence among intestinal-type GC patients using Kaplan-Meier curves and the log-rank test. There were no statistically significant differences in rs4072037 polymorphism in intestinal-type GC. According to the multivariate analysis of tumor recurrence rate using Cox proportional hazard modeling (**Table 5**), AG genotype of rs4072037 polymorphism (P=0.048), large tumor size (P=0.016), deeper tumor invasion (P≤0.001), and lymph node metastasis (P=0.002) were independent risk factors influencing tumor recurrence in diffuse-type GC. Age (P=0.030), large tumor size (P=0.011), and lymph node metastasis (P<0.001) were independent risk factors influencing the tumor recurrence in intestinal-type GC.

### Disease-related survival according to rs4072037 polymorphism

The median (interquartile range) follow-up period was 61.7 (0.2–262.1) months. The univariate analysis of disease-related death rate in diffuse-type GC using Kaplan-Meier analysis with the log-rank test is shown in **Fig. 3**. The disease-related death rate was higher among patients with the AG genotype of the rs4072037 polymorphism in diffuse-type GC (P<0.05). **Fig. 4** shows the univariate analysis of disease-related death rate associated with intestinal-type GC using Kaplan-Meier analysis with the log-rank test. There were no statistically significant differences in rs4072037 polymorphism (P>0.05). The multivariate analysis of factors influencing disease-related death using Cox proportional hazard modeling is summarized in **Table 6**. The AG genotype of the rs4072037 polymorphism (P=0.030),

**Table 3.** Association between the rs4072037 genotype in *MUC1* and gastric adenocarcinoma, overall and according to the histological sub type

Gastric cancer	Model	Genotype	Cases (n=803)	Controls (n=816)	Frequency in cases	Frequency in controls	OR (95% CI)	P-value*
All sites	Genotype	AA	638	600	0.79	0.74	1.00	
		AG	154	199	0.19	0.24	0.73 (0.57–0.92)	0.009
		GG	11	17	0.01	0.02	0.61 (0.28–1.31)	0.204
	Dominant	AA	638	600	0.79	0.74	1.00	
		AG+GG	165	216	0.21	0.26	0.72 (0.57–0.91)	0.005
	Recessive	AA+AG	792	799	0.99	0.98	1.00	
		GG	11	17	0.01	0.02	0.65 (0.30–1.40)	0.274
	Allele <sup>†</sup>	A	1,430	1,399	0.89	0.86	1.00	
		G	176	233	0.11	0.14	0.74 (0.60–0.91)	0.004
Diffuse-type	Genotype	AA	347	600	0.82	0.74	1.00	
		AG	72	199	0.17	0.24	0.63 (0.46–0.85)	0.002
		GG	4	17	0.01	0.02	0.41 (0.14–1.22)	0.108
	Dominant	AA	347	600	0.82	0.74	1.00	
		AG+GG	76	216	0.18	0.26	0.61 (0.45–0.82)	8.84×10 <sup>-4</sup>
	Recessive	AA+AG	419	799	0.99	0.98	1.00	
		GG	4	17	0.01	0.02	0.45 (0.15–1.34)	0.152
	Allele <sup>†</sup>	A	766	1,399	0.91	0.86	1.00	
		G	80	233	0.09	0.14	0.63 (0.48–0.82)	6.14×10 <sup>-4</sup>
Intestinal-type	Genotype	AA	261	600	0.76	0.74	1.00	
		AG	77	199	0.22	0.24	0.89 (0.66–1.20)	0.445
		GG	7	17	0.02	0.02	0.95 (0.39–2.31)	0.904
	Dominant	AA	261	600	0.76	0.74	1.00	
		AG+GG	84	216	0.24	0.26	0.89 (0.67–1.20)	0.450
	Recessive	AA+AG	338	799	0.98	0.98	1.00	
		GG	7	17	0.02	0.02	0.97 (0.40–2.37)	0.953
	Allele <sup>†</sup>	A	599	1,399	0.87	0.86	1.00	
		G	91	233	0.13	0.14	0.91 (0.70–1.18)	0.489

MUC1 = Mucin 1; OR = odds ratio; CI = confidence interval.

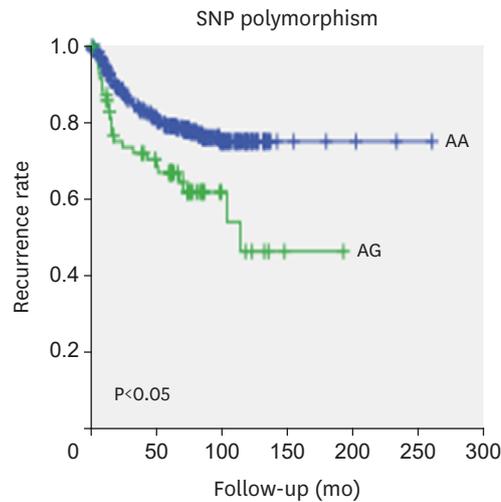
\*P-values were calculated using logistic regression; <sup>†</sup>P-values were calculated using the  $\chi^2$  test.

**Table 4.** Clinical outcomes according to rs4072037 polymorphism in all histologic types

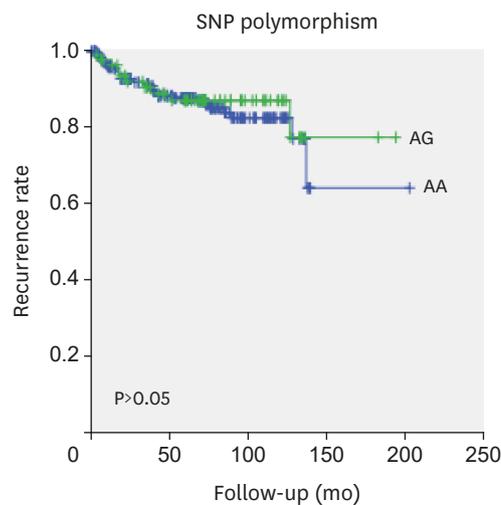
Characteristics	AA (n=638)	AG (n=154)	GG (n=11)	P-value
Sex				NS
Male	420	113	8	
Female	218	41	3	
Age (yr)				NS
≤50	454	120	9	
>50	184	34	2	
Tumor size (mm)				<0.05*
≤50	356	75	11	
>50	268	68	0	
Histologic types				<0.05*
Diffuse	347	72	4	
Intestinal	261	77	7	
Mixed	30	5	0	
Location of tumor				NS
Non-cardia	579	145	0	
Cardia	59	9	9	
Depth of invasion				<0.05 <sup>†</sup>
Invasion of serosa	458	106	9	
Non-invasion of serosa	151	48	2	
Lymph node metastasis				<0.05*
No	363	83	11	
Yes	275	71	0	

NS = non-specific.

\*Comparing GG with the 2 other types, <sup>†</sup>comparing AG with the other 2 types.



**Fig. 1.** Univariate analysis of tumor recurrence in diffuse-type GC using Kaplan-Meier analysis and log-rank test according to rs4072037 polymorphism. GC = gastric cancer; SNP = single nucleotide polymorphism.



**Fig. 2.** Univariate analysis of tumor recurrence in intestinal-type GC using Kaplan-Meier analysis and log-rank test according to rs4072037 polymorphism. GC = gastric cancer; SNP = single nucleotide polymorphism.

large tumor size ( $P=0.011$ ), deeper tumor invasion ( $P<0.001$ ), and lymph node metastasis ( $P=0.001$ ) were independent risk factors influencing disease-related death in diffuse-type GC. Age ( $P=0.025$ ), large tumor size ( $P=0.007$ ), and lymph node metastasis ( $P<0.001$ ) were independent risk factors influencing the disease-related death in intestinal type-GC.

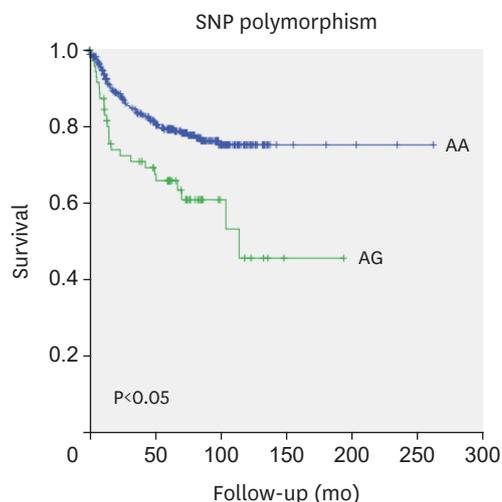
## DISCUSSION

To the best of our knowledge, this is the first report on the association between rs4072037 polymorphism and long-term clinical outcomes of GC. We found that heterozygosity (AG) in rs4072037 is an independent prognostic factor for disease-related death in diffuse type-GC. Additionally, this heterozygosity (AG) of rs4072037 is an independent risk factor for tumor recurrence in diffuse type-GC. Mucin family members can be classified into two types

**Table 5.** Cox proportional hazards analysis of factors potentially influencing the tumor recurrence

Characteristics	Diffuse type			Intestinal type		
	Hazard ratio (95% CI)	Time to recurrence (median mo, 95% CI)	P-value	Hazard ratio (95% CI)	Time to recurrence (median mo, 95% CI)	P-value
Sex			0.884			0.070
Female	1.00			1.00		
Male	0.96			1.15		
Age (years)			0.074			0.030
≤50	1.000			1.00	19.6 (14.7–24.5)	
>50	1.627			2.02 (1.07–3.82)	16.6 (7.2–26.4)	
rs4072037 polymorphism			0.048			0.742
AA	1.00	21.4 (13.8–29.1)		1.00		
AG	1.69 (1.00–2.87)	15.6 (11.4–20.1)		0.78		
Tumor size (mm)			0.016			0.011
≤50	1.00	29.2 (0.0–87.5)		1.00	20.7 (11.1–29.0)	
>50	2.15 (1.15–4.02)	19.2 (11.9–26.4)		3.04 (1.28–7.16)	17.2 (12.9–22.3)	
Depth of invasion			<0.001			0.326
Non-invasion of serosa	1.00	27.2 (7.8–48.5)		1.000		
Invasion of serosa	2.95 (1.73–5.03)	17.0 (10.7–23.2)		1.411		
Lymph node metastasis			0.002			<0.001
No	1.00	24.1 (0.0–64.2)		1.00	48.4 (0.0–100.2)	
Yes	3.08 (1.5–6.23)	19.2 (12.6–25.7)		8.46 (3.24–22.05)	17.2 (13.0–18.7)	

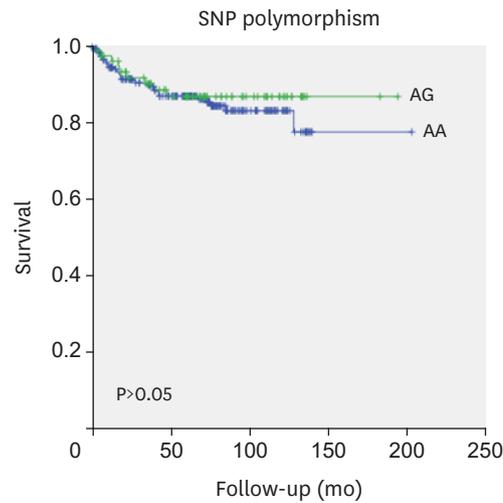
CI = confidence interval.



**Fig. 3.** Univariate analysis of disease-related death in diffuse-type GC using Kaplan-Meier analysis and log-rank test according to rs4072037 polymorphism. GC = gastric cancer; SNP = single nucleotide polymorphism.

(secreted or membranous) based on their localization, and MUC1 is a transmembrane mucin [18]. MUC1 is overexpressed in breast, ovarian, lung, pancreatic, and prostate cancers and is a marker of poor prognosis in GC [19,20].

Recently, a few meta-analysis studies were performed to evaluate the relationship between MUC1 rs4072037 polymorphism and GC susceptibility [21,22]. Luca et. al reported an association between MUC1, *H. pylori*, and gastric cancer in a review of twenty-one studies [21]. The meta-analysis on the MUC1 rs4072037 polymorphism and GC risk reported an OR of 0.66 (95% CI, 0.57–0.78) for the dominant model (AG/GG vs. AA). When stratifying for ethnicity, an OR of 0.73 (95% CI, 0.62–0.86) was reported for the Asian population and an OR of 0.48 (95% CI, 0.38–0.61) was reported for the white population. Further, a



**Fig. 4.** Univariate analysis of disease-related death in intestinal-type GC using Kaplan-Meier analysis and log-rank test according to rs4072037 polymorphism. GC = gastric cancer; SNP = single nucleotide polymorphism.

**Table 6.** Cox proportional hazards analysis of factors influencing disease-related death

Characteristics	Diffuse type			Intestinal type		
	Hazard ratio (95% CI)	Time to survival (median mo, 95% CI)	P-value	Hazard ratio (95% CI)	Time to survival (median mo, 95% CI)	P-value
Sex			0.772			0.777
Female	1.000			1.00		
Male	0.936			0.89		
Age (yr)			0.090			0.025
≤50	1.00			1.00	22.6 (17.4–26.9)	
>50	1.58			2.09 (1.09–3.98)	28.9 (5.9–51.9)	
rs4072037 polymorphism			0.030			0.373
AA	1.00	20.4 (14.3–26.5)		1.00		
AG	1.77 (1.05–2.97)	15.6 (13.2–18.0)		0.68		
Tumor size (mm)			0.011			0.008
≤50	1.00	47.4 (9.0–85.8)		1.00	35.1 (3.1–67.1)	
>50	2.26 (1.20–4.26)	20.4 (13.8–27.1)		3.31 (1.36–8.07)	22.1 (15.7–28.5)	
Depth of invasion			<0.001			0.808
Non-invasion of serosa	1.00	37.1 (9.9–64.3)		1.00		
Invasion of serosa	30.6 (1.79–5.21)	16.3 (14.7–17.8)		1.09		
Lymph node metastasis			0.001			<0.001
No	1.00	50.4 (0.0–108.1)		1.00	48.9 (29.0–68.0)	
Yes	3.30 (1.59–6.82)	16.9 (13.2–20.6)		12.5 (4.11–38.22)	21.5 (16.8–26.3)	

CI = confidence interval.

protective effect of MUC1 rs4072037 polymorphism in the risk of GC was confirmed under the dominant model. In another meta-analysis study [22], Ye et al. reviewed 12 research publications comprising of 18 studies and reported that rs4072037 polymorphism was associated with decreased risk of GC. Stratification analyses of ethnicity indicated that rs4072037 decreased the risk of GC among white populations, but no significant relationship was observed among Asian populations. Further, no significant associations were observed in subgroups of Lauren classification and anatomical classification.

The finding that the MUC1 rs4072037 polymorphism protects against GC has been validated in Asian [2,10,12-15] and Caucasian populations [16,17]. Recently, large GWASs, including three conducted in Asian populations, have reported associations between GC

and *MUC1* rs4072037 polymorphisms [2,10,12,15]. A recent meta-analysis of 6,580 cases and 10,324 controls reported a summary estimate for rs4072037 of 0.72 (95% CI, 0.68–0.77;  $P=7.82 \times 10^{-25}$ ) [14]. In the most recent GWAS with >1,000 cases each of cardia and non-cardia GC of Asian ethnicity, including our current data, rs4072037 showed similar associations for cardia and non-cardia tumors [15]. Moreover, an association between *MUC1* gene polymorphisms and GC has also been reported by other groups even before the GWAS era. The *MUC1* gene contains a central region with a variable number of tandem repeat (VNTR), which is visible as an electrophoretic pattern following restriction enzyme digestion. When the polymorphic allele is divided into large (L) and small (S) alleles, the latter are shown to associate with GC among Caucasians [20,23]. The A allele of rs4072037, identified through GWAS work, is in linkage disequilibrium with the S allele in Japanese and Caucasian patients [9,10]. The results strongly support the idea that *MUC1* is a GC susceptibility gene.

The fact that the associations that we observed in this study were most apparent in the major-allele homozygotes and in the heterozygote is worth some consideration. Although this could be because of a heterozygote advantage, it is likely that these findings reflect power limitations due to the limited number of minor allele homozygote cases in the study groups. In this study, the minor allele occurred at such a low frequency in the study populations that we cannot exclude the possibility that we were unable to detect an association due to a lack of power. In support of this explanation, our dominant models are in accordance with the observed associations for heterozygotes. However, reports on the specificity of the association by Lauren classification are not consistent. Palmer et al. [16] reported an association between rs4072037 (G>A) in *MUC1* and intestinal-type GC, whereas Saeki et al. [10] reported its association with diffuse-type GC. The discrepancy might be due to an ethnic difference in the allele frequency between Asian (0.15–0.19 for G allele) and Caucasian populations (0.53–0.56 for G allele). However, studies consistently found that the A allele elevated risk compared to the G allele. Additionally, there was a sample size difference between the two studies. Only 56 cases with intestinal-type GC were evaluated in the report by Palmer et al. [16], but 1,367 cases with diffuse-type GC were assessed in the Japanese study [10]. Our study, which consisted of 423 cases with diffuse-type and 345 cases with intestinal-type GC, replicated the Japanese finding that the association of rs4072037 was specific to diffuse-type GC.

Depth of invasion is a well-known prognostic factor. In our study, depth of invasion was not an independent factor for tumor recurrence or disease-related death in intestinal-type GC. Two factors may have influenced this finding. Firstly, the sample size was relatively small, with only 53 cases with serosal invasion. Secondly, in the intestinal-type GC, there was a strong association between depth of invasion and lymph node metastasis. Nineteen out of 53 cases with serosal invasion experienced tumor recurrence; all the 19 cases also had lymph node metastasis. Twenty-six cases with non-invasion of the serosa experienced tumor recurrence, and 20 of these 26 cases had lymph node metastasis. Also, 26 cases with non-invasion of the serosa died of disease progression. Twenty-two of these 26 had a lymph node metastasis. Eighteen cases with serosal invasion died of disease progression. All 18 cases had lymph node metastasis. These 2 factors may have influenced the multivariate analysis in intestinal-type GC.

In this study, the A allele of the *MUC1* rs4072037 polymorphism was found to be a risk allele for GC. For diffuse-type GC, an association was observed for heterozygotes, and in a dominant model. However, no associations were observed with intestinal type-GC.

Heterozygosity (AG) of rs4072037 was an independent risk factor influencing disease-related death in diffuse type-GC. However, it was not a risk factor influencing the tumor recurrence or disease-related death in intestinal type-GC.

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