

Hepatocyte Growth Factor in Blood and Gastric Cancer Risk: A Nested Case–Control Study

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ABSTRACT

Background: Potential of hepatocyte growth factor (HGF)–stimulating signaling pathways related to cytotoxin-associated gene A (CagA) to predict gastric cancer development has not been fully investigated.

Methods: We conducted a nested case–control study consisting of 238 gastric cancer cases and 238 matched controls within the Korean Multicenter Cancer Cohort. Plasma HGF concentrations were measured with a human HGF ELISA. Odds ratios (OR) and 95% confidence intervals (CI) for gastric cancer development according to HGF level were calculated using conditional logistic regression model.

Results: Sequential elevation of gastric cancer risk according to HGF level increase was observed (OR, 10.99; 95% CI, 4.91–24.62) for highest quartile HGF (≥ 364 pg/mL) versus lowest quartile HGF (< 167 pg/mL). A significantly increased gastric cancer risk associ-

ated with high HGF level measured even 6 or more years prior to cancer diagnosis was also found. The group with both high risk of HGF and CagA-related genetic variants was associated with highest gastric cancer risk compared with the group with both low risk of HGF and genetic variants ($P_{\text{interaction}} = 0.05$). Model performance using HGF and CagA-related genetic variants to discriminate gastric cancer was fair [area under the curve of receiver operating characteristic (AUC-ROC), 0.71; 95% CI, 0.64–0.78] and significantly higher than that of model not including those biomarkers.

Conclusions: Our results suggest HGF as a potential biomarker to predict gastric cancer development.

Impact: These findings suggest HGF as a useful biomarker to predict gastric cancer risk. Further research to assess gastric cancer risk based on useful biomarkers, including HGF, may contribute to primary prevention of gastric cancer.

Introduction

Hepatocyte growth factor (HGF) is a paracrine cellular cytokine and acts as a growth factor by binding to c-MET, the HGF receptor, to form the c-Met–HGF complex (1, 2). Binding of HGF to c-Met activates phosphorylation of tyrosine kinase residue within the c-Met and contributes to carcinogenesis through sequential c-Met signaling pathways, which lead to induce cell proliferation, cell-cycle progression, survival, transformation, and invasion (1, 2).

The c-Met signaling pathway is also activated by the cytotoxin-associated gene A (CagA; ref. 3), which is a protein secreted into host gastric epithelial cells by specific *Helicobacter pylori* (*H. pylori*) strains (4, 5). The CagA directly binds to the c-Met receptor, and the c-Met–CagA complex eventually leads to the c-Met signaling pathway (3). Therefore, both the c-Met–HGF and the c-Met–CagA interaction can be major inducers of gastric carcinogenesis.

The possibility of c-Met, HGF, or c-Met–HGF complex as a tissue or circulating biomarker for gastric cancer progression or drug response

has been proposed (6). The circulating c-Met was also proposed as a predictive or diagnostic biomarker for gastric cancer. In our previous study, we found that among healthy cohort subjects prior to the diagnosis of gastric cancer, subjects with lower levels of blood c-Met had a higher risk of gastric cancer development than subjects with higher levels of blood c-Met. In addition, the ability to predict gastric cancer development was higher in the gastric cancer risk model including both c-Met and CagA-related genetic markers compared with the gastric cancer risk model consisting of only c-Met (7).

From the ability of c-Met as a biomarker for prediction of gastric cancer development, HGF, a ligand for c-Met, may be able to be used as a biomarker for prediction of gastric cancer development. Previous studies reported higher blood HGF concentration in patients with gastric cancer compared with those in the control group, showing the possibility of HGF as a biomarker of gastric cancer diagnosis (8–10). However, all these studies above were limited to a small number of cases and controls. In addition, the relationship between blood HGF levels and gastric cancer development with consideration of the temporal sequence of exposure and outcome has not been reported to date.

Therefore, this study aimed (i) to assess a performance of blood HGF as a predictive biomarker for gastric cancer development by investigating the association between circulating HGF concentration in normal condition before cancer diagnosis and subsequent gastric cancer development, and (ii) to evaluate the increase in discrimination ability of model including blood HGF for gastric cancer when genetic variants on signaling pathways that are triggered by CagA were considered together.

Materials and Methods

Study population and study design

This study was based on a nested case–control study within the Korean Multicenter Cancer Cohort (KMCC). A detailed description for the rationale and methods of the KMCC is described elsewhere (11, 12).

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Briefly, KMCC is a community-based prospective cohort study designed to investigate risk factors for cancers in the Korea from 1993 to 2004. In total, 20,636 subjects with a median age of 56 years [interquartile range (IQR), 45–64 years] participated in the KMCC after providing written informed consent. Information on demographic, lifestyle, dietary factors, medical history, and environmental factors as potential risk factors of cancers was collected from the cohort participants through personal interviews, using a standardized questionnaire. Blood and urine samples were collected from the participants at enrollment and stored at -70°C and -20°C , respectively.

In total, 304 gastric cancer cases were identified by linking the Korean National Cancer Registry and national death certificates with the KMCC data, following the definition of gastric cancer (C16) by the International Classification of Diseases for Oncology, third edition until 2008. Among 304 patients with gastric cancer, 249 patients yielded sufficient plasma samples and 55 patients yielded no or insufficient plasma samples to measure HGF concentration. After eliminating 11 prevalent cases among 249 cases yielding sufficient plasma samples, 238 gastric cancer cases were included in this study. No significant differences in characteristic were observed, including median age (62 years vs. 62 years), median follow-up period (4.6 years vs. 4.2 years), proportion of female (31.9% vs. 32.7%), cigarette smoker (62.2% vs. 69.1%), and past gastritis/ulcer history (17.6% vs. 23.4%) between the included ($N = 238$) and nonincluded ($N = 55$) patients with gastric cancer (Supplementary Table S1). In total, 238 cancer-free subjects yielding sufficient plasma sample, 1:1 matched to the gastric cancer cases for age (± 5 year), sex, residence area, and year of enrollment, were selected as controls for this study. Finally, 238 patients with gastric cancer and 238 matched cancer-free controls were included in this study. In total, 238 gastric cancer cases were subclassified to three groups on the basis of the period from enrollment to gastric cancer diagnosis, including (i) case group diagnosed with gastric cancer after 6 years, (ii) case group diagnosed with gastric cancer after 2–5 years, (iii) case group diagnosed with gastric cancer within 2 years from enrollment.

Ethics statement

The KMCC and the current study protocols were approved by the Institutional Review Boards of the Seoul National University Hospital (Seoul, South Korea; H-0110-084-002, 0907-044-286, and 1701-062-823) and performed in accordance with the principles of the Declaration of Helsinki.

Measurement of plasma HGF concentration

Plasma HGF concentration was measured using a Human HGF ELISA kit following the manufacturer's instructions (Invitrogen Corp.). Minimal detectable dose of this kit is 20 pg/mL and there is no cross-reactivity with human antibodies according to the manufacturer's guidance.

Assessment of stability and reliability of blood HGF concentration

We verified whether the exposure time at room temperature, freeze-thaw cycle, or storage period affects the stability of HGF in blood or not. To confirm no differences in the plasma HGF concentration according to the exposure time of samples at room temperature, four different plasma samples were repetitively measured five times according to different exposure times at room temperature (0, 1, 3, 5, and 24 hours).

Because some samples of gastric cancer cases have been used in previous studies, the number of freeze-thaw cycle was different among specimens. Thus, we investigated whether different freeze-thaw cycles

of the samples affect plasma HGF stability or not through repeatedly measuring HGF concentration in four gastric case-control sets by increasing the number of freeze-thaw cycles from one to four times. Difference in repeatedly measured HGF concentration was evaluated using the repeated-measures ANOVA (RMANOVA).

Different sample collection time point in the cohort can affect the stability of the protein and the feasibility of the assay because of the differences in sample collection, processing, and storage period. Thus, we compared the plasma HGF levels of the cancer-free controls with analysis of covariance (ANCOVA) according to the various time periods from blood extraction to HGF measurements (7–8, 9–11, 12–14, and 15–17 years).

In addition, duplicate samples of 88 cohort members were prepared and newly coded to blind the operator to evaluate reproducibility of HGF concentration. Pearson correlation analysis was used to assess the reproducibility of HGF measurements.

Assessment of *H. pylori* infection and CagA immunoglobulin G seropositivity

H. pylori is a major carcinogen for gastric cancer, and the CagA protein is a virulence factor derived from *H. pylori*, which is associated with gastric carcinogenesis (13–16). To control the confounding effects of *H. pylori* or CagA protein, we determined *H. pylori* infection status and CagA immunoglobulin G (IgG) seropositivity using the immunoblot kit (Helico Blot 2.1, MP Biomedicals Asia Pacific). Helico Blot 2.1 has high sensitivity and specificity for *H. pylori* infection (sensitivity over 98% and specificity over 80%) and CagA IgG seropositivity (sensitivity over 97% and specificity over 87%; refs. 17–19).

CagA-related genetic variants risk score assessment

In our previous study to investigate single-nucleotide polymorphism (SNP) in CagA-related genes, which is associated with increased gastric cancer risk, five SNPs (rs6122566 and rs6124914 in *SRC*, rs41739 and rs41737 in *c-MET*, and rs7208768 in *CRK*) within CagA-interacting genes (20) and six SNPs (rs5999749 in *ERK*, rs4635002 in *Dock180*, rs7853122 and rs10901081 in *C3G*, rs530801 in *Rap1*, and rs747182 in *Src*) within CagA-interfering genes were found (21). Total CagA-related genes were defined by summing both CagA-interacting and CagA-interfering genes. Some subjects in this nested case-control study (133 gastric cancer cases and 140 controls) had information on genetic variants in CagA-interacting or CagA-interfering genes. We calculated genetic risk scores (GRS) based on the total number of risk alleles from the number of significant SNPs by summing the code number for the risk alleles (homozygous risk allele genotype = 2, heterozygous genotype = 1, and homozygous nonrisk allele genotype = 0). The GRS of the five SNPs in CagA-interacting genes, six SNPs related to CagA-interfering genes, and eleven SNPs in total CagA-related genes were each 0–10, 0–12, and 0–17, respectively. Each cut-off point of GRS was chosen by finding score, which is associated with highest gastric cancer risk, when we classified the score as binary variable and calculated OR of high GRS group compared with low GRS group. The GRS cut-off point of CagA-interacting genes, CagA-interfering genes, and total CagA-related genes were each 5 (high risk: ≥ 5 ; low risk: < 5), 9 (high risk: ≥ 9 ; low risk: < 9), and 12 (high risk: ≥ 12 ; low risk: < 12).

Statistical analysis

Differences in the frequency or distribution between gastric cancer cases and controls were assessed using the χ^2 test and *t* test in accordance with categorical and continuous explanatory variables, respectively. Plasma HGF levels were classified into 2, 3, and 4 groups

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in accordance with median (251 pg/mL), tertiles (200 and 320 pg/mL), and quartiles (167, 251, and 364 pg/mL, respectively) of HGF levels in the cancer-free controls. The associations between gastric cancer risk and blood HGF concentration were evaluated by calculating odds ratios (OR) and 95% confidence intervals (CI) using the conditional logistic regression model, adjusted for three potential confounders including age, CagA seropositivity (negative vs. positive), and cigarette smoking status (never vs. ever).

Potential confounders were selected as follows. Cigarette smoking status, which was associated with blood HGF level and with the difference in the distribution between gastric cancer cases and controls in our study, was considered a primary potential confounder. Furthermore, age, displaying a different distribution among case subgroups in accordance with the time of gastric cancer onset, and seropositivity of CagA, which is the specific virulence factor of *H. pylori* defined as a Group I carcinogen of gastric cancer by the International Agency for Research on Cancer (IARC), were considered as additional potential confounders.

To evaluate differences in plasma HGF levels among gastric cancer-free controls during cohort follow-up and the three gastric cancer groups classified by period from subjects' cohort entry to gastric cancer ascertainment (≥ 6 , 2–5.9, and 6 months–1.9 years), ANCOVA adjusted for potential confounders including cigarette smoking and CagA serotypes were conducted. *Post hoc* analysis was conducted using the Student–Newman–Keuls method to identify time points when the HGF levels in cases were significantly greater than those of the controls.

Polytomous logistic regression analysis with adjustment for age, sex, cigarette smoking, and CagA seropositivity was performed to evaluate the association between HGF concentration and gastric cancer risk according to period from enrollment and gastric cancer diagnosis.

Furthermore, the combinatorial effect of HGF and CagA-related genetic variants on gastric cancer development was also assessed. Subjects were classified as four groups in accordance with a combination of risk condition of plasma HGF level and CagA-related genetic variants. Group with both low-risk conditions in plasma HGF and CagA-related genetic markers were defined as reference group, and OR and 95% CI values were estimated using the logistic regression model adjusted for age, sex, cigarette smoking, and CagA seropositivity. *P* values for interaction between HGF and CagA-related genetic variants on gastric cancer development were estimated by adding interaction term in the logistic regression model.

The predictive performance of HGF and the combination of HGF and genetic susceptibility markers on the CagA signal transduction pathway previously considered as a biomarker for differentiating gastric cancer cases and controls was determined via AUC-ROC based on various logistic regression models. Statistical analyses were conducted using the SAS software ver. 9.4 (SAS Institute) and Cran-R ver. 3.5.0 (<https://cran.r-project.org/>).

Results

Stability and reproducibility in blood HGF concentration

We assessed the stability and reliability of blood HGF levels at different conditions in accordance with different exposure duration at room temperature, freeze–thaw cycles, and storage durations. HGF protein concentration was stable regardless of the duration of exposure at room temperature or individual subject (*P* for different time exposures = 0.84 and *P* for between-subject effects = 0.66; Supplementary Fig. S1A). No significant changes were observed in HGF levels based on the number of freeze–thaw cycles (*P* for different freeze–thaw cycles = 0.24 and *P* for between-subject effects = 0.08; Supplementary

Fig. S1B). Furthermore, we assessed the difference in plasma HGF levels in accordance with storage duration by comparing mean plasma HGF levels among groups with different follow-up periods. Mean plasma HGF concentration decreased slightly but not significantly with an increase in the follow-up duration (*P* = 0.85; Supplementary Fig. S2). To assess reproducibility of HGF levels, we measured HGF concentration in duplicated samples from 88 subjects and observed a strong correlation coefficient of 0.91 (*P* < 0.01; Supplementary Fig. S3).

Baseline characteristics in gastric cancer cases and controls

The proportion of cigarette smokers in gastric cancer cases (62.2%) was significantly higher than that in the controls (51.3%) in the nested case–control study (*P* = 0.02). No significant differences in other variables such as *H. pylori* infection, seropositivity of CagA and VacA IgG, and past history of gastritis or ulcer were detected between cases and controls. The median follow-up time of gastric cancer cases was 4.6 years (IQR, 2.2–7.3 years) and that of controls was 11.3 years (IQR, 8.3–12.5 years) in the nested case–control study (Supplementary Table S2).

Association between HGF concentration and gastric cancer development

When we used the cut-off point of median HGF levels, relative to the reference group with the low HGF levels (< 251 pg/mL), the group with the high HGF levels (≥ 251 pg/mL) displayed a 3.77-fold increased risk of gastric cancer (95% CI, 2.28–6.22). Compared with the reference group with the lowest HGF concentration (< 200 pg/mL, of the three categories; < 167 pg/mL, of the four categories), the group with the highest HGF levels (≥ 320 pg/mL of the three categories; ≥ 364 pg/mL of the four categories) displayed a greater than 8-fold increased risk of gastric cancer (OR, 8.71; 95% CI, 4.30–17.64 for three categories; OR, 10.99; 95% CI, 4.91–24.62 for four categories, respectively; **Table 1**).

Plasma HGF concentration according to period from cohort entry to gastric cancer diagnosis

The geometric mean of plasma HGF levels in incident gastric cancer cases diagnosed at least 6 months after enrollment was significantly higher than that in controls (238.9 pg/mL for controls vs. 322.1 pg/mL for gastric cancer cases; *P* < 0.01). In the nested case–control study, HGF levels were the lowest in gastric cancer-free controls, and the

Table 1. Gastric cancer risk according to plasma HGF levels in a nested case–control study within the KMCC.

Plasma HGF levels (pg/mL)	Gastric cancer cases (N = 238)	Controls (N = 238)	OR (95% CI) ^a
Median			
< 251	62 (26.1)	119 (50.0)	1.00
≥ 251	176 (73.9)	119 (50.0)	3.77 (2.28–6.22)
Tertiles			
< 200	33 (13.9)	78 (32.8)	1.00
200–319.9	60 (25.2)	81 (34.0)	2.38 (1.31–4.31)
≥ 320	145 (60.9)	79 (33.2)	8.71 (4.30–17.64)
Quartiles			
< 167	23 (9.7)	59 (24.8)	1.00
167–250.9	39 (16.4)	60 (25.2)	2.01 (0.99–4.05)
251–363.9	64 (26.9)	60 (25.2)	3.95 (1.94–8.08)
≥ 364	112 (47.0)	59 (24.8)	10.99 (4.91–24.62)

^aAdjusted for cigarette smoking and CagA IgG serotype.

Table 2. Plasma HGF concentrations at the cohort enrollment according to period from cohort entry to gastric cancer diagnosis in a nested case-control study within the KMCC.

Groups	N	Geometric mean (95% CI) ^a			
Gastric cancer-free group over cohort follow-up (controls)	238	238.9 (220.6–258.6)	$P < 0.01$		
Total new gastric cancer cases ^b	222	322.1 (301.8–343.9)		P^a	P^a
Gastric cancer-free group over cohort follow-up (controls)	238	238.9 (220.6–258.6)	Ref.		
New gastric cancer cases ^b subgroup by period from cohort entry to diagnosis					
≥ 6 years	85	286.8 (261.5–314.5)	**	Ref.	
2–5.9 years	106	327.9 (296.4–362.7)	**	ns	Ref.
6 months–1.9 years	31	417.0 (356.9–487.1)	**	**	** Ref.
Prevalent cases and gastric cancer cases diagnosed within 6 months from cohort entry	27	396.3 (318.5–493.1)	**	**	ns ns

Abbreviations: N, number; Ref., reference.

^aPost hoc pairwise multiple comparison corrected *P* values based on Fisher LSD (least significant difference) method. Comparisons significant at the 0.05 level are indicated by **. The "ns" means comparisons statistically not significant.

^bNew gastric cancer cases were defined as incident gastric cancer cases diagnosed at least 6 months later from cohort entry.

shorter the time from enrollment to gastric cancer onset, the greater the HGF levels among patients with gastric cancer. HGF concentrations among the four groups in the cohort (three gastric cancer case groups diagnosed with gastric cancer after 6 months–1.9 years, after 2–5.9 years and after 6 years, and gastric cancer-free controls during follow-up) displayed significant difference on ANCOVA ($P < 0.01$). Post hoc analysis revealed no significant difference in HGF levels between two case groups composing patients diagnosed after 6 years and after 2–5.9 years from enrollment. However, HGF levels in cancer-free control group and gastric cancer group diagnosed after 6 months–1.9 years were significantly different from those of other gastric cancer groups ($P < 0.01$ between control and each case groups; $P = 0.02$ between the two case groups diagnosed after 2–5.9 years and after 6 months–1.9 years; $P < 0.01$ between the two case groups diagnosed after 6 years and after 6 months–1.9 years; Table 2).

Association between HGF concentration and gastric cancer risk according to period from cohort entry to gastric cancer diagnosis

Compared with the reference group with the lowest HGF levels, the group with the highest HGF levels had an increased gastric cancer risk, irrespective of the time from enrollment to gastric cancer diagnosis. The gastric cancer risk of the group with the highest HGF levels relative to that in the group with the lowest HGF levels (using the threshold of tertiles and quartiles) was approximately 3-fold higher when the gastric cancer occurred even after 6 years from the cohort enrollment (OR, 3.08 for ≥ 320 pg/mL vs. < 200 pg/mL; OR, 3.41 for ≥ 364 pg/mL vs. < 167 pg/mL). The shorter the interval between cohort registration and the gastric cancer diagnosis, the greater the gastric cancer risk in the group with high HGF levels: the group with the highest HGF levels relative to the group with the lowest HGF levels was associated with approximately 4-fold and 5-fold higher risk for gastric cancer upon gastric cancer diagnosed after 2–5 years (OR, 3.97 for ≥ 320 pg/mL vs. < 200 pg/mL; OR, 4.51 for ≥ 364 pg/mL vs. < 167 pg/mL) and within 2 years from the enrollment (OR, 10.95 for ≥ 320 pg/mL vs. < 200 pg/mL; OR, 9.30 for ≥ 364 pg/mL vs. < 167 pg/mL), respectively (Table 3).

Combined effect between HGF concentration and CagA-related genetic variants on gastric cancer risk

We previously evaluated single-nucleotide polymorphisms (SNP) in the CagA signal transduction pathway for the genetic susceptibility

to gastric cancer. Eleven genetic susceptibility markers, including five SNPs in CagA-interacting genes (*SRC* rs6122566, rs6124914, *MET* rs41739, rs41737, and *CRK* rs7208768) and six SNPs in CagA-interfering genes on ERK pathway activated by the subsequent signals of CagA (*MAPK1* rs5999749, *Dock180* rs4635002, *RAPGEF1* rs7853122, rs10901081, *RAP1A* rs530801, and *SRC* rs747182) were significantly associated with gastric cancer risk. The gastric cancer risks in subjects with both low-risk conditions in plasma HGF (< 251 pg/mL) and CagA-related genetic markers (GRS < 12), and that in subjects with low-risk condition in HGF and high-risk condition in genetic markers or those having high-risk condition in HGF and low-risk condition in genetic markers were not significantly different (OR, 1.21; 95% CI, 0.47–3.10 for low-risk HGF and high-risk genetic markers; OR, 1.20; 95% CI, 0.55–2.60 for high-risk HGF and low-risk genetic markers, respectively). However, those with both high-risk conditions of HGF and CagA-relating genetic markers had at least 4.98-fold higher risk for gastric cancer (95% CI, 2.04–12.15; Table 4).

Performance of multivariable model using HGF to discriminate gastric cancer case and control

In this study, the discriminative performance for distinguishing patients with gastric cancer diagnosed at least 6 months after enrollment from gastric cancer-free controls (measured by AUC-ROC) was 0.57 (95% CI, 0.52–0.62) and was determined using a basic model comprising age, CagA serotypes, and cigarette smoking (Model 1). For Model 2 based on plasma HGF levels (tertiles) added to Model 1, the discriminative performance was 0.68 (95% CI, 0.63–0.73; $P < 0.01$ for Model 1 vs. Model 2). Inclusion of the 11 SNPs in CagA-related genes in Model 2 increased the discriminative performance to 0.71 (95% CI, 0.64–0.78; Model 3; $P = 0.44$ for Model 2 vs. and 3; Fig. 1A). Point estimates based on predictors within each model (Model 1–3) are presented in Supplementary Table S3. Although potential confounders including age, cigarette smoking, and CagA IgG seropositivity were considered in the model, there were no significant associations of those factors with gastric cancer risk in this study. Each HGF level and CagA-related genetic variants was significantly associated with gastric cancer risk when potential confounders were simultaneously adjusted in the model (HR, 3.59; 95% CI, 1.67–7.73 for HGF ≥ 320 pg/mL vs. < 200 pg/mL; HR, 2.72; 95% CI, 1.47–5.07 for CagA-related genetic variants high-risk group vs. low-risk group).

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Table 3. Association between plasma HGF concentration and the gastric cancer risk in the three groups, classified by period from cohort entry to gastric cancer diagnosis (polytomous logistic regression model) in a nested case-control study within the KMCC.

Plasma HGF levels (pg/mL)	Gastric cancer cases classified according to the time of gastric cancer diagnosis after the cohort entry						
	Gastric cancer-free controls (N = 238)	Gastric cancer cases diagnosed after ≥ 6 years (N = 85)	OR (95% CI) ^a	Gastric cancer cases diagnosed after 2–5 years (N = 106)	OR (95% CI) ^a	Incident gastric cancer cases diagnosed within < 2 years (N = 47)	OR (95% CI) ^a
Median							
< 251	119 (50.0)	27 (31.8)	1.00	29 (27.3)	1.00	6 (12.8)	1.00
≥ 251	119 (50.0)	58 (68.2)	2.20 (1.29–3.76)	77 (72.7)	2.60 (1.57–4.29)	41 (87.2)	6.44 (2.60–15.96)
Tertiles							
< 200	78 (32.8)	13 (15.3)	1.00	17 (16.0)	1.00	3 (6.4)	1.00
200–319.9	81 (34.0)	34 (40.0)	2.56 (1.24–5.27)	19 (17.9)	1.06 (0.51–2.20)	7 (14.9)	1.78 (0.43–7.33)
≥ 320	79 (33.2)	38 (44.7)	3.08 (1.49–6.33)	70 (66.1)	3.97 (2.13–7.40)	37 (78.7)	10.95 (3.19–37.61)
Quartiles							
< 167	59 (24.8)	8 (9.4)	1.00	12 (11.3)	1.00	3 (6.4)	1.00
167–250.9	60 (25.2)	19 (22.4)	2.23 (0.90–5.54)	17 (16.0)	1.37 (0.60–3.14)	3 (6.4)	0.87 (0.17–4.57)
251–363.9	60 (25.2)	31 (36.5)	3.76 (1.58–8.95)	22 (20.8)	1.73 (0.78–3.84)	11 (23.4)	3.05 (0.79–11.79)
≥ 364	59 (24.8)	27 (31.7)	3.41 (1.41–8.24)	55 (51.9)	4.51 (2.17–9.36)	30 (63.8)	9.30 (2.64–32.76)

^aAdjusted for age, sex, cigarette smoking, and CagA IgG serotypes.

The discriminative performances of Model 1–3 were persistent even when we restricted gastric cancer cases diagnosed at least 2 years after enrollment (AUC-ROC, 0.58 for Model 1; AUC-ROC, 0.66 for Model 2; AUC-ROC, 0.70 for Model 3, respectively; Fig. 1B).

Discussion

The present nested case-control study reports that plasma HGF levels are different between gastric cancer cases and controls, and higher the HGF levels, the greater the gastric cancer risk. When gastric cancer cases were classified in accordance with the period from enrollment to gastric cancer diagnosis, HGF measured in blood sampled before gastric cancer diagnosis (≥ 6 years) was also positively

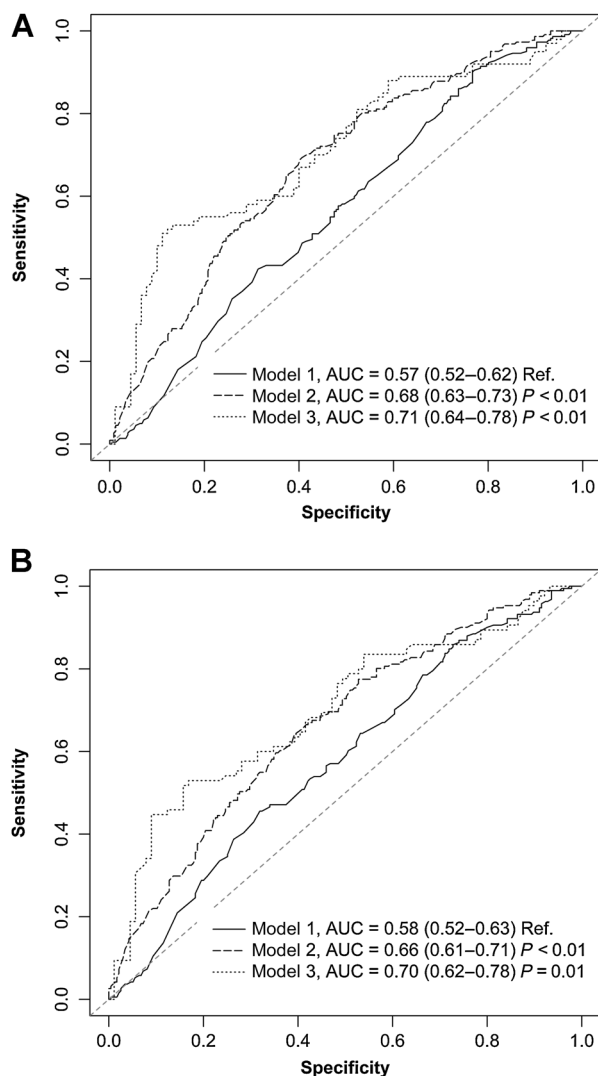
associated with the gastric cancer risk. The model based on HGF level and 11 genetic markers selected from among genes associated with CagA transduction pathway added to the basic gastric cancer prediction model further increased the discriminant performance for distinguishing patients with gastric cancer and controls up to 70% (AUC-ROC, 0.71).

Thus far, previous studies have assessed the association between HGF level and gastric cancer progression or prognosis. These studies have reported poor survival and advanced progression in patients with gastric cancer with high serum HGF level (10, 22, 23). Furthermore, HGF has been considered a therapeutic target to treat gastric cancer on the basis of the role of HGF in tumor progression, for example, regulation of cell proliferation, survival, and motility. A monoclonal

Table 4. Association with the combined effects between CagA-related genetic variants and HGF concentrations on gastric cancer risk in a nested case-control study within the KMCC.

HGF levels ^a	GRS levels ^b	Gastric cancer cases	Controls	OR (95% CI) ^c	P _{interaction}
Low	CagA-interacting genes ^b				0.77
	Low	25 (20.1)	49 (37.7)	1.00	
High	High	11 (8.9)	12 (9.2)	1.73 (0.67–4.47)	0.49
	Low	58 (46.8)	55 (42.3)	2.18 (1.21–3.91)	
High	High	30 (24.2)	14 (10.8)	4.52 (2.04–10.02)	0.05
	Low	28 (23.7)	39 (38.2)	1.00	
High	High	7 (5.9)	9 (8.8)	1.08 (0.35–3.29)	0.05
	Low	67 (56.8)	48 (47.1)	1.99 (1.07–3.71)	
High	High	16 (13.6)	6 (5.9)	3.60 (1.23–10.52)	0.05
	Low	18 (17.0)	26 (28.6)	1.00	
High	High	15 (14.1)	16 (17.6)	1.21 (0.47–3.10)	0.05
	Low	31 (29.3)	37 (40.7)	1.20 (0.55–2.60)	
High	High	42 (39.6)	12 (13.2)	4.98 (2.04–12.15)	0.05
	Low	15 (14.1)	16 (17.6)	1.21 (0.47–3.10)	

^aThe risk status cut-off point for HGF protein level was 251 pg/mL (high risk: ≥ 251 pg/mL; low risk: < 251 pg/mL).^bThe GRS of the five SNPs in CagA-interacting genes were 0–10 and the cutoff for risk status was 5 (high risk: ≥ 5; low risk: < 5). The GRS of six SNPs in CagA-interfering genes were 0–12 and the risk status cutoff was 9 (high risk: ≥ 9; low risk: < 9). Total CagA-related genes were both CagA-interacting and CagA-interfering genes. GRS of total CagA-related genes were 0–17 and the risk status cutoff was 12 (high risk: ≥ 12; low risk: < 12).^cAdjusted for age, sex, cigarette smoking, and CagA IgG serotypes.

**Figure 1.**

AUC-ROC of model using plasma HGF levels for predicting gastric cancer risk. **A**, Gastric cancer cases diagnosed at least 6 months after enrollment versus gastric cancer-free controls. **B**, Gastric cancer cases diagnosed at least 2 years after enrollment versus gastric cancer-free controls (Model 1) logistic regression model for gastric cancer risk by age, cigarette smoking, and CagA serotypes; (Model 2) Model 1 + plasma HGF levels (classified by HGF tertiles); (Model 3) Model 2 + CagA-related GRS.

anti-HGF antibody, rilotumumab, has been developed (24) and randomized clinical trials have attempted to assess efficacy of this agent in patients with gastric cancer (25–27), although these trials were terminated owing to adverse event in treatment group. HGF has received increasing attention with respect to its prognostic or therapeutic potential; however, its predictive value in gastric cancer development has not been determined through prospective studies. However, our previous study on soluble c-Met and gastric cancer development suggests the possibility of HGF being a marker to predict gastric cancer risk (7).

HGF may be associated with gastric cancer risk and may help to predict gastric cancer development. c-Met–HGF binding stimulates various signal transduction pathways including MAPK/ERK, PI3K/

AKT, and JACK/STAT pathways and this signaling transduction regulates cell proliferation, survival, motility, and invasion, which involve in tumor development and progression (28). Thus, HGF levels are potential markers to assess the gastric cancer risk.

The association between inflammation and HGF release might offer yet another explanation. Increase in IL1 β and HGF release in the enlarged fold gastritis patients with *H. pylori* infection was reported, and significant decrease in HGF after *H. pylori* eradication or treatment with an IL1 β antagonist was observed (29). In other words, HGF production can result from inflammation due to *H. pylori* infection; hence, HGF might reflect the inflammatory status, which is crucial to induce gastric carcinogenesis.

Herein, we observed the combinatorial effect of HGF level and genetic variants in signaling molecules related to CagA on gastric cancer risk along with improved predictability of the model including both HGF and those genetic variants in comparison with that of the model including only HGF level, although the difference was not significant. These findings above may be owing to the interaction between c-Met receptor and CagA protein. *In vitro* studies have reported that CagA protein stimulates PI3K/AKT and MAPK/ERK pathways by binding to the c-Met receptor, and finally induces cell proliferation, proinflammatory response (30), and cell motility (31). Because the impact of CagA protein on host cells depends on signaling proteins in pathways stimulated by CagA and the activity of these signaling proteins is regulated by their host genes, genetic polymorphisms in these genes may induce individual susceptibility to gastric cancer development.

This study has several limitations. First, the samples used to quantify HGF levels were obtained upon enrollment, hence, these samples do not reflect variations in HGF levels in accordance with the progression of gastric cancer. Second, the follow-up was performed by linking data with the cancer registry and death certificates. Thus, specific information regarding cancer, such as histologic type and Tumor–Node–Metastasis stage, could not be obtained. Owing to limited information regarding gastric cancer, a stratified analysis based on the cancer type could not be performed. Third, the number of subjects in this study was not enough to have sufficient statistical power and external validation could not be carried out. Hence, we cannot generalize these results and there is possibility of overestimating the performance of model based on HGF and CagA-related genetic variants to predict gastric cancer development. External validation based on large-scale prospective cohort is needed to establish HGF as a useful marker to predict gastric cancer development.

Despite of aforementioned limitations, the current results are based on a nested case–control study in a prospective cohort study, wherein the patient samples were obtained for HGF quantification and information on confounding factors was obtained before gastric cancer diagnosis. Thus, the current results are not as likely to be affected by reverse causality in explaining the association between HGF and gastric cancer. Furthermore, this study verified the availability and high potential of HGF as a biomarker for predicting gastric cancer development and reported an improved performance when other markers associated with HGF were included in the model.

In conclusion, these results indicate that blood HGF levels and the GRS of significant genetic variants on CagA–cMET–ERK signaling pathways may serve as better biomarkers to predict gastric cancer development. Further clinical studies are needed to verify the clinical utility and validity of our proposed biomarkers to select groups at a high risk of gastric cancer development and different clinical indications in a prospective cohort.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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