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FcγRIIA and *IIIA* polymorphisms predict clinical outcome of trastuzumab-treated metastatic gastric cancer

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Abstract: Trastuzumab has substantial antitumor activity in metastatic gastric cancer. One such mechanism by which it exerts its antitumor activity is antibody-dependent cell-mediated cytotoxicity, which has been reported to be influenced by *FcγRIIA* and *IIIA* polymorphisms. This study is the first to assess their impact on trastuzumab efficacy in patients with metastatic gastric cancer. We retrospectively examined 42 Her-2-positive patients receiving fluorouracil and platinum-based chemotherapy and trastuzumab, and 68 Her-2-negative patients receiving fluorouracil and platinum-based chemotherapy only as the first-line treatment. *FcγRIIA* and *IIIA* polymorphisms were assessed, and their associations with efficacy in both settings were analyzed. In patients treated with trastuzumab, the *FcγRIIA* H/H genotype was associated with significantly superior progression-free survival (PFS) (hazard ratio [HR] [95% CI]: 0.36 [0.16–0.82], adjusted HR [95% CI]: 0.18 [0.07–0.48], P=0.001). When combining *FcγRIIA* and *IIIA* polymorphisms, the *FcγRIIA* H/H or *FcγRIIIA* V/V genotype was associated with a significantly improved disease control rate (P=0.04) and PFS (HR [95% CI]: 0.29 [0.13–0.67], adjusted HR [95% CI]: 0.17 [0.07–0.45], P<0.001). As expected, no association of *FcγRIIA* and *IIIA* polymorphisms with efficacy was found in patients receiving chemotherapy only. We concluded that *FcγRIIA* and *IIIA* polymorphisms might predict disease control rate and PFS in metastatic gastric cancer patients receiving trastuzumab treatment.

Keywords: gastric cancer, trastuzumab, antibody-dependent cell-mediated cytotoxicity, *FcγRIIA* polymorphism, *FcγRIIIA* polymorphism, human epidermal growth factor receptor-2

Introduction

Gastric cancer (GC) is one of the leading malignancies of the digestive system. There were an estimated 26,370 new cases of GC in the USA in 2016, making it fourth in rank among all cancers of the digestive system.[1] The incidence of GC is much higher in China, where it ranks second among all cancers as estimated in 2015.[2] The prognosis of GC is relatively poor.[3] GC is also the second leading cause of cancer-specific death in China.[2] The median overall survival for stage IV patents is about 1 year. The use of targeted therapy prolongs survival for ~2–4 months.[4–7] Trastuzumab, the main targeted drug for GC, was approved by the Food and Drug Administration in 2010 for the treatment of epidermal growth factor receptor 2 (ERBb2 or Her-2)-overexpressed metastatic GC or gastroesophageal junction adenocarcinoma.

The major mechanism of the antitumor effect of trastuzumab involves the growth signaling pathways transduced by the transmembrane protein, Her-2, as indicated by in vitro and in vivo laboratory and clinical studies.[8–10] However, another Her-2-targeted drug, lapatinib, has not been found to significantly improve survival in advanced or

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metastatic GC.[11] This drug is a small-molecule tyrosine kinase inhibitor and is different from trastuzumab, which is an IgG1 monoclonal antibody that mediates antibody-dependent cell-mediated cytotoxicity (ADCC) and the subsequent lysis of targeted tumor cells.[12,13] This efficacy disparity may emphasize the importance of ADCC in the antitumor effect of trastuzumab in GC.

The ADCC effect is mediated by the binding of the fragment C (Fc) portion of the antibody and the Fc γ receptor (FcγR) on immunologic effector cells.[13] Genetic polymorphisms of FcγR have been demonstrated to result in differences in binding affinity, with the *FcγRIIA* and *IIIA* alleles most frequently reported. For *FcγRIIA*, a single-nucleotide substitution (A519G, rs1801274) results in the substitution of histidine (H) by arginine (R) at amino acid position 131. Likewise, for *FcγRIIIA*, a single-nucleotide substitution (A559C, rs396991) leads to the substitution of phenylalanine (F) by valine (V) at amino acid position 158. Both the *FcγRIIA* 131H/H and *FcγRIIIA* 158V/V genotypes are identified as having the strongest binding affinity.[14–16]

An association between patient outcomes and *FcγRIIA* and/or *IIIA* genotypes has been discovered in lymphoma treated with rituximab[17] and colorectal cancer treated with cetuximab,[18,19] and both these drugs are IgG1 monoclonal antibodies. The prognostic value of *FcγRIIA* and *IIIA* genotypes in breast cancer treated with trastuzumab was found to be inconsistent in several previous investigations, but these genotypes seemed to be relevant in cases of advanced and metastatic breast cancer.[20–23] Only about half of Her-2-positive GC patients respond to trastuzumab,[7] and thus, the identification of biomarkers predicting its efficacy has potential clinical application. ADCC may constitute a significant portion of the antitumor mechanism of trastuzumab in GC. To our knowledge, this is the first study to evaluate the clinical and prognostic relevance of *FcγRIIA* and/or *IIIA* polymorphisms in trastuzumab-treated metastatic GC.

Materials and methods

Ethics statement

All patients provided written informed consent for their information to be used in our hospital database. Study approval was obtained from independent ethics committees at Sun Yat-sen University Cancer Center. This study was conducted in accordance with the ethical standards of the World Medical Association Declaration of Helsinki.

Study population

This research was retrospectively conducted at Sun Yat-sen University Cancer Center. We included two sets of patients pathologically diagnosed with metastatic gastric adenocarcinoma in this study. Set A included patients with Her-2-positive GC, who received trastuzumab combined with chemotherapy as the first-line treatment. Set B included patients with Her-2-negative GC, who received only chemotherapy as the first-line treatment. The assessment of Her-2 status followed the criteria of the National Comprehensive Cancer Network guidelines. HER-2 positive was defined as HER-2(+++) by immunohistochemistry or HER-2(++) by immunohistochemistry and HER-2(positive) by fluorescence in situ hybridization. There were 228 patients pathologically diagnosed with metastatic GC from May 1, 2011, to August 30, 2015, in our center, including 58 patients with Her-2-positive cancer and 170 patients with Her-2-negative cancer. We included only those with an efficacy assessment and follow-up information, as well as those who signed informed consent forms and had qualified blood samples. With these eligibility criteria, there were 42 patients enrolled in set A. All of them received trastuzumab combined with fluorouracil and platinum-based chemotherapy as the first-line treatment. As a comparison, enrolled in set B were 68 patients who received only fluorouracil and platinum-based chemotherapy as the first-line treatment. The Consolidated Standards of Reporting Trials (CONSORT) diagram is shown in Figure S1. The clinicopathologic data and follow-up information were retrospectively collected.

FcγRIIA and *IIIA* polymorphism genotyping

Blood samples were collected before treatment. Archived peripheral blood mononuclear cells were used for DNA extraction with the QIAamp DNA blood mini kit (Qiagen). Nested polymerase chain reaction (PCR) was conducted to detect single-nucleotide polymorphisms in *FcγRIIA* and/or *IIIA* using the same primers as in a previous study.[21] PCR was carried out using the HiFi HotStart ReadyMix (KAPA Biosystems) and optimized protocols. The PCR products were purified using the PCR clean kit (Qiagen) and then sequenced on an ABI3730XL (Applied Biosystems) with the BigDye Terminator v3.1 Cycle Sequencing Kit. The PCR products of 110 samples were also analyzed on MassARRAY analyzer (Sequenom) with the iPLEX Gold assay (Sequenom) using primers, as in a previous study,[21] to identify the A559C polymorphism in *FcγRIIIA* and the A519G polymorphism in *FcγRIIA*.

Statistical analysis

All statistical analyses were performed using the SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA).

A two-tailed *P*-value of <0.05 was considered statistically significant. The associations between *FcγRIIA* and/or *IIIA* genotypes and clinicopathologic characteristics were analyzed with a chi-square test or Kruskal–Wallis H-test, based on the type of the data. Comparisons of tumor responses between *FcγRIIA* and/or *IIIA* genotypes were also conducted with chi-square test or Kruskal–Wallis H-test. Survival curves were plotted by the Kaplan–Meier method and compared using the log-rank test. The prognostic value of *FcγRIIA* and/or *IIIA* genotypes was evaluated using univariate and multivariate Cox regression analyses.

Results

Patient characteristics

Patient characteristics according to treatment settings are shown in Table S1. All 42 patients in set A were Her-2 positive; by contrast, all 68 patients in set B were Her-2 negative. Accordingly, differences in some clinicopathologic features existed between the two sets. For example, tumors in set A presented with more intestinal types compared with those in set B (33.3% vs 2.9%). The correlations between *FcγRIIA* and/or *IIIA* genotypes and clinicopathologic features are shown in Table 1. The frequency of *FcγRIIA* and/or *IIIA* genotypes did not differ between the two study sets, as demonstrated by the lack of associations between *FcγRIIA* and/or *IIIA* genotypes and Her-2 status (negative/positive). In addition, we did not find any correlations between other clinicopathologic features including age (<52/≥52 years), gender (male/female), Eastern Cooperative Oncology Group score (0/1), gastrectomy (yes/no), location of tumor (proximal/middle/distal/others), degree of differentiation (well-differentiated adenocarcinoma/moderately differentiated adenocarcinoma/poorly differentiated/signet ring cell carcinoma/mucinous adenocarcinoma), Lauren classification (intestinal type/diffuse type/mixed type), smoking status (present or previous/never), drinking status (present or previous/never), and metastasis (single/multiple).

FcγR genotypes and tumor responses

The frequencies of the *FcγRIIA* H/H, H/R, and R/R genotypes were 45.2%, 38.1%, and 16.7%, respectively, in set A (patients receiving trastuzumab and chemotherapy) and 54.4%, 35.3%, and 10.3%, respectively, in set B (patients receiving chemotherapy only). The frequencies of the *FcγRIIIA* V/V, V/F, and F/F genotypes were 11.9%, 45.2%, and 42.9%, respectively, in set A and 20.6%, 47.1%, and 32.4%, respectively, in set B. The objective response rate (ORR) and the disease control rate (DCR) were 59.5% and 83.3%, respectively, in set A, but were lower in set B (32.4% and 72.1%, respectively). We did not find any significant associations between the *FcγRIIA* H/H, H/R, and R/R genotypes and the *FcγRIIIA* V/V, V/F, and F/F genotypes with tumor response in set B. However, we observed a trend of higher DCR in the *FcγRIIA* H/H genotype compared with the H/R or R/R genotypes (94.7% vs 73.9%, *P*=0.07) in set A. We further classified patients based on their combination of *FcγRIIA* and *IIIA* into two groups: the H/H or V/V group, which represented the group with higher affinity, and all the others, which represented the group with lower affinity. Still, no association between *FcγRIIA* and *IIIA* genotypes and tumor response was found in set B, but the H/H or V/V genotype was associated with a significantly higher DCR in set A (95.2% vs 71.4%, *P*=0.04). The detailed information is presented in Table 2.

FcγR genotypes and progression-free survival

The median follow-up time was 13.15 (range: 3.5–73.63) and 7.30 (range: 1.3–63.1) months in set A and set B, respectively. The median progression-free survival (PFS) was estimated to be 7.23 (95% CI: 4.93–9.53) and 5.27 (95% CI: 3.66–6.87) months in set A and set B, respectively.

In set A, univariate analysis showed that *FcγRIIA* polymorphism with the H/H genotype was associated with significantly superior PFS compared with the H/R or R/R genotype (hazard ratio [HR] [95% CI]: 0.36 [0.16–0.82], *P*=0.02). At the same time, *FcγRIIIA* polymorphism had no impact on PFS (HR [95% CI]: 0.20 [0.03–1.49], *P*=0.12). When considering the prognostic value of the combination of *FcγRIIA* and *IIIA* polymorphisms, we found that the H/H or V/V genotype was also related to significantly better PFS compared with other genotypes (HR [95% CI]: 0.29 [0.13–0.67], *P*=0.004). The prognostic value of *FcγRIIA* and/or *IIIA* was further adjusted for the following factors in the multivariate analysis: age (<52/≥52 years), gender (male/female), Eastern Cooperative Oncology Group score (0/1), degree of differentiation (well-differentiated adenocarcinoma/moderately differentiated adenocarcinoma/poorly differentiated/signet ring cell carcinoma/mucinous adenocarcinoma), and Lauren classification (intestinal type/diffuse type/mixed type). *FcγRIIA* polymorphism and the combination of *FcγRIIA* and *IIIA* polymorphisms remained independent and significant prognostic factors for PFS in set A (adjusted HR [95% CI]: 0.18 [0.07–0.48] and 0.17 [0.07–0.45], *P*=0.001 and *P*<0.001, respectively). *FcγRIIIA* polymorphism also showed a tendency to be prognostic in the multivariate analysis in set A (adjusted HR [95% CI]: 0.15 [0.02–1.31], *P*=0.09).

Table 1 Patient characteristics by genotypes in metastatic gastric cancer

Features	*FcγRIIA*				*FcγRIIIA*				*FcγRIIA* and *IIIA*		
	H/H (%), n=56	R/R (%), n=14	H/R (%), n=40	*P*-value	V/V (%), n=19	F/F (%), n=40	V/F (%), n=51	*P*-value	H/H or V/V, n=61	Others, n=49	*P*-value
Her-2 status				0.52				0.38			0.37
Positive	19 (45.2)	7 (16.7)	16 (38.1)		5 (11.9)	18 (42.9)	19 (45.2)		21 (50.0)	21 (50.0)	
Negative	37 (54.4)	7 (10.3)	24 (35.3)		14 (20.6)	22 (32.4)	32 (47.1)		40 (58.8)	28 (41.2)	
Age (years, median 52)				0.52				0.97			0.38
<52	23 (45.1)	7 (13.7)	21 (41.2)		9 (17.6)	19 (37.3)	23 (45.1)		26 (51.0)	25 (49.0)	
≥52	33 (55.9)	7 (11.9)	19 (32.2)		10 (16.9)	21 (35.6)	28 (47.5)		35 (59.3)	24 (40.7)	
Gender				0.08				0.08			0.56
Male	33 (50.0)	5 (7.6)	28 (42.4)		7 (10.6)	26 (39.4)	33 (50.0)		35 (53.0)	31 (47.0)	
Female	23 (52.3)	9 (20.5)	12 (27.3)		12 (27.3)	14 (31.8)	18 (40.9)		26 (59.1)	18 (40.9)	
ECOG score				0.25				0.08			
0	42 (48.3)	10 (11.5)	35 (40.2)		15 (17.2)	36 (41.4)	36 (41.4)		47 (54.0)	40 (46.0)	0.56
1	14 (60.9)	4 (17.4)	5 (21.7)		4 (17.4)	4 (17.4)	15 (65.2)		14 (60.9)	9 (39.1)	
Gastrectomy				0.47				0.27			0.99
Yes	43 (51.8)	12 (14.5)	28 (33.7)		12 (14.5)	33 (39.8)	38 (45.8)		46 (55.4)	37 (44.6)	
No	13 (48.1)	2 (7.4)	12 (44.4)		7 (25.9)	7 (25.9)	13 (48.1)		15 (55.6)	12 (44.4)	
Location of tumor				0.87				0.36			0.81
Proximal	14 (60.9)	3 (13.0)	6 (26.1)		3 (13.0)	9 (39.1)	11 (47.8)		14 (60.9)	9 (39.1)	
Middle	16 (50.0)	4 (12.5)	12 (37.5)		8 (25.0)	10 (31.3)	14 (43.8)		18 (56.3)	14 (43.8)	
Distal	23 (46.9)	7 (14.3)	19 (38.8)		6 (12.2)	21 (42.9)	22 (44.9)		25 (51.0)	24 (49.0)	
Others	3 (50.0)	0 (0.0)	3 (50.0)		2 (33.3)	0 (0.0)	4 (66.7)		4 (66.7)	2 (33.3)	
Degree of differentiation				0.53				0.16			0.50
Well-differentiated adenocarcinoma	1 (33.3)	0 (0.0)	2 (66.71)		0 (0.0)	3 (100.0)	0 (0.0)		1 (33.3)	2 (66.7)	
Moderately differentiated adenocarcinoma	13 (65.0)	2 (10.0)	5 (25.0)		2 (10.0)	7 (35.0)	11 (55.0)		13 (65.0)	7 (35.0)	
Poorly differentiated/signet ring cell carcinoma/mucinous adenocarcinoma	42 (48.3)	12 (13.8)	33 (37.9)		17 (19.5)	30 (34.5)	51 (46.0)		47 (54.0)	40 (46.0)	
Lauren classification				0.48				0.81			0.83
Intestinal type	10 (62.5)	0 (0.0)	6 (37.5)		2 (12.5)	6 (37.5)	8 (50.0)		10 (62.5)	6 (37.5)	
Diffuse type	34 (50.0)	9 (13.2)	25 (36.8)		14 (20.6)	23 (33.8)	31 (45.6)		37 (54.4)	31 (45.6)	
Mixed type	12 (46.2)	5 (19.2)	9 (34.6)		3 (11.5)	11 (42.3)	12 (46.2)		14 (53.8)	12 (46.2)	
Smoke				0.53				0.69			0.98
Present or previous	20 (52.6)	3 (7.9)	15 (39.5)		5 (13.2)	15 (39.5)	18 (47.4)		21 (55.3)	17 (44.7)	
Never	36 (50.0)	11 (15.3)	25 (34.7)		14 (19.4)	25 (34.7)	33 (45.8)		40 (55.6)	32 (44.4)	
Drink				0.69				0.51			0.94
Present or previous	15 (48.4)	3 (9.7)	13 (41.9)		4 (12.9)	10 (32.3)	17 (54.8)		17 (54.8)	14 (45.2)	
Never	41 (51.9)	11 (13.9)	27 (34.2)		15 (19.0)	30 (38.0)	34 (43.0)		44 (55.7)	35 (44.3)	
Metastasis				0.95				0.86			0.38
Single	26 (51.0)	7 (13.7)	18 (35.3)		8 (15.7)	18 (35.3)	25 (49.0)		26 (51.0)	25 (49.0)	
Multiple	30 (50.8)	7 (11.9)	22 (37.3)		11 (18.6)	22 (37.3)	26 (44.1)		35 (59.3)	24 (40.7)	

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Table 2 FcγR polymorphisms and tumor responses in metastatic GC

	Set A (n=42)									Set B (n=68)							
	No (%)	Response						*P*-value for ORR	*P*-value for DCR	No (%)	Response						
		CR no (%)	PR no (%)	SD no (%)	PD no (%)	ORR no (%)	DCR no (%)				CR no (%)	PR no (%)	SD no (%)	PD no (%)	ORR no (%)	DCR no (%)	*P*-value for ORR	*P*-value for DCR
FcγRIIA																	
H/H	19 (45.2)	1 (5.3)	11 (57.9)	6 (31.6)	1 (5.3)	12 (63.2)	18 (94.7)			37 (54.4)	0 (0.0)	14 (37.8)	13 (35.1)	10 (27.0)	14 (37.8)	27 (73.0)		
H/R	16 (38.1)	0 (0.0)	8 (50.0)	2 (12.5)	6 (37.5)	8 (50.0)	10 (62.5)			24 (35.3)	0 (0.0)	6 (25.0)	13 (54.2)	5 (20.8)	6 (25.0)	19 (79.1)		
R/R	7 (16.7)	0 (0.0)	5 (71.0)	2 (28.6)	0 (0.0)	5 (71.0)	7 (100.0)			7 (10.3)	0 (0.0)	2 (28.6)	1 (14.3)	4 (57.1)	2 (28.6)	3 (42.9)		
H/R or R/R	23 (54.8)	0 (0.0)	13 (56.5)	4 (17.4)	6 (26.1)	13 (56.5)	17 (73.9)			31 (45.6)	0 (0.0)	8 (25.8)	14 (45.2)	9 (29.0)	8 (25.8)	22 (71.0)		
H/H vs H/R vs R/R								0.58	0.02								0.57	0.18
H/H vs H/R or R/R								0.66	0.07								0.29	0.85
FcγRIIIA																		
V/V	5 (11.9)	0 (0.0)	4 (80.0)	1 (20.0)	0 (0.0)	4 (80.0)	5 (100.0)			14 (20.6)	0 (0.0)	7 (50.0)	1 (7.1)	6 (42.9)	7 (50.0)	8 (57.0)		
V/F	19 (45.2)	1 (5.3)	10 (52.6)	3 (15.8)	5 (26.3)	11 (57.9)	14 (73.7)			32 (47.1)	0 (0.0)	9 (28.1)	15 (46.9)	8 (25.0)	9 (28.1)	24 (75.0)		
F/F	18 (42.9)	0 (0.0)	10 (55.6)	6 (33.3)	2 (11.1)	10 (55.6)	16 (88.9)			22 (32.4)	0 (0.0)	6 (27.3)	11 (50.0)	5 (22.7)	6 (27.3)	17 (77.3)		
V/F or F/F	37 (88.1)	0 (0.0)	21 (56.8)	9 (24.3)	7 (18.9)	21 (56.8)	30 (81.0)			54 (79.4)	0 (0.0)	15 (27.8)	26 (48.1)	13 (24.1)	15 (27.8)	41 (75.9)		
V/V vs V/F vs F/F								0.61	0.27								0.29	0.38
V/V vs V/F or F/F								0.32	0.29								0.11	0.16
FcγRIIA and *IIIA*								0.35	0.04								0.28	0.65
H/H or V/V	21 (50.0)	1 (61.9)	13 (66.7)	6 (28.6)	1 (4.8)	14 (66.7)	20 (95.2)			40 (58.8)	0 (0.0)	15 (37.5)	13 (32.50)	12 (30.0)	15 (37.5)	28 (70.0)		
Others	21 (50.0)	0 (0.0)	11 (52.4)	4 (19.0)	6 (28.6)	11 (52.4)	15 (71.4)			28 (41.2)	0 (0.0)	7 (25.0)	14 (50.0)	7 (25.0)	7 (25.0)	21 (75.0)		

Notes: Set A: patients with Her-2–positive GC who received trastuzumab combined with chemotherapy as the first-line treatment. Set B: patients with Her-2–negative GC who received chemotherapy only as the first-line treatment.
Abbreviations: CR, complete response; DCR, disease control rate; FcγR, Fc γ receptor; GC, gastric cancer; ORR, objective response rate; PD, progressed disease; PR, partial response; SD, stable disease.

However, in set B, no associations between *FcγRIIA* and/or *IIIA* polymorphisms and PFS were identified in either univariate or multivariate analysis. Detailed information on the univariate and multivariate survival analyses is given in Table 3. The prognostic differences in PFS between *FcγRIIA* and/or *IIIA* polymorphisms in set A and set B were analyzed by the Kaplan–Meier method, as shown in Figure 1 (*FcγRIIA*), Figure 2 (*FcγRIIIA*), and Figure 3 (the combination of *FcγRIIA* and *IIIA*).

Discussion

To our knowledge, this is the first study to assess the impact of *FcγRIIA* and/or *IIIA* polymorphisms on tumor response and PFS in metastatic GC patients receiving chemotherapy and trastuzumab or chemotherapy alone. We found that the *FcγRIIA* H/H genotype was associated with a trend of higher DCR and significantly superior PFS, compared with the H/R or R/R genotype. In addition, when combining *FcγRIIA* and *IIIA* polymorphisms together, the H/H or V/V genotype was related to significantly higher DCR and better PFS compared with other genotypes.

Her-2 overexpression is identified to be a negative prognostic factor, and anti-Her-2–targeted therapy with trastuzumab could remedy the survival gap between Her-2-positive GC and Her-2-negative GC, as suggested by a cohort study.[10] In ToGA trial, the addition of trastuzumab to cisplatin-based chemotherapy significantly prolonged PFS from 5.5 to 6.7 months and improved ORR from 35% to 47% in Her-2-positive GC.[7] However, primary resistance to trastuzumab was also observed in a small portion of patients. The identification of predictive markers for screening of patients for the efficacy of trastuzumab is important to avoid unnecessary costs.

ADCC has been suggested as another antitumor mechanism in addition to blocking the signal transduction pathway.[24,25] *FcγRIIA* and *IIIA* polymorphisms have a substantial impact on the binding affinity in ADCC.[15,16] Consistent with some previous findings, we identified that the *FcγRIIA* H/H or *FcγRIIIA* V/V genotype benefits more from trastuzumab than other genotypes. Although it had no impact on ORR, the H/H or V/V genotype was associated with significantly improved DCR and PFS. This impact did not exist in patients receiving chemotherapy-only treatment. This finding is consistent with the mechanism of ADCC. Lymphocyte infiltration of tumors might not lead to obvious shrinkage of tumor size, and sometimes even results in enlargement of the tumor size, as observed in

Table 3 The prognostic value of *FcγRIIA* and *FcγRIIIA* polymorphisms for PFS in patients with metastatic GC: univariate and multivariate analyses

	Set A (n=42)					Set B (n=68)				
	Median PFS (months)	Univariate		Multivariate		Median PFS (months)	Univariate		Multivariate	
		Hazard ratio (95% CI)	*P*-value	Adjusted hazard ratio (95% CI)	*P*-value		Hazard ratio (95% CI)	*P*-value	Adjusted hazard ratio (95% CI)	*P*-value
General	7.23					5.27				
FcγRIIA polymorphism			0.02		0.001			0.83		0.78
H/R or R/R	4.93	1 (reference)		1 (reference)		5.67	1 (reference)		1 (reference)	
H/H	11.7	0.36 (0.16–0.82)		0.18 (0.07–0.48)		4.60	1.06 (0.61–1.86)		0.91 (0.50–1.69)	
FcγRIIIA polymorphism			0.12		0.09			0.96		0.94
F/V or F/F	6.87	1 (reference)		1 (reference)		6.07	1 (reference)		1 (reference)	
V/V		0.20 (0.03–1.49)		0.15 (0.02–1.31)		2.93	1.02 (0.50–2.06)		0.97 (0.45–2.09)	
FcγRIIA and *IIIA*	–		0.004		<0.001			0.62		0.94
Others	4.33	1 (reference)		1 (reference)		5.67	1 (reference)		1 (reference)	
H/H or V/V	15.93	0.29 (0.13–0.67)		0.17 (0.07–0.45)		4.60	1.15 (0.66–2.03)		1.03 (0.56–1.90)	

Notes: Set A: patients with Her-2-positive GC who received trastuzumab combined with chemotherapy as the first-line treatment. Set B: patients with Her-2–negative GC who received chemotherapy only as the first-line treatment. Multivariate analysis for the prognostic value of *FcγRIIA* and *FcγRIIIA* polymorphisms was adjusted by age (<52/≥52 years), gender (male/female), ECOG score (0/1), degree of differentiation (well-differentiated adenocarcinoma/moderately differentiated adenocarcinoma/poorly differentiated/signet ring cell carcinoma/mucinous adenocarcinoma), and Lauren classification (intestinal type/diffuse type/mixed type).

Abbreviations: ECOG, Eastern Cooperative Oncology Group; FcγR, Fc γ receptor; GC, gastric cancer; ORR, objective response ratio; PFS, progression-free survival.

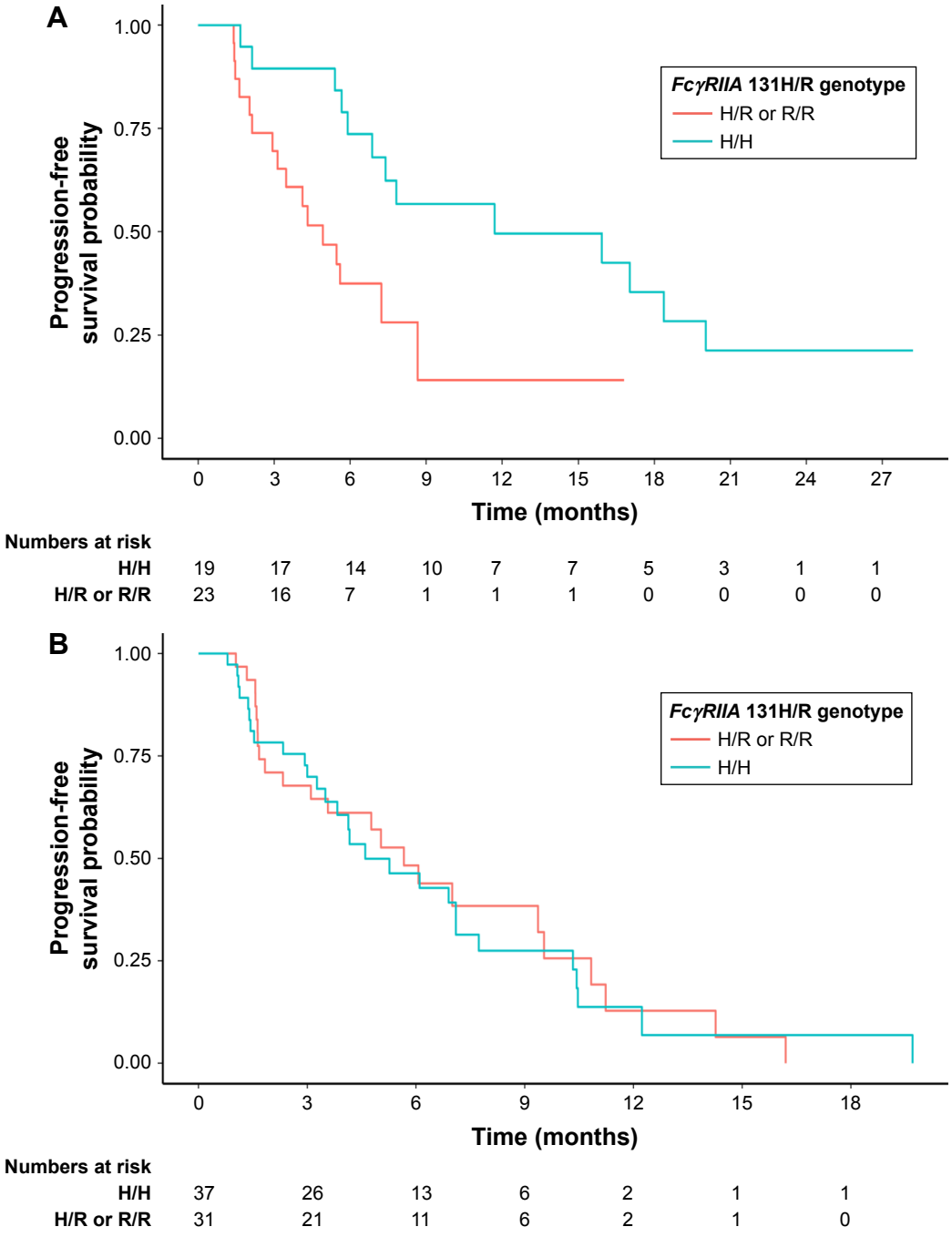

Figure 1 Progression-free survival for patients with metastatic gastric cancer receiving chemotherapy and trastuzumab (**A**, *P*=0.02) or chemotherapy only (**B**, *P*=0.83) as the first-line treatment, according to *FcγRIIA* polymorphisms (H/R or R/R vs H/H).
Abbreviation: FcγR, Fc γ receptor.

immune checkpoint blockage therapy. However, once this treatment is effective, patients might gain a durable benefit for a long time.[26]

In this study, we suggested that the *FcγRIIA* H/H genotype, but not the *FcγRIIIA* V/V genotype, predicts a benefit in PFS from trastuzumab treatment in Her-2-positive metastatic GC. This is in accordance with the findings on metastatic breast cancer. In addition, we demonstrated that the combination of *FcγRIIA* and *IIIA* polymorphisms in the *FcγRIIA* H/H or *FcγRIIIA* V/V genotype definitely results in benefits from trastuzumab treatment, with a significant improvement in both DCR and ORR. Thus, the *FcγRIIIA* polymorphism might also have a predictive role in trastuzumab treatment in metastatic GC. Actually, as shown

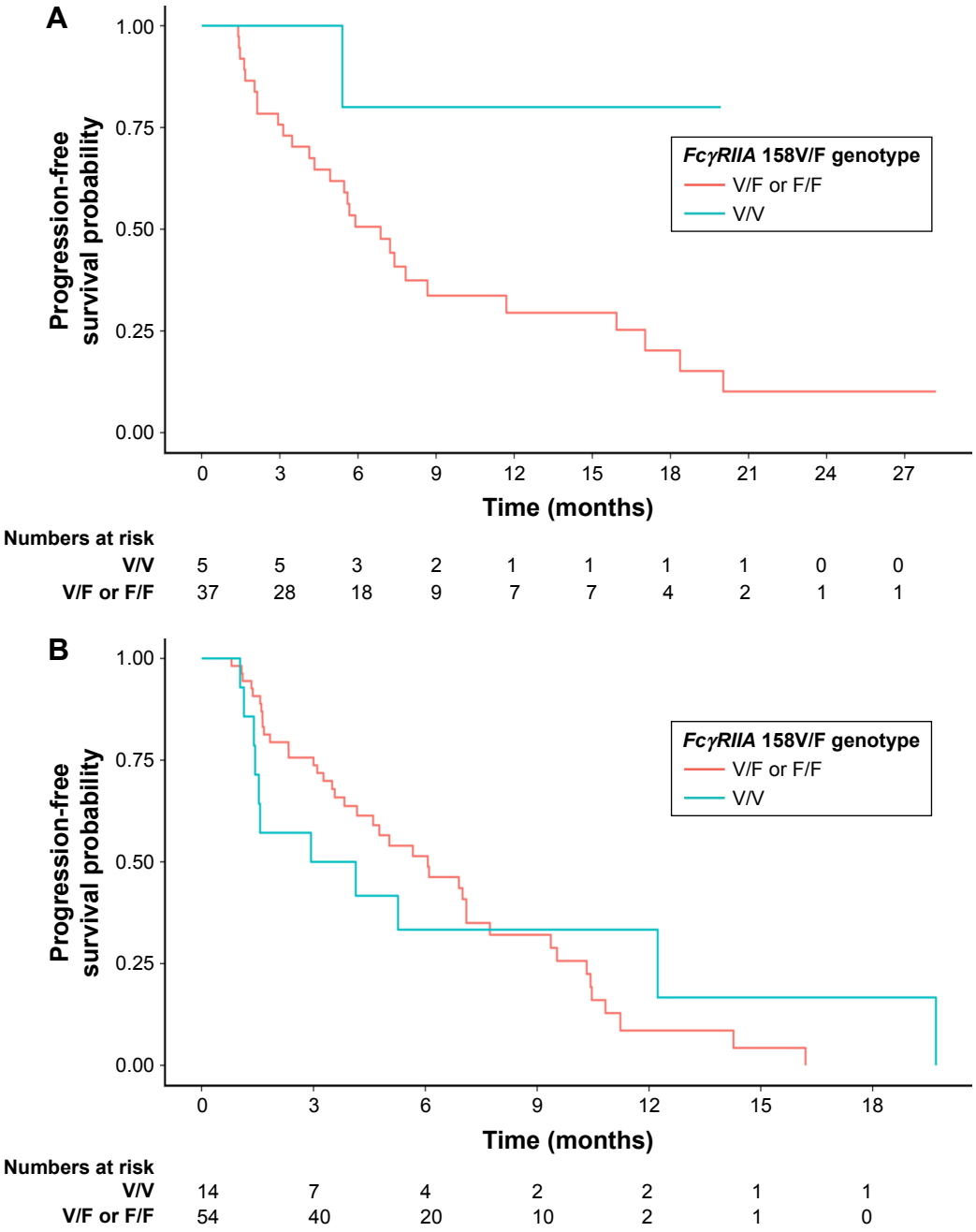

Figure 2 Progression-free survival for patients with metastatic gastric cancer receiving chemotherapy and trastuzumab (**A**, *P*=0.12) or chemotherapy only (**B**, *P*=0.96) as the first-line treatment, according to *FcγRIIIA* polymorphisms (F/V or F/F vs V/V).
Abbreviation: FcγR, Fc γ receptor.

in Table 2, FcγR *IIIA* V/V seemed to demonstrate higher ORR and DCR, but a statistically significant difference was not obtained. However, in the combined analysis, positive results were demonstrated when the sample size was enlarged. Thus, our study is limited by a small sample size, and verification by further studies is warranted.

The major limitation of our study was that it was a retrospective, single-center study. Trastuzumab was approved for Her-2-positive metastatic GC in late 2012, and the frequency of Her-2 overexpression was relatively low (~20%) in GC. In addition, trastuzumab was not covered by health insurance in most areas in China. Thus, the sample size was relatively small. Our conclusions need to be verified by larger studies. Nevertheless, to our knowledge, this study demonstrated the predictive value of *FcγRIIA* and/or *IIIA* polymorphisms for benefits from trastuzumab

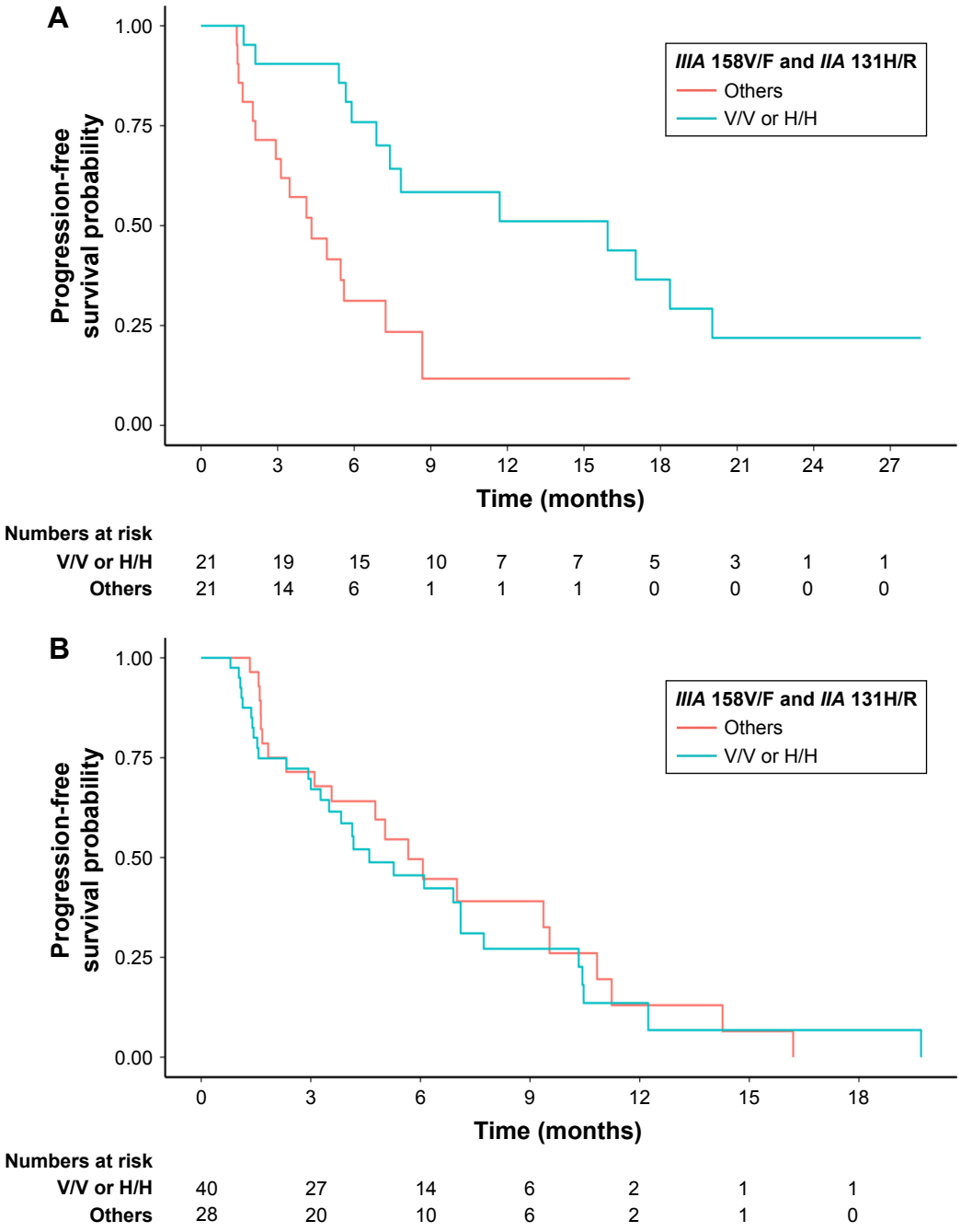

Figure 3 Progression-free survival for patients with metastatic gastric cancer receiving chemotherapy and trastuzumab (**A**, *P*=0.004) or chemotherapy only (**B**, *P*=0.62) as the first-line treatment, according to *FcγRIIA* and *IIIA* polymorphisms (others vs H/H or V/V).
Abbreviation: FcγR, Fc γ receptor.

treatment among patients with metastatic GC for the first time.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

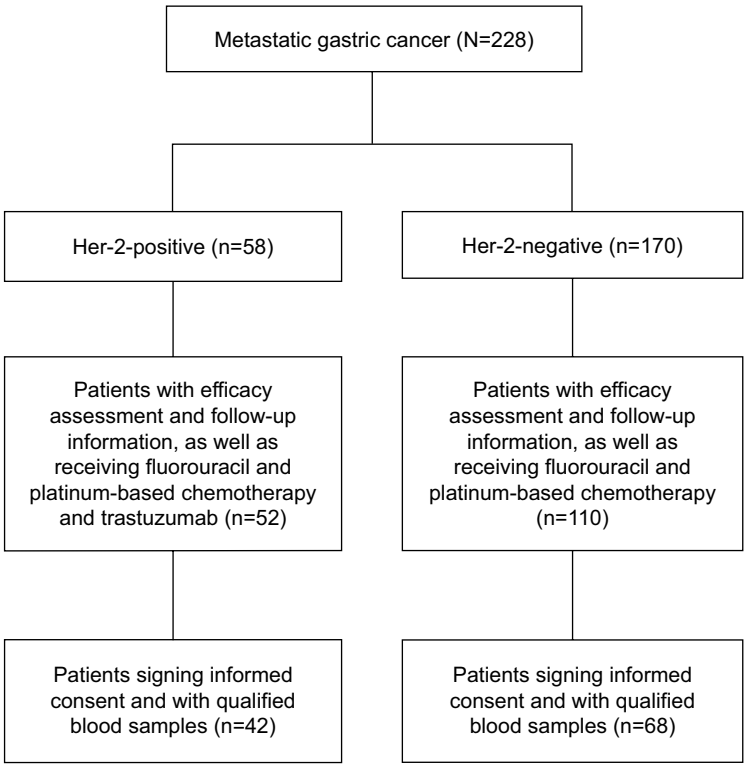

Figure S1 The CONSORT diagram.

Table S1 Patient characteristics by treatment settings

Characteristics	Set A, no (%)	Set B, no (%)
Her-2 status		
Positive	42 (100.0)	0
Negative	0	68 (100.0)
Age (years)		
Median (range)	56 (25–72)	51 (22–73)
Gender		
Male	32 (76.2)	34 (50.0)
Female	10 (23.8)	34 (50.0)
Gastrectomy		
Yes	33 (78.6)	50 (73.5)
No	9 (21.4)	18 (26.5)
Location of tumor		
Proximal	12 (28.6)	11 (16.2)
Middle	15 (35.7)	17 (25.0)
Distal	14 (33.3)	35 (51.5)
Others	1 (2.4)	5 (7.4)
Degree of differentiation		
Well-differentiated adenocarcinoma	2 (4.8)	1 (1.5)
Moderate differentiated adenocarcinoma	13 (31.0)	7 (10.3)
Poorly differentiated/signet ring cell carcinoma/mucinous adenocarcinoma	27 (64.3)	60 (88.2)
Lauren classification		
Intestinal type	14 (33.3)	2 (2.9)
Diffuse type	23 (54.8)	45 (66.2)
Mixed type	5 (11.9)	21 (30.9)

Notes: Setting A: patients with Her-2-positive GC who received trastuzumab combined with chemotherapy as the first-line treatment. Setting B: patients with Her-2-negative GC who received chemotherapy only as the first-line treatment.
Abbreviation: GC, gastric cancer.