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HER2 Pro1170Ala polymorphism is associated with decreased survival rate in HER2-negative breast cancer

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Abstract. The Pro1170Ala polymorphism is one of the most common polymorphisms of human epidermal growth factor receptor 2 (HER2) and may affect the clinical outcome in breast cancer. Therefore, in the present study, the incidence of the HER2 Pro1170Ala polymorphism was determined in 3,305 female patients with operable primary breast cancer using a DNA-sequencing assay, and the potential association with survival was investigated. Of these 3,305 patients, 29% (955/3,305) were homozygous for the Pro/Pro genotype, 51% (1,679/3,305) were heterozygous for the Pro/Ala genotype and 20% (671/3,305) were homozygous for the Ala/Ala genotype. The frequency of this polymorphism conformed to the Hardy-Weinberg equilibrium (P=0.175). No significant association between the HER2 Pro1170Ala polymorphism and recurrence-free survival (RFS) or distant recurrence-free survival (DRFS) was identified in the entire cohort of 3,305 patients. HER2 status was available for 3,170/3,305 patients; no significant association between the HER2 Pro1170Ala polymorphism and survival was identified in HER2-positive patients (n=728). However, among the HER2-negative patients (n=2,442), those with the Pro/Ala or Ala/Ala genotype had a significantly decreased RFS [unadjusted hazard ratio (HR), 1.45; 95% confidence interval (CI), 1.03-2.04; P=0.033] and DRFS (unadjusted HR, 1.65; 95% CI, 1.11-2.44; P=0.012) compared with those with the Pro/Pro genotype. Multivariate analysis revealed that the Pro/Ala or Ala/Ala genotype was an independent unfavorable factor for DRFS (adjusted HR, 1.63; 95% CI, 1.05-2.53; P=0.029) in the subgroup of HER2-negative patients. The results of the present study suggest that patients with HER2-negative breast cancer with the HER2 Pro1170Ala polymorphism variant exhibit a decreased survival outcome.

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Abbreviations: HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; ER, estrogen receptor; PR, progesterone receptor; RFS, recurrence-free survival; DRFS, distant recurrence-free survival; HR, hazard ratio; CI, confidence interval

Key words: human epidermal growth factor receptor 2 Pro1170Ala, polymorphism, breast cancer, prognosis

Introduction

The human epidermal growth factor receptor 2 (HER2) gene (also known as erbB-2 or neu) is a member of the epidermal growth factor receptor (EGFR) family (1,2). Located on chromosome 17q21, it codes for a transmembrane glycoprotein with tyrosine kinase activity (3). HER2 is markedly homologous with EGFR, which serves an important role in the regulation of cell growth, differentiation and invasion (2,3). It is well documented that HER2 amplification and/or overexpression is associated with a worse clinical outcome in breast cancer (4-9). HER2-targeted therapy, i.e., using trastuzumab, markedly improves the survival outcome of patients with HER2-positive breast cancer (10-13).

We recently reported that the HER2 Ile655Val polymorphism is significantly associated with the survival outcome of patients with HER2-positive breast cancer, indicating that patients with the Val variant exhibit breast cancer with an aggressive phenotype, but are more sensitive to trastuzumab treatment (14). In the present study, another common polymorphism (HER2 Pro1170Ala) of HER2 was investigated. This polymorphism is located in the tail region of the HER2 protein, and codes for either Pro (CCC) or Ala (GCC) at position 1170 (15). Previous studies have suggested that this polymorphism affects HER2 activity by altering tail construction and function (2,16-18). A previous study indicated that the HER2 Pro1170Ala variant is associated with an increased risk of developing lung cancer in Korean women (19). However, no association between the HER2 Pro1170Ala polymorphism and the risk of developing breast or endometrial cancer was identified in further studies (15,20-23). A recent study identified that the Pro1170Ala polymorphism is associated with a risk of trastuzumab cardiotoxicity (24). Currently, to the best of our knowledge, no investigation has been conducted into whether the HER2 Pro1170Alapolymorphism affects breast cancer survival.

Therefore, in the present study, the incidence of the HER2 Pro1170Ala polymorphism was determined in a cohort of 3,305 women with operable primary breast cancer, and the association between the HER2 Pro1170Ala polymorphism and survival was investigated; it was further determined whether the HER2 Pro1170Ala polymorphism was associated with survival in HER2-positive and HER2-negative patients, respectively.

Materials and methods

Patients. A total of 3,430 female patients with operable primary breast cancer (stages I-III) were treated at the Breast Center, Peking University Cancer Hospital (Beijing, China) between January 2005 and October 2011. Of these 3,430 patients, 125 were excluded from the present study, as theHER2 Pro1170Ala genotype was not identified due to the poor quality of the DNA samples of 77 patients and as survival data were not available for 48 patients. Consequently, 3,305 patients were included in the present study. Ages at diagnosis of the patients ranged between 21 and 90 years, with a median age of 50 years. The stage of the tumors was classified according to the tumor-node-metastasis classification of the Union Internationale Contre Le Cancer (25,26). Tumor size was defined as the maximum tumor diameter measured using ultrasound at the time of diagnosis. Tumor grade, tumor size, estrogen receptor (ER) status, progesterone receptor (PR) status and adjuvant therapy were obtained from the review of medical records, and are presented in Table I. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and approved by the Research and Ethics Committee of Peking University Cancer Hospital. The project number was 2011KT12. All patients provided written informed consent.

HER2 Pro1170Ala genotyping. Genomic DNA was isolated from peripheral blood leukocytes using phenol-chloroform. In brief, peripheral blood leukocytes were mixed with equal volumes of a phenol-chloroform mixture to remove protein contaminants, then precipitated with 100% ethanol. Amplification of DNA fragments was performed using polymerase chain reaction (PCR) using a Gene Cycler™ thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) in a 20-μl solution containing 30 ng genomic DNA, 2.5 mM $MgCl_2$, 0.8 mM dNTPs, 1.0X PCR buffer, 0.5 μM forward and reverse primers, and 1.25 units AmpliTaq DNA polymerase (Promega Corporation, Madison, WI, USA). The reaction conditions were an initial 94˚C for 5 min to activate Taq DNA polymerase, followed by 35 cycles of denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 45 sec, and a final extension at 72˚C for 10 min. The forward primer sequence was 5'-CCTGCCCTCTGAGACTGATG-3' and the reverse primer sequence was 5'-GTTCCTCTTCCAACGAGG CT-3'. The HER2 Pro1170Ala genotype was detected by direct sequencing in our laboratory. All fragments were sequenced using a BigDye Terminator Cycle Sequencing kit and ABI 3730 automated sequencer (both Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). HER2 gene (ID 2064) was used as the reference gene with primer sequences 5'-ATGGAGCTGGCGGCCTTGT-3'. In order to avoid potential contamination, each set of PCR contained a negative and 3 positive controls, a negative control without DNA template and 3 positive controls (known Pro/Pro, Pro/Ala or Ala/Ala genotype, respectively) performed simultaneously. A total of 30% of the cases were genotyped in duplicate and results were fully concordant.

Assessment of HER2 status. HER2 status was obtained from a review of pathology reports. The HER2 status was determined using immunostaining according to a standard method (27): A score of 0 and 1+ was considered negative and score of 3+ was considered positive; a score of 2+ was further evaluated using fluorescence *in situ* hybridization using a Vysis CLL FISH Probe kit (Abbott Laboratories, Abbott Park, IL, USA), according to the manufacturer's protocol.

Statistical analysis. Statistical analysis was performed using SPSS software for Windows (version 20.0; IBM SPSS, Armonk, NY, USA). The associations between the HER2 Pro1170Ala genotype variants and clinicopathological characteristics in the entire cohort were evaluated using Pearson's χ^2 test. Survival curves were derived from Kaplan-Meier estimator analysis and the differences between the curves were compared using log-rank tests. Recurrence-free survival (RFS) was defined as the time between the date of pathological diagnosis and the date of locoregional recurrence or metastasis, distant metastasis or mortality from breast cancer. Distant recurrence-free survival (DRFS) was defined as the time between diagnosis and the occurrence of distant metastasis or mortality, for which breast cancer was the primary or underlying cause. Multivariate survival analysis was performed to identify independent prognostic variables in the patients with HER2-negative breast cancer using tumor grade, tumor size, lymph node status, ER status, PR status, whether adjuvant therapy was used or not and the HER2 Pro1170Ala genotype as covariates. All statistical tests were two-sided, and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. The clinicopathological characteristics of the 3,305 patients examined are presented in Table I. The incidence of the HER2 Pro1170Ala genotype was determined in these 3,305 patients: 29% (955/3,305) were homozygous for the Pro/Pro genotype, 51% (1,679/3,305) were heterozygous for the Pro/Ala genotype and 20% (671/3,305) were homozygous for the Ala/Ala genotype. The frequency of the variants conformed to the Hardy-Weinberg equilibrium (P=0.175).

No significant association between the Pro1170Ala polymorphism and age at diagnosis, tumor size, tumor grade, lymph node status, ER status, PR status, HER2 status, adjuvant therapy or trastuzumab treatment was identified in this cohort of 3,305 patients (Table I).

Survival outcome is not associated with the HER2 Pro1170Ala genotype for the entire cohort. Follow-up data were available for all 3,305 patients; the median follow-up period was 53 months (range, 2-110 months). The estimated 5-year RFS and DRFS in the 3,305 patients were 90.9% [95% confidence interval (CI), 89.7-92.1) and 92.4% (95% CI, 91.4-93.4), respectively. No significant difference in survival was identified between the HER2 Pro1170Ala genotypes in

Table I. Association between the HER2 Pro1170Ala genotype and clinicopathological characteristics.

Characteristic	Overall	Pro/Pro, n (%)	Pro/Ala, n (%)	Ala/Ala, n (%)	P-value
Total	3,305	955 (29)	1,679 (51)	671 (20)	0.175[a]
Age at diagnosis, years					0.771[b]
≤40	593	174 (18)	305 (18)	114 (17)	
>40	2,712	781 (82)	1,374 (82)	557 (83)	
Tumor size, cm					0.547[b]
≥2	1,733	499 (55)	872 (54)	362 (57)	
<2	1,412	403 (45)	734 (46)	275 (43)	
Unknown	160				
Tumor grade					0.312[b]
I	414	130 (16)	199 (14)	85 (15)	
II	2,045	583 (72)	1,033 (72)	429 (73)	
III	381	99 (12)	210 (14)	72 (12)	
Unknown	465				
Lymph nodes					0.239[b]
Positive	1,154	323 (35)	580 (36)	251 (39)	
Negative	2,030	605 (65)	1,031 (64)	394 (61)	
Unknown	121				
ER status					0.699[b]
Positive	2,290	662 (72)	1,154 (70)	474 (72)	
Negative	934	261 (28)	486 (30)	187 (28)	
Unknown	81				
PR status					0.636[b]
Positive	2,040	594 (64)	1,027 (62)	419 (63)	
Negative	1185	328 (36)	615 (38)	242 (37)	
Unknown	80				
HER2 status					0.941[b]
Positive	728	205 (23)	370 (23)	153 (23)	
Negative	2,442	696 (77)	1247 (77)	499 (77)	
Unknown	135				
Trastuzumab use					0.374[b]
Yes	146	44 (5)	79 (5)	23 (3)	
No	3,159	911 (95)	1,600 (95)	648 (97)	
Adjuvant therapy					0.495[b]
C	1,133	313 (33)	593 (35)	227 (34)	
E	623	189 (20)	316 (19)	118 (18)	
C+E	1,248	357 (37)	620 (37)	271 (40)	
None	301	96 (10)	150 (9)	55 (8)	

P-value, [a]Hardy-Weinberg equilibrium; [b]Pro/Pro vs. Pro/Ala vs. Ala/Ala. HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; C, chemotherapy; E, endocrine therapy.

the entire cohort of 3,305 patients; patients with the Pro/Ala or Ala/Ala genotype exhibited a similar survival outcome to those with the Pro/Pro genotype [RFS: unadjusted hazard ratio (HR), 1.26; 95% CI, 0.96-1.65; P=0.102; Fig. 1A; DRFS: unadjusted HR, 1.31; 95% CI, 0.97-1.79; P=0.082; Fig. 1B].

Survival outcome is associated with the HER2 Pro1170Ala genotype for HER2-negative breast cancer, but not HER2-positive breast cancer. HER2 status was available for 3,170/3,305 patients. Of these, 728 (23%) were diagnosed withHER2-positive breast cancer and 2,442 (77%) were diagnosed with HER2-negative breast cancer. The association between the HER2 Pro1170Ala polymorphism and survival was analyzed in patients with HER2-positive and HER2-negative tumors, respectively. Among the patients with HER2-positive breast cancer, no significant association was identified between the HER2 Pro1170Ala genotype and survival (RFS: Pro/Ala or Ala/Ala genotype vs. Pro/Pro

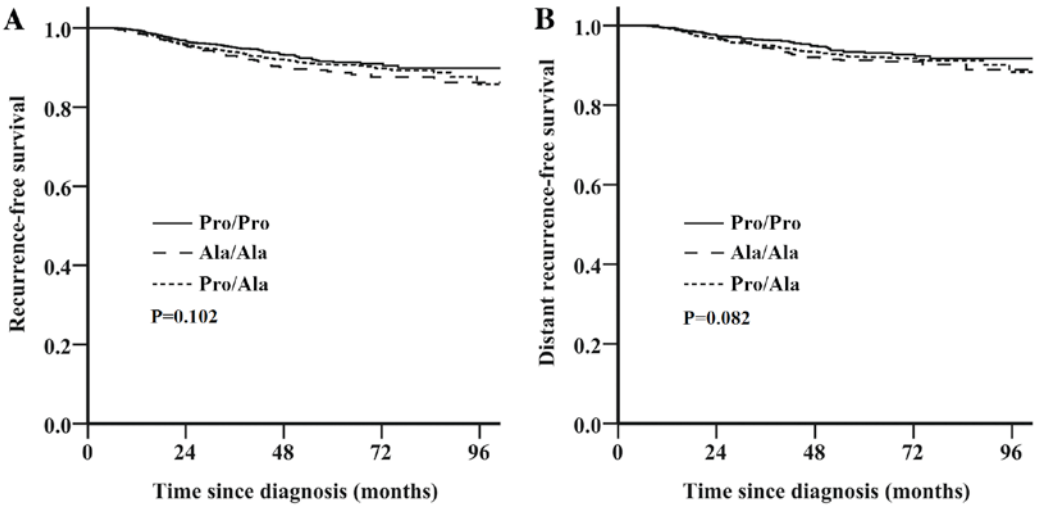


Figure 1. Kaplan-Meier estimator survival curves of (A) recurrence-free survival (P=0.102) and (B) distant recurrence-free survival (P=0.082) in the entire study population of 3,305 patients with breast cancer, according to the human epidermal growth factor receptor 2 Pro1170Ala genotype.

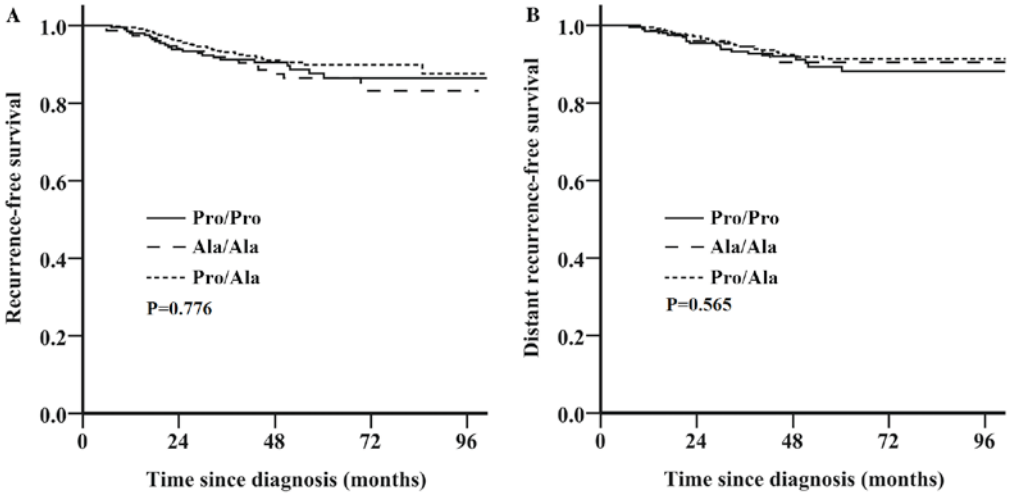


Figure 2. Kaplan-Meier estimator survival curves of (A) recurrence-free survival (P=0.776) and (B) distant recurrence-free survival (P=0.565) in the 728 patients with HER2-positive breast cancer, according to human epidermal growth factor receptor 2 Pro1170Ala genotype.

genotype; unadjusted HR, 0.93; 95% CI, 0.56-1.54; P=0.776; Fig. 2A; DRFS: Unadjusted HR, 0.85; 95% CI, 0.49-1.48; P=0.565; Fig. 2B). By contrast, among patients with HER2-negative tumors, those with the Pro/Ala or Ala/Ala genotype exhibited decreased RFS (unadjusted HR, 1.45; 95% CI, 1.03-2.04; P=0.033; Fig. 3A) and DRFS (unadjusted HR, 1.65; 95% CI, 1.11-2.44; P=0.012; Fig. 3B) compared with those with the Pro/Pro genotype. Furthermore, multivariate analysis revealed that the Pro/Ala or Ala/Ala genotype was a near significant unfavorable factor for RFS (adjusted HR, 1.46; 95% CI, 1.00-2.15; P=0.053; Table II) and a significantly unfavorable factor for DRFS (adjusted HR, 1.63; 95% CI, 1.05-2.53; P=0.029; Table II) after adjustment for age, tumor grade, tumor size, lymph node status, ER status, PR status and adjuvant therapy.

Discussion

To the best of our knowledge, the present study is the first to investigate the association between the HER2 Pro1170Ala polymorphism and survival outcome in a large cohort of patients with breast cancer. Although the HER2 Pro1170Ala genotype was not identified to be associated with survival outcome in the entire cohort of 3,305 patients with breast cancer or in the 728 patients with HER2-positive breast cancer, this polymorphism was identified to be significantly associated with survival outcome in the 2,442 patients with HER2-negative breast cancer. The Pro/Ala or Ala/Ala genotype was associated with a decreased RFS and DRFS compared with the Pro/Pro genotype in the HER2-negative-subgroup, and the Pro/Ala or Ala/Ala genotype was identified as an independent unfavorable factor for DRFS, indicating that the Ala variant led to a more aggressive phenotype compared with the Pro variant among HER2-negative patients.

HER2 protein consists of four domains: The extracellular region, the transmembrane domain, the tyrosine kinase domain and the C-terminal tail (28). The Pro1170Ala polymorphism is located in the tail coding region of HER2. The C-terminal tail serves a critical role in the regulation of the enzyme activity of the kinase (2,16,17,29,30). As a non-synonymous coding

Table II. Multivariate analysis of RFS and DRFS in the 2,442 patients with HER2-negative breast cancer.

	RFS		DRFS	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
Pro1170Ala genotype				
Pro/Ala or Ala/Ala vs. Pro/Pro	1.46 (1.00-2.15)	0.053	1.63 (1.05-2.53)	0.029
Age, years				
≤40 vs. >40	1.40 (0.96-2.04)	0.081	1.42 (0.94-2.14)	0.101
Tumor size, cm				
≥2 vs. <2	2.71 (1.84-4.00)	<0.001	2.58 (1.69-3.94)	<0.001
Tumor grade				
III vs. I/II	1.17 (0.71-1.93)	0.548	1.13 (0.66-1.96)	0.653
Lymph nodes				
Positive vs. negative	3.57 (2.52-5.06)	<0.001	4.29 (2.89-6.36)	<0.001
ER status				
Negative vs. positive	1.64 (1.04-2.57)	0.033	1.66 (1.00-2.72)	0.047
PR status				
Negative vs. positive	1.89 (1.22-2.90)	0.004	1.82 (1.13-2.94)	0.014
Adjuvant therapy				
Therapy vs. none	1.63 (0.78-3.37)	0.191	2.10 (1.00-4.39)	0.048

RFS, recurrence-free survival; DRFS, distant recurrence-free survival; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor.

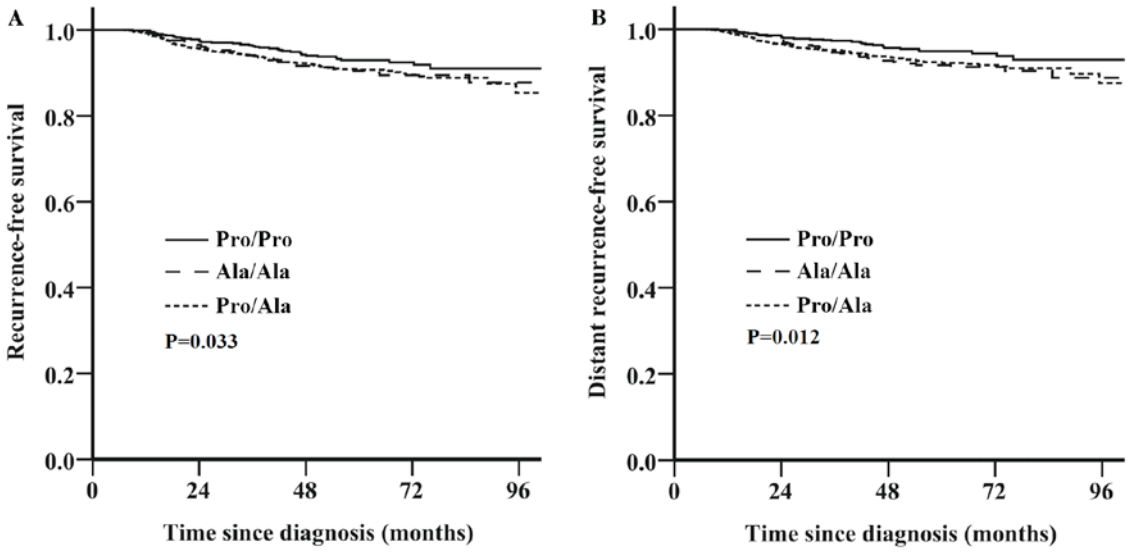


Figure 3. Kaplan-Meier estimator survival curves of (A) recurrence-free survival (P=0.033) and (B) distant recurrence-free survival (P=0.012) in the 2,442 patients with HER2-negative breast cancer, according to human epidermal growth factor 2 Pro1170Ala genotype.

variant, the Ala variant of the HER2 Pro1170Ala genotype may alter the spatial conformation of the tail region and may affect tyrosine kinase activity (18,31).

A previous study suggested that the Ala variant increased the risk of lung cancer (19), indicating that the Ala variant may promote HER2 activity. In the present study, an association between the HER2 Pro1170Ala polymorphism and survival was identified in patients with HER2-negative breast cancer, but not in patients with HER2-positive breast cancer. The underlying molecular mechanism for this difference remains unclear; however, one possibility is that, although the HER2 Pro1170Ala polymorphism may alter the HER2 activity, it is not sufficient to influence HER2 activity when the HER2 gene is amplified or overexpressed. Although patients with

HER2-negative breast cancer have a more favorable survival outcome compared with that of patients with HER2-positive breast cancer, a minority of patients with HER2-negative breast cancer may exhibit metastases after treatment (5-7). Therefore, genotyping of the HER2 Pro1170Ala polymorphism may be useful for identifying the relatively high-risk patients among all patients with HER2-negative breast cancer. In conclusion, the results of the present study demonstrated that, among the patients with HER2-negative breast cancer, the HER2 Pro1170Ala polymorphism is significantly associated with survival, with the Ala variant exhibiting an aggressive phenotype and decreased survival outcome. Further independent studies are required to confirm these findings.

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