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Research Paper

Evaluation of GWAS-Identified Genetic Variants for Gastric Cancer Survival

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ABSTRACT

Backgrounds: Genome-wide association studies (GWASs) have identified several gastric cancer (GC) susceptibility loci in Asians, but their effects on disease outcome are still unknown. This study aimed to investigate whether these GWAS-identified genetic variants could serve as robust prognostic biomarkers for GC.
Methods: A multistage clinical cohort, including a total of 2432 GC patients in the Chinese population, was used to identify the association between GWAS-identified risk variants and overall survival of GC. Hazard ratios (HRs) and 95% confidence intervals (CIs) were computed by Cox regression analysis, and the log-rank *P* was calculated by the log-rank test with the Kaplan-Meier method.
Results: We found that rs2274223 A>G in *PLCE1* was associated with increased GC survival in both training set ($P = .011$), which was independently replicated in validation set 1 ($P = .045$), but not in validation set 2. The area under the curve (AUC) from receiver-operator characteristic (ROC) curve showed this clinical relevance with onset age-dependence, especially in the subgroup of early-onset cases. Moreover, a significant improvement in overall survival prediction was identified when the rs2274223 genetic effect was included in the estimation; this result was also supported by the prognostic nomogram. In addition, patients with lower expression of *PLCE1* showed benefits *via* longer survival, potentially due to the functional effect of rs2274223.
Interpretation: This preliminary study suggests that a GWAS-identified genetic variant in *PLCE1* may serve as a potential biomarker for GC survival. Additional replication with larger samples size is warranted to further investigation.

1. Introduction

Gastric cancer is one of the most common malignancies dangerous to human health, being fifth in morbidity and third in mortality among cancers worldwide [1]. Notably, the Asian region has the highest rate of gastric cancer incidence, which is partly attributed to its differences in diverse hereditary backgrounds, behavioral factors and *Helicobacter pylori* infection [2, 3]. Although the diagnosis and therapy of gastric cancer have been greatly improved in recent years, the 5-year survival rate remains poor at approximately 30% [4]. To date, clinical staging has been widely applied to determine tumor aggression and prognosis, but a wide heterogeneity of prognosis still exists, mainly due to deficiencies in the staging system. Therefore, a number of studies

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have been devoted to discovering new biomarkers to combine with traditional tumor diagnosis, staging and prognosis and thus to improve early diagnosis and prognostic prediction [5].

In recent years, emerging evidence has demonstrated the significant genetic effects of single nucleotide polymorphisms (SNPs) on gastric cancer development and progression [6, 7]. Genome-wide association studies (GWASs) are now well known as a powerful approach to explore complex disease-risk-related variants. Recently, five significant gastric-cancer-related GWASs from Asian populations have identified a moderate number of independent loci and SNPs with genome-wide statistical significance, including rs2294008 in *PSCA* at 8q24 [8], rs2274223 in *PLCE1* at 10q23 [9], rs4072037 in *MUC1* at 1q21 [9], rs98401504 in *ZBTB20* at 3q13 [10] and rs13361707 in *PTGER4* at 5q13 [10]. These genetic variants have been further studied in diverse ethnic backgrounds, and some have been identified as high-quality biomarkers for screening gastric cancer susceptibility [11].

However, few studies have focused on the effects of genetic factors on gastric cancer clinical outcomes. Considering the difference between gastric cancer etiology and its developmental mechanism, we hypothesized that these GWAS-identified susceptibility SNPs were associated with survival time in gastric cancer patients. In this study, we evaluated the association between the risk variants for gastric cancer found in previous GWASs and patients' survival based on large, multistage clinical cohorts in Chinese populations, and we assessed the potential of these variants as prognostic biomarkers for gastric cancer.

2. Methods

2.1. Study Population

A two-stage follow-up study was designed to investigate the effect of gastric cancer risk SNPs on patients' survival. In the first stage, we enrolled patients from Yixing People's Hospital, Yixing city, as a training set, for which detailed population information has been described in our previous publication [7]. In the second stage, patients from Nantong city and Nanjing city were considered as validation sets 1 and 2, respectively. For validation set 1, a total of 480 patients were recruited from Nantong Tumor Hospital from December 2000 to July 2006, and 471 of them were successfully followed up, with 113.0 months for the maximum follow-up time and 41.1 for the median. For validation set 2, a total of 1021 patients with adequate follow-up information were enrolled from January 2005 to December 2009, with 87.8 months maximum follow-up time and 34.0 months median. In each cohort, the clinical pathological variables, including tumor size, tumor site (cardia or noncardia), histological type, invasion, lymph node, distant metastasis, and TNM stage (American Joint Commission for Cancer Staging, 6th ed., 2002), were collected from the medical records of the patients. All subjects signed an informed consent, and our study was approved by the Institutional Review Board of Nanjing Medical University, Nanjing, China.

2.2. SNP Genotyping

Genomic DNA was extracted from paraffin sections of tumor tissues according to the detailed method reported previously [7]. A TaqMan PCR Genotyping Assay using the ABI 7900HT Real Time PCR System (Applied Biosystems, Foster City, CA) was utilized to perform candidate SNP genotyping. For quality control, all genotype analyses were performed by two blinded individuals who did not know the subjects' status. Approximately 10% of all samples were selected randomly for genotype confirmation, and the results were 100% concordant.

2.3. Statistical Analysis

The overall survival time was the primary outcome in this study, and it was calculated from the day of gastric cancer diagnosis until death or the last follow-up. Median survival time (MST) was used to compare the life span associated with each variable; if the median was not available, the mean survival time was used as an alternative. A multivariate Cox regression analysis was utilized to evaluate the adjusted hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) with adjustments for age, sex, tumor size, histological type and TNM stage. A Cox stepwise regression analysis was performed to determine what factors could be used as an independent factor for gastric cancer prognosis, with $P < .05$ for entering and $P > .10$ for removing the model. The genetic effects of each SNP were estimated using additive, dominant, recessive and codominant models. The association between survival time and each included variable was measured using the Kaplan-Meier method and the log-rank test. Subsequently, a survival model including genetic effect was built to assess the prognostic efficacy by using a time-dependent receiver-operator characteristic (ROC) curve analysis and calculating the area under the curve (AUC) of the ROC curve. In addition, a nomogram was formulated based on the results of the Cox stepwise regression analysis, and its performance was evaluated by the concordance index (C-index) and assessed by comparing nomogram predictions to Kaplan-Meier estimates of survival probability; bootstrap analyses with 1000 resamples were applied to these activities. All tests within two-sided were performed using the SAS software (version 9.2, SAS Institute, Cary, NC) and R version 3.1.3.

3. Results

3.1. Patient Characteristics

The demographic and clinical pathological characteristics of each cohort are shown in Supplementary Table 1. Briefly, a total of 2432 gastric cancer patients from three independent cohorts were enrolled for survival analysis, and in each group, patients with larger tumor size, diffuse type and late TNM stages (including invasion depth, lymph node and distant metastasis) had shorter survival times than other patients did (all log-rank $P < .001$).

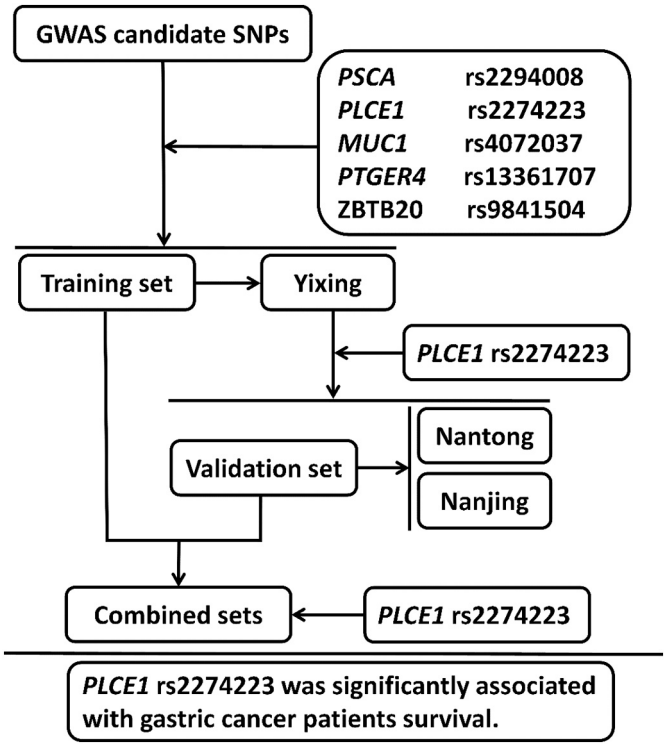

Fig. 1. The flow chart for association analysis of the GWAS-identified SNPs and gastric cancer survival.