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Correlation study on chromogranin A genetic polymorphism and prognosis of critically ill patients☆,☆☆

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ABSTRACT

Objective: The objective was to investigate the correlation between single nucleotide polymorphism (SNP) of chromogranin A (CHGA) and prognosis of critically ill patients.
Methods: We screened 357 critically ill patients consecutively admitted to our intensive care unit. The −89/−415/−462 SNP locus in the promoter region and the +9559/+9578/+9590/+9611 SNP locus in exon 7 coding of CHGA were genotyped by polymerase chain reaction and DNA sequencing technology. Subsequently, the correlation between genotype and prognosis of patients was analyzed.
Results: (1) Three hundred critically ill Chinese Han patients were enrolled in the study. CHGA−415/−462/+9559/+9611 SNPs were polymorphically distributed. Phenotypes of the 4 SNPs were shown not to be in linkage disequilibrium, and there were no significant differences in the minor allele frequencies (MAFs) of the 4 SNPs between participants of this study and healthy people in Asia. (2) The CHGA−415 T/C MAF of the nonsurvival group was significantly higher than that of the survival group (MAF 0.3813 and 0.2864, respectively; $P = .026$). Survival analysis showed that there were significant differences between the CHGA−415 T/C mutation group (including TC and CC genotypes) and the wild-type group (TT genotype) (log rank = 8.887, $P = .003$). The mortality in the mutant group was significantly higher than that in the wild-type group (0.3333 and 0.1852, respectively; $P = .004$). (3) Binary logistic analysis showed that CHGA−415 T/C polymorphism was an independent risk factor for the mortality of critically ill patients (odds ratio, 2.286; 95% confidence interval, 1.165-4.484; $P = .016$).
Conclusions: Critically ill patients with CHGA−415 T/C mutant genotype display higher 30-day mortality than those with the wild-type group. CHGA−415 T/C polymorphism is an independent risk factor of poor prognosis in critically ill Chinese Han patients.

Critically ill patients with different etiologies share a common pathophysiological process of systemic inflammatory response syndrome, which may induce multiple organ damage and death. Systemic inflammatory response syndrome causes a stress response characterized by activation of the sympathetic adrenergic system, which adjusts the functions of body systems under severe diseases. This stress response is an important compensatory reaction during the pathological process of critical illness and is closely related to the prognosis of critically ill patients.

Chromogranin A (CHGA) is the major prohormone stored and secreted with catecholamine by stimulated chromaffin cells in the adrenal medulla. It is a precursor of biologically active peptides and can be derived to a series of polypeptide fragments with multiple vital physiological functions such as regulating the secretion of catecholamine, improving the function of vascular endothelial cells, antibiosis, immune chemotaxis, and regulation of cardiac function [1]. Our previous studies show a significant increase of serum CHGA levels in critically ill patients on admission, and CHGA is a good biomarker of prognosis in the early

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evaluation of critically ill patients [2,3]. The FINNSEPSIS study, a large multicenter study in Europe, obtained similar results [4]. These findings suggest that the stress protein CHGA may participate in core mechanisms of the compensatory response to the pathological process in critically ill patients' stress reaction.

Increasingly, studies focus on the possible link between genetic polymorphisms and the prognosis and susceptibility of critical illness. Recent studies have shown that [5,6] CHGA genetic polymorphisms may affect the activities of the sympathetic nervous system, leading to hypertension and hypertension-related nephropathy. Whether the prognosis of critically ill patients is related to CHGA genetic polymorphism has never been explored.

In this cohort study, we chose SNP loci with relatively high minor allele frequencies (MAFs) or that contribute functional variation at CHGA, including 2 SNP loci of the promoter region, −462 T/C (rs9658634) and −415 A/G (rs9658635), and 4 SNP loci of the exon 7 coding region of CHGA, +9559 A/G (rs9658667), +9578 C/T (rs9658668), +9590 A/G (rs9658669), and +9611 T/C (rs729940). Then we investigated the relationship between CHGA genetic polymorphism and the prognosis of critically ill patients.

1. Methods

1.1. Patients

This study is a prospective and observational cohort study, and the sample size was estimated by Quanto software (the MAF of CHGA−415 T/C SNP on healthy people in Asia is 0.384, estimated mortality: 15%-25%, power = 0.8, $P = .05$; then the estimated sample: 360-530). From July 2012 to March 2013, 357 patients consecutively admitted to the intensive care unit (ICU) of the First Affiliated Hospital of Chongqing Medical University were included to the study.

According to the exclusion criteria, we excluded 57 patients: 24 cases who stayed in the ICU less than 24 hours, 5 cases who were non–Chinese Han nationality, 2 cases with incomplete medical records, 2 cases who had been admitted to ICU repeatedly, 17 cases of neuroendocrine tumor and advanced stage of tumor, 2 cases after cardio-pulmonary resuscitation, and 5 cases of failure in DNA extraction and DNA amplification. Finally, 300 patients (167 male and 133 female) fulfilled the criteria of this study, and the most frequent causes of critical illness were acute pancreatitis, pneumonia, and poisoning.

This study was approved by hospital ethical committees, and informed consents were signed by the patients or their family members.

1.2. DNA extraction and genotyping of CHGA genetic polymorphisms

Five milliliters of venous peripheral blood was collected from patients within 24 hours after admission to the ICU. Total DNA extraction kit of whole blood (Tiangen Biotech Co, Ltd, Beijing, China; blood genome extraction kit, item no. DP319) was used to extract total genomic DNA. Genotype detection is a blind study determined through polymerase chain reaction (PCR) amplification of target fragments and gene sequencing. Primers and conditions of PCR are shown in Table 1. Shanghai Majorbio Bio-pharm Technology Co, Ltd, of China performed the genomic fragment sequencing of PCR amplification and genotype identification of the CHGA SNP loci (Table 1).

1.3. The characteristics of patients

Clinical data, including demographic information, infection, presence of acute respiratory distress syndrome (ARDS) and shock, mechanical ventilation, oxygenation index (Pao_2/Fio_2), and routine laboratory test results, were recorded. The Acute Physiology and Chronic Health Evaluation (APACHE) II Score, the Simplified Acute Physiology Score (SAPS) II, and the Sequential Organ Failure Assessment (SOFA) score were obtained for all the patients at 24 hours after ICU admission. The primary end point of outcome was mortality in 30 days.

1.4. Statistical analysis

Categorical variables are expressed as frequencies and percentages, with χ^2 tests conducted for comparison among groups. Normal distribution variables are expressed as a median ± SD, with t tests conducted for comparison among groups. Nonnormal distribution variables are expressed as median and interquartile range, with Wilcoxon-Mann-Whitney tests conducted for comparison among groups.

Genotype frequencies and MAF statistics of both the survivor and nonsurvivor groups were recorded. Linkage disequilibrium analysis was conducted through analysis software provided by Cell Res [7], with $D' > 0.8$, $r^2 > 1/3$ indicating linkage disequilibrium.

In our study, participants were divided into a wild-type group and a mutation group according to whether there was a mutation of the significant loci. We compared clinical characteristics between the groups. Kaplan-Meier survival analysis was used for comparing the 30-day survival conditions of patients in both groups. Binary logistic regression analysis was used to evaluate the influence of genotype and other factors on the patient outcome. For the results above, differences were considered significant when $P < .05$. Statistical analyses were performed using the SPSS (Chicago, IL) 12.0 package. The sample size was estimated by Quanto software.

2. Results

2.1. Patient demographics

Three hundred critically ill Chinese Han patients were included in this study. The mortality rate at 30 days was 26.67% (220 patients in the survivor group vs 80 patients in the nonsurvivor group). The incidence of septic shock in this study in which the diagnosis criteria were based on the sepsis definition of The Surviving Sepsis Campaign in 2002 was 11.67% (35 patients). The main diagnoses of the recruited patients included 52 cases of severe acute pancreatitis (SAP), 47 cases of severe pneumonia and acute respiratory failure, 41 cases of gastrointestinal disease, 35 cases of multiple trauma, 35 cases of poisoning, 34 cases of craniocerebral disease, 18 cases of urinary infection or acute renal insufficiency, 12 cases of diabetic ketoacidosis and hyperosmotic state, 12 cases of obstetric diseases, and 14 cases of other diseases (Table 2, Supplementary Table 1).

Table 1
SNPs of the CHGA locus and the primer for the PCR experiment in this study

Domain	Ref SNP no. (dbSNP)	SNP identity	Amino acid variation	Primer	Size of PCR products	Amplification conditions
Promoter	rs9658635	T-415C	–	5′-AGGGAAGGGAGAACAG-3′	353 bp	94°C 30 s, 59°C 60 s, 72°C 45 s; 35 repeats
	rs9658634	A-462G	–	3′-CGTTGTCCAGAAGGCAGTAGG-5′		
Exon 7 coding	rs9658667	A + 9559G	Gly364Ser	5′-AGGGAAGGGAGAACAGGAAGC-3′	690 bp	
	rs9658668	C + 9578 T	Pro370Leu	3′-CCTACTGCCTTCTGGACAACG-5′		
	rs9658669	A + 9590G	Arg374Gln			
	rs729940	T + 9611 C	Trp381Arg			