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SH3D21 rs34416442 genetic variation is associated with pancreatic cancer patients' overall survival and their response to gemcitabine

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ABSTRACT

Pancreatic cancer remains one of the most lethal malignancies worldwide due to late diagnosis and limited therapeutic response. Gemcitabine is widely used as a first-line chemotherapeutic agent, and *SH3D21* has been identified as a gemcitabine sensitizer in pancreatic cancer cells. Here, we investigated whether rs34416442 polymorphism of *SH3D21* is associated with overall survival in a cohort of Iranian patients with pancreatic cancer. Peripheral blood samples were collected from 26 patients, and genotyping was performed. Patients were followed and survival outcomes were analyzed using Kaplan–Meier curves and the log-rank test. Three genotypes—TTT/TTT, TTT/T, and T/T—were identified with frequencies of 38%, 54%, and 8%, respectively. Significant differences in survival were observed among genotypes (p = 0.004). Patients carrying the *T/T* genotype exhibited markedly shorter mean overall survival (135 days) compared with those harboring at least one TTT allele (395 and 450 days). A recessive model further confirmed the reduced survival associated with the homozygous T/T genotype (p = 0.001). Stratified analyses showed that this association persisted in both gemcitabine-received (p = 0.006) and not-received (p = 0.016) subgroups. While different genotype-based survival patterns were observed among patients of the two groups. The TTT/T genotype showed the best survival in patients receiving gemcitabine, while TTT/TTT genotype did so in patients not receiving gemcitabine. These findings suggest *SH3D21* rs34416442 as a potential predictive biomarker for pancreatic cancer.

Keywords: Pancreatic cancer; *SH3D21*; rs34416442; Gemcitabine

INTRODUCTION

According to the latest data from Globocan, published under the supervision of the International Agency for Research on Cancer (IARC), pancreatic cancer was the twelfth most common cancer among men and women in 2022 [1]. Based on the same source, pancreatic cancer ranks sixth in terms of mortality rate, implying its high mortality rate [1]. In fact, pancreatic ductal adenocarcinoma (PDAC) has now become one of the deadliest types of cancer, having overtaken breast cancer as the third leading cause of cancer-related death in the

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United States [2]. Projections indicate that by 2040, it will surpass colorectal cancer to become the second leading cause of cancer death, following only lung cancer [2]. The high mortality rate is mainly because diagnosis typically happens at stage 4 [2], by which time the cancer has spread to other organs such as the liver, lungs, or peritoneum and treatment is often aimed at alleviating symptoms and prolonging life [3].

Gemcitabine has long been considered the first-line chemotherapeutic agent for pancreatic cancer treatment and *SH3D21* gene has appeared as a gemcitabine sensitizer in pancreatic cancer cells in a genome-wide CRISPR/Cas9 screening [4]. *SH3D21* gene, also known as *C1orf113*, is a human gene located on chromosome 1 that encodes a protein consisting of 756 amino acids. This protein contains three SH3 domains, which play a crucial role in protein-protein interactions [5]. A study has shown that impairing *SH3D21* expression by either CRISPR/Cas9 or siRNA sensitizes pancreatic cancer cells to gemcitabine [4]. Another study revealed that *SH3D21* is highly expressed in hepatocellular carcinoma tumors. This gene contributes significantly to the growth and invasion of liver cancer cells via activating the PI3K/AKT/mTOR signaling pathway [6].

As *SH3D21* is a gemcitabine sensitizer we hypothesized that its genetic variations might lead to the differences in the survival of pancreatic cancer patients. The rs34416442 genetic variation within the *SH3D21* gene was selected to test the hypothesis due to its relatively high minor allele frequency (MAF = 0.26). In this study, we investigated whether rs34416442 polymorphism of *SH3D21* associates with overall survival among Iranian patients with pancreatic cancer.

MATERIALS AND METHODS

Blood Sampling and Patient Data: Peripheral blood samples were collected from 26 patients diagnosed with pancreatic cancer at medical centers in Zanjan city, Zanjan province, Iran. Prior to sampling, written informed consent was obtained. There was no criteria for excluding the patients form the study. All the patients with confirmed diagnosis of pancreatic cancer who were willing to participate in the study were included. The disease of all the patients were in metastatic stage at the time of the diagnosis. Each patient was assigned a coded medical file containing demographic data, clinical history, cancer subtype, treatment modalities (chemotherapy, radiotherapy, surgery), and outcomes. Follow-up was conducted every three months. In cases where patients were unavailable, data were collected from close relatives or caregivers. Data were anonymized for statistical analysis. The study was approved by the Ethics Committee of Zanjan University of Medical Sciences.

PCR Amplification and Sequencing: Polymerase chain reaction (PCR) was carried out in a total volume of 20 µL using the following primers forward primer: TGTGAAGTGCCCAGT TGCT, reverse primer: GTCTCCCCTGTTCAAACGCT. Phusion high-fidelity DNA polymerase was used for amplification of the target DNA and PCR reaction was performed under the following thermal cycling conditions: initial denaturation at 98 °C for 30 s, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 68.8 °C for 20 s, and extension at 72 °C for 10 s, with a final extension step at 72 °C for 5 min. PCR products were examined by electrophoresis on a 2% agarose gel to confirm the validity of the product. The expected amplicon size was 429 bp. PCR products were purified and subjected to Sanger sequencing.

Statistical Analysis: All statistical analyses were performed using IBM SPSS Statistics version 27. Kaplan–Meier survival analysis was applied to analyze patients' survival, and the log-rank test was used to compare survival distributions among patients. Cox regression analysis was used to calculate Hazard Ratio. Descriptive statistics (mean and relative frequency) were computed for all variables. A p-value ≤ 0.05 was considered statistically significant in all analyses.

RESULTS

The demographic and clinical characteristics of the 26 patients enrolled in this study are presented in Table1. The age of participants ranged from 34 to 79 years, with a median age of 64 years (interquartile range: 57.75–74), and mean of 63.73. Of the total patients, 15 patients (57.69%) were male and 11 (42.30%) were female. The majority of participants had Azeri ethnicity (76.92%), while 23.07% were of Kurd ethnicity (Table 1).

Table 1: Summary of the patients' information

Variable	Value/Number (%)
Age (year)	
-Range	34-79
-Median	64
-Mean	63.73
Sex	
-Male	15 (58)
-Female	11(42)
Ethnicity	
-Azeri	20 (77)
-Kurd	6 (23)
Tobacco	
-Yes	7 (27)
-No	19 (73)
Opioid	
-Yes	8 (31)
-No	18 (69)
Alcohol	
-Yes	5 (19)
-No	21 (81)
Diabetes mellitus	12 (46)
Hypertension	11 (42)
Histopathology	
-Pancreatic ductal adenocarcinoma	25 (96)
-Pancreatic squamous cell carcinoma	1 (4)
Tumor location	
-Head of pancreas	10 (38)
-Body/tail of pancreas	2 (8)
-Not specified	14 (54)
Surgical intervention	6 (23)
Radiotherapy	5 (19)
Family history of cancer	12 (46)

Sequencing analysis identified three major genotypes in the paitients: TTT/TTT, TTT/T, and T/T (Fig. 1), with respective frequencies of 38%, 54%, and 8%. Allelic frequency of TTT and T alleles among patients were 0.654 and 0.346, respectively.

The analysis of the survival duration from diagnosis to death (overall survival) revealed a minimum interval of 60 days and a maximum of 930 days. The interquartile range (IQR) indicated that 50% of patients had a survival duration between 233 and 585 days, with a median overall survival of 300 days and mean of 393 days.

Further analysis of mean overall survival based on genotypes demonstrated notable differences among groups. Patients with the TTT/T genotype exhibited the longest mean survival of 450 days, followed by those with the TTT/TTT genotype with a mean survival of 395 days. In contrast, individuals carrying the T/T genotype had a substantially shorter mean survival of only 135 days (Fig. 2C).

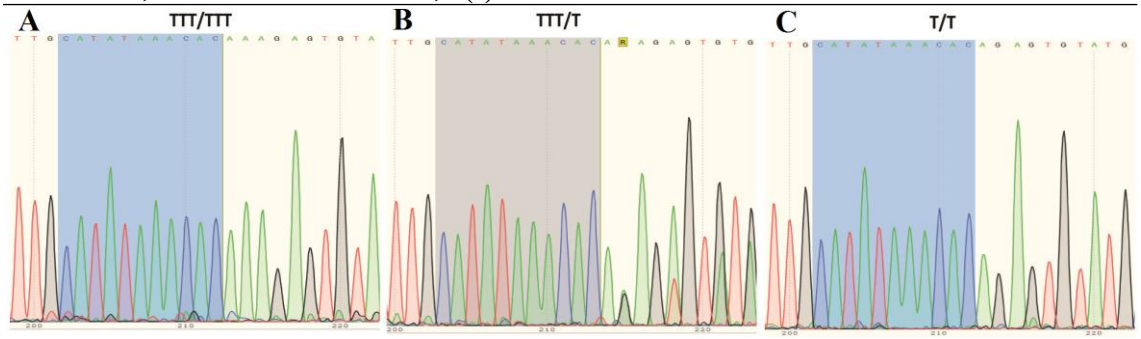

Figure 1: Genotyping. (A), (B) and (C) show the sequencing results of TTT/TTT, TTT/T and T/T genotypes, respectively. The sequence right to the highlighted area is rs34416442. The reverse primer is used for sequencing so the result is complementary to the reference sequence.

Kaplan–Meier analysis was conducted to analyze survival outcomes across the three identified genotypes. The log-rank test indicated a statistically significant difference in survival among different genotypes (log-rank, $p = 0.004$) (Fig. 2A). Cox regression analysis showed that T/T genotype had a HR of 8.14 (95% CI = 1.4-47.4, $p = 0.020$).

To further investigate the impact of the *rs34416442* genetic variation on patient survival, a recessive inheritance model was applied. In this model, individuals carrying two copies of the minor allele (T/T genotype) were grouped together and compared against those with at least one major allele (TTT/TTT or TTT/T genotypes). Kaplan–Meier survival analysis revealed that patients having two T alleles (T/T genotype) had a significantly shorter overall survival compared to those with at least one *TTT* allele, log-rank $p = 0.001$ (Fig. 2B). Cox regression analysis was also performed for the recessive model, HR = 9.8, 95% CI = 1.8-54.4, $p = 0.009$. A multivariable Cox analysis was performed to adjust for surgery status, sex, treatment regimen and age. The factors included in the model did not change the result and only genotypes effects were statistically significant.

The patients were divided into two groups based on their treatment regimen: those who received gemcitabine (either as monotherapy or in combination with 5-FU, n = 18), and those who received no gemcitabine (treated mainly with 5-FU, n = 8). Kaplan–Meier survival analysis within the gemcitabine received and not-received subgroups revealed that the survival differences among genotypes remained statistically significant, log-rank $p = 0.006$ and log-rank $p = 0.016$, respectively. However, the survival pattern was different among the two groups (Fig. 2D and E).

DISCUSSION

Kaplan–Meier survival analysis demonstrated a statistically significant difference in survival across genotypes and Cox regression analysis showed that T/T genotype had a HR of 8.14 (Fig. 2A). Furthermore, the recessive model analysis showed that patients with the T/T genotype had significantly shorter survival compared to those carrying at least one TTT allele (Fig. 2B and C). These findings suggest that the T allele exerts a recessive effect, influencing survival only in the homozygous state. Mean overall survivals varied by genotype: 395 days for TTT/TTT, 450 days for TTT/T, and 135 days for T/T. This further supports the recessive mode of action of T allele in overall survival of patients seen in Figure 2B.

As *SH3D21* had been reported as a gemcitabine sensitizer [4], we hypothesized that its genetic variations might influence pancreatic cancer patients' response to gemcitabine. The majority of the patients (69%) enrolled in the study received gemcitabine, and the rest did not, treated mainly with 5-FU. The survival analysis results indicated that, in total, T/T genotype was associated with lower overall survival of the patients (Fig. 2A). Even after splitting the patients to gemcitabine receiving/not-receiving groups the outcome did not change and patients with T/T genotype showed a significantly lower overall survival in both groups, log-rank 0.006

and 0.016, respectively. However, the patterns of the patients' responses to the drugs were different. In gemcitabine receiving group, the best survival outcome was for patients carrying TTT/T genotype (Fig. 2D). While in gemcitabine not-receiving group, the best survival outcome was for TTT/TTT genotype (Fig. 2E). The mean overall survival among TTT/T patients receiving and not-receiving gemcitabine were 524 and 214 days, respectively (Fig. 2F), revealing that the patients carrying TTT/T genotype respond better to gemcitabine.

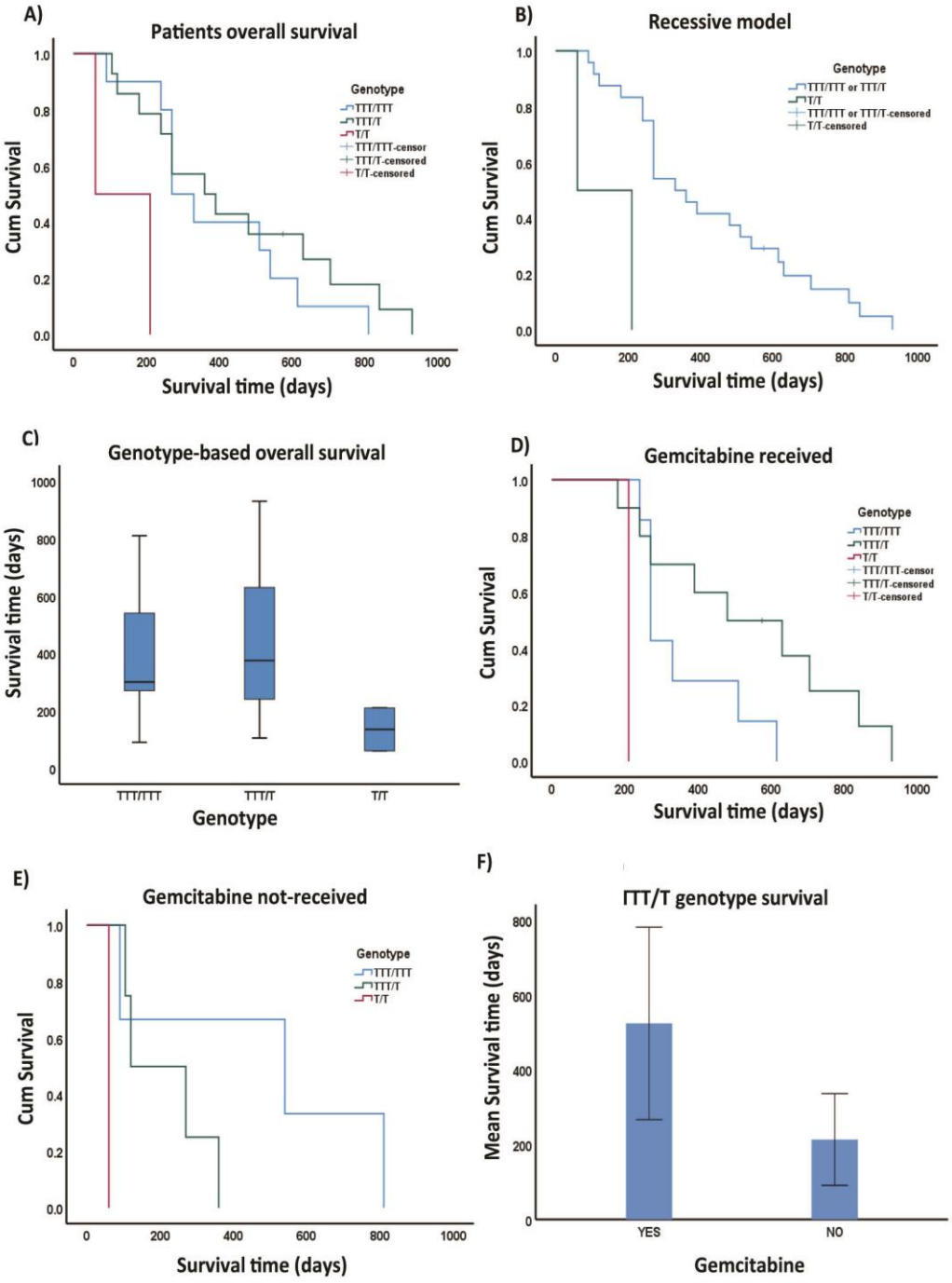

Figure 2: Survival plots of the pancreatic cancer patients. (A) Patients overall survival based on the different genotypes (log-rank p = 0.004). (B) Survival plot of the recessive model (log-rank p = 0.001). (C) Survival time distribution of the patients with different genotypes. (D) Survival plot of the patients received gemcitabine (log-rank p = 0.006). (E) Survival plot of the patients not-received gemcitabine (log-rank p = 0.016). (F) Mean overall survival of TTT/T carrying patients based on their gemcitabine treatment.

This study is the first of its kind about the association of *SH3D21* rs34416442 with pancreatic cancer and has its own limitations including sample size and performance status of the patients. For future studies bigger sample size and including the performance status of patients should be considered.

In conclusion, our findings indicate that rs34416442 genotypes are associated with differences in overall survival of pancreatic cancer patients, in a way that T/T genotype has the lowest overall survival. In addition, our data shows that patients with heterozygote genotype TTT/T respond better to gemcitabine, while patients with TTT/TTT genotype respond better to no-gemcitabine regimen, 5-FU. Altogether, our findings suggest rs34416442 as a potential pancreatic cancer predictive biomarker.

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Conflict of Interest: The Authors declare that they have no conflicts of interest.

Authors' Contribution: MM conceptualized, and supervised the study. MM designed the experiments, analyzed the data and visualized it. NA collected the patients' blood sample and information and performed the experiments. HA advised about and supervised the clinical aspect of the project. MM and NA wrote the original draft. All the authors reviewed and approved the last version of the manuscript.

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