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Analyzing TCGA Data to Identify Gene Mutations Linked to Hepatocellular Carcinoma in Asians

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Keywords
Asian · Hepatocellular carcinoma · The Cancer Genome Atlas · Bioinformatics · Prognostic marker

Abstract
Introduction: Liver cancer is the sixth most common and second most fatal type of cancer worldwide. Few treatment options are available as patients with liver cancer are often diagnosed in an advanced stage due to a lack of clinical symptoms. Effectively preventing and treating liver cancer relies heavily on early diagnosis; early diagnosis results from identifying and monitoring high-risk patients. Epigenetic risk factors, such as hepatitis B, hepatitis C, cirrhosis, nonalcoholic fatty liver disease, and alcohol/tobacco abuse, are highly prevalent in Asia and likely cause Asians to have a higher incidence and mortality rate of liver cancer. While these acquired risk factors are relatively well understood, the underlying genetic background of liver cancer in Asians has not been well established or correlated with clinical outcomes. ***Methods:*** In this study, we accessed The Cancer Genome Atlas (TCGA) hepatocellular carcinoma clinical and mutation data through TCGAbiolinksGUI. ***Results:*** We found that mutations in five genes (*TP53, TTN, OBSCN, MUC5B, CSMD1*) were statistically linked with increased mortality in Asians compared to non-Asians, four of which (*TTN, OBSCN, MUC5B, CSMD1*) were also more prevalent in the Asian population. Within the Asian cohort, two gene mutations (*TTN, HMCN1*) were statistically linked with worse outcomes. We also found that the *TP53* mutation predicts worse outcomes within the non-Asian cohort but not within the Asian cohort. ***Discussion/Conclusion:*** Our findings can improve cancer care in the Asian population through better disease prognostication, evaluations for potential targeted therapy, and a deeper understanding of liver cancer pathogenesis.

Introduction

Liver cancer is the sixth most common and second most fatal type of cancer [1]. From 1990 to 2015, the incidence rate of liver cancer has increased by 75% worldwide [2]. The overall 5-year relative survival rate, based on the Surveillance, Epidemiology, and End Results Program, is 34% for localized disease, 12% for locally advanced or with lymph node involvement, and 3% for distant metastases [3]. The average 5-year survival rate of liver cancer is 19%, as determined by the American Cancer Society using data from 2010 to 2016 [3, 4].

The most common types of liver cancer are hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC). These two subtypes make up 90% and 10% of liver cancer, respectively [5]. The primary acquired risk

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factors for developing HCC and ICC described in the literature are hepatitis B, hepatitis C, parasite infection, nonalcoholic fatty-liver disease, liver cirrhosis, and alcohol and tobacco use [6, 7]. An exposure to hepatic carcinogens (e.g., aflatoxins, arsenic) is also strongly correlated with an onset of liver cancer [8].

A cancer incidence report published by Ferlay et al.[1] in 2015 concluded that liver cancer is significantly more prevalent in less-developed regions, such as Southeast Asia and Africa [1, 9]. These regions have significantly higher reported cases of hepatitis B, hepatitis C [10], liver fluke infection [5], and aflatoxins exposure [11] than developed countries. Recently NAFDL prevalence in Asia has approached similar levers to those in Western countries and was reported higher in certain areas and obese children [12, 13]. Even though these risk factors are endemic to Asia and Africa, liver cancer is nearly twice as common and fatal in Asian Americans than non-Asian Americans [14, 15]. This phenomenon suggests that regardless of exposure to environmental risk factors, Asians are likely still highly susceptible to liver cancer, possibly due to genetic background. This study mines The Cancer Genome Atlas (TCGA) for gene mutations linked with worse outcomes in Asian patients with HCC.

Table 1. HCC patient demographic information

Race	Gender	Vital status		
		Alive	Dead	Total
American Indian or Alaska Native	Male	2	0	2
	Female	0	0	0
Asian	Male	91	35	126
	Female	25	9	34
Black or African American	Male	8	5	13
	Female	2	1	3
Not reported	Male	4	3	7
	Female	1	2	3
White	Male	67	38	105
	Female	43	39	82
Total		243	132	375

Materials and Methods

Data Download and Analysis

TCGA is a publicly available database that provides cancer genomics data to researchers. Docker Desktop was launched in order to provide a local server for the RStudio virtual environment. Powershell was used to run the RStudio local server, which was in turn used to run TCGAbiolinksGUI. Patient mutation and clinical data from TCGA were downloaded through TCGAbiolinksGUI on July 3, 2020. These data were uploaded to the GenePattern Jupyter Notebook server and analyzed using the pandas, NumPy, Matplotlib, seaborn, and SciPy libraries in Python.

SciPy, a Python library used for scientific computing, was used to analyze the prognostic significance of these 24 common genes on survival using a two-tailed test. KaplanMeierFitter, a program that utilizes the Kaplan-Meier method, was used to graph the 5-year survival rates.

Selection of Patient Sample

The number of HCC patients registered in TCGA is 375, most of whom are White and Asian (187 and 160, respectively) (shown in Table 1). The overall survival rates of Asian and non-Asian patients were compared; at around 750 days since diagnosis, the Asian survival curve began to flatten noticeably. Furthermore, the estimated 5-year survival rates of Asian and non-Asian patients were 2–3 times higher than published statistics [4]. These discrepancies suggest poor follow-up with living patients (shown in Fig. 1).

When only the survival rates of deceased patients were compared, the resulting survival graphs better represented known trends (shown in Fig. 2) [4]. Accordingly, this study only analyzed deceased Asian and non-Asian HCC patients.

Survival Analysis of Asian and Non-Asian

Seaborn was used to analyze the survival rate differences between all Asian and non-Asian HCC patients.

Survival Analysis of Deceased Asian and Non-Asian Patients

Kaplan-Meier and Matplotlib were used to investigate the survival rate differences between deceased Asian and non-Asian HCC patients.

Gene Mutation Effects on the Survival Rate in Deceased Asian and Non-Asian Patients

The 24 most common mutated genes in deceased Asian HCC patients were identified. Kaplan-Meier and Matplotlib were used to compare the survival rates of Asian and non-Asian patients with these genes.

Gene Mutation Effects on the Survival Rate in Deceased Asian Patients

The 24 genes were used to determine their effect on Asian prognosis. Kaplan-Meier and Matplotlib were used to compare the survival rate of Asian patients with these genes and Asian patients without these genes.

Gene Mutation Effects on the Survival Rate in Deceased Non-Asian Patient

The 24 genes were used to determine their effect on non-Asian prognosis. Kaplan-Meier and Matplotlib were used to compare the survival rate of non-Asian patients with these genes and non-Asian patients without these genes.

Statistical Analysis

SciPy was used to conduct Welch's *t* test (unequal variances *t* test), with p values <0.05 considered to be statistically significant.

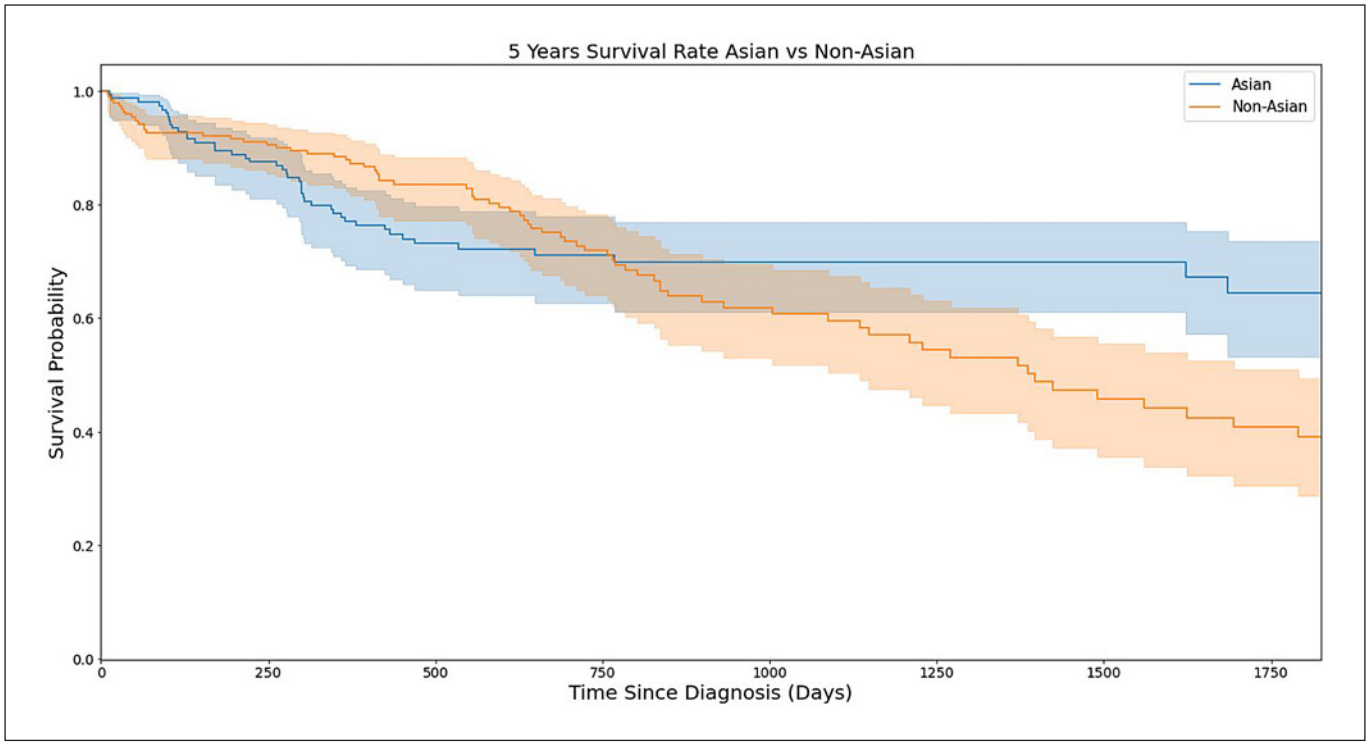


Fig. 1. The overall survival rate of Asian versus non-Asian HCC patients.

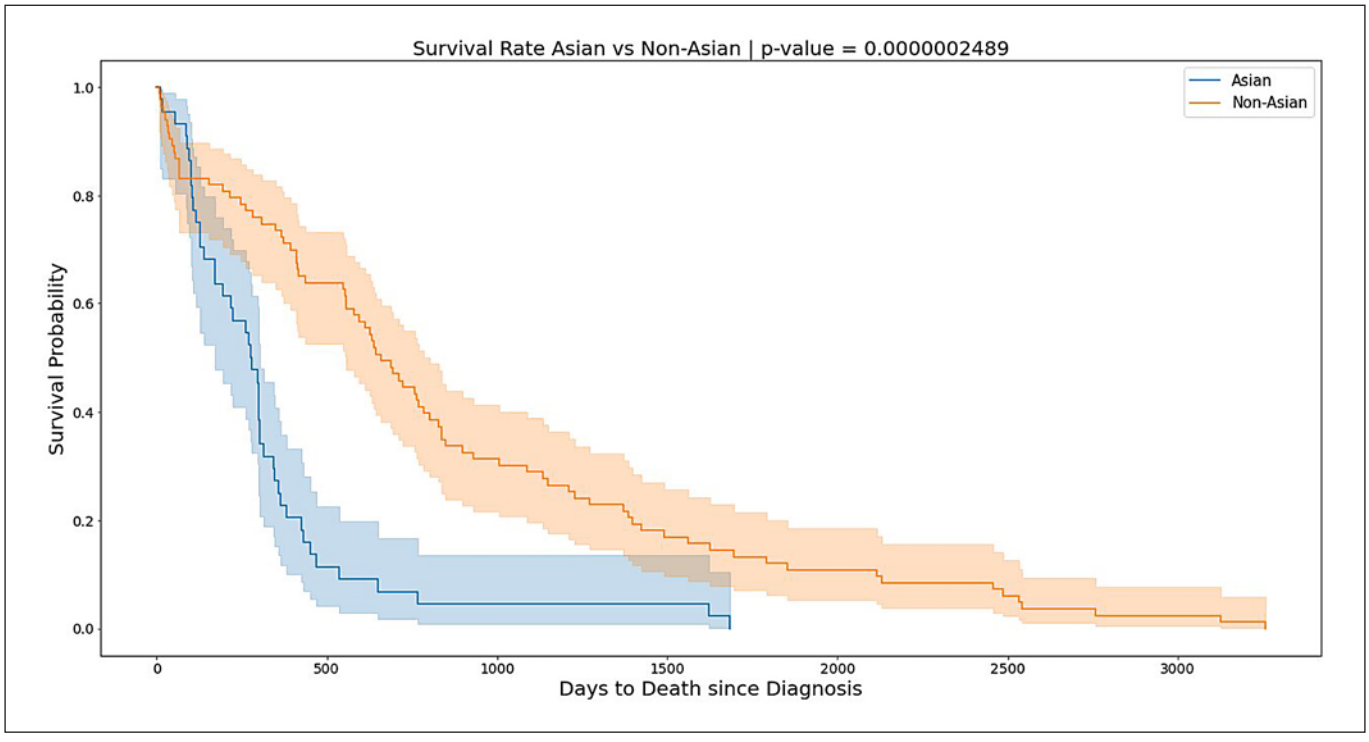


Fig. 2. The overall survival rate of deceased Asian versus deceased non-Asian HCC patients.

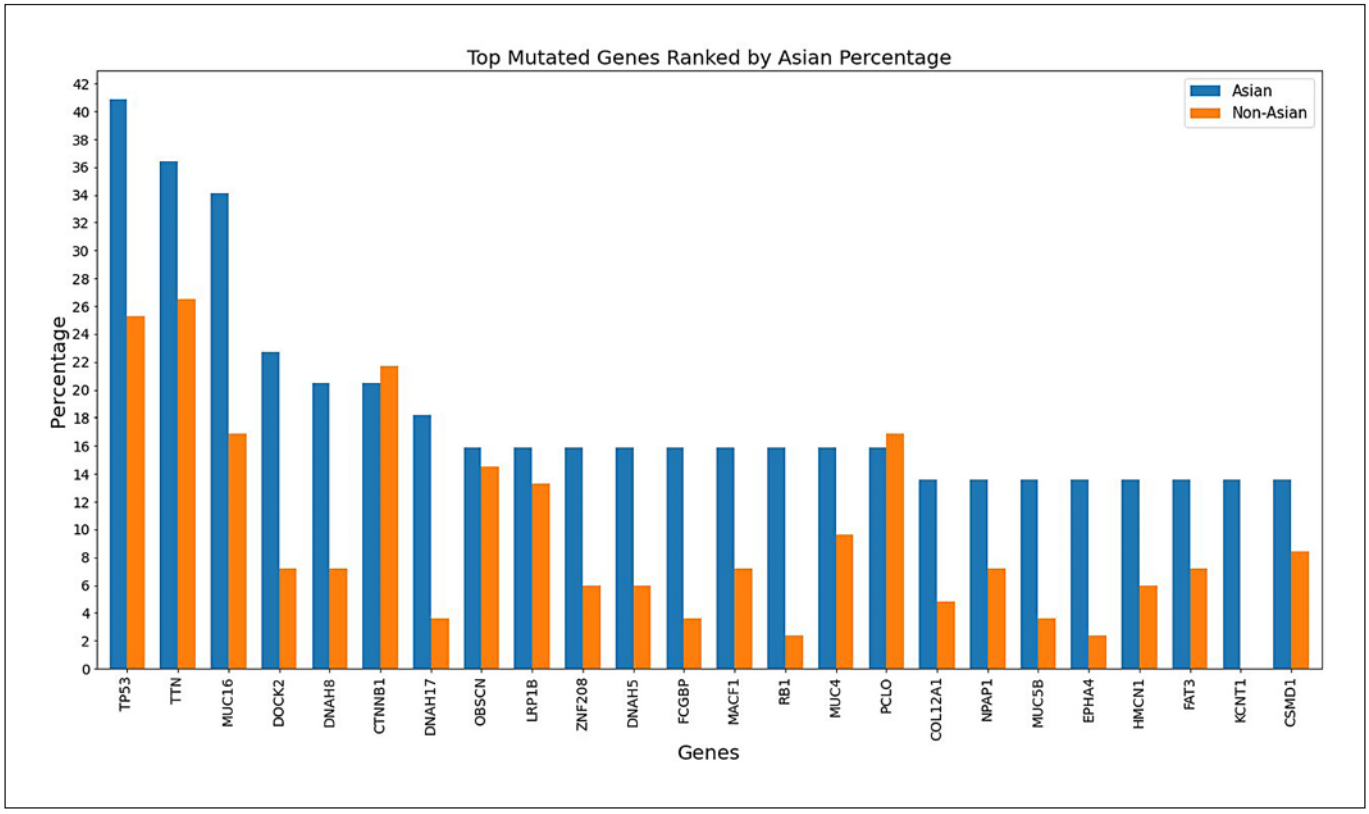


Fig. 3. The 24 most frequently mutated genes in deceased Asian and non-Asian HCC patients ranked by deceased Asian HCC patient percentage.

Results

Survival Analysis of Deceased Asian and Non-Asian Patients

The clinical data showed that deceased Asian HCC patients had a significantly lower survival rate than deceased non-Asian HCC patients (shown in Table 2, shown in Fig. 2, $p < 0.000001$).

Table 2. Days to death in deceased Asian and deceased non-Asian HCC patients

	Asian ($n = 44$)	Non-Asian ($n = 83$)
Mean	325	841
Standard deviation	335	762
Min	12	9
Max	1,685	3,258

Gene Mutation Effects on the Survival Rate in Deceased Asian and Non-Asian Patients

The 24 most frequently mutated genes in deceased Asian HCC patients were identified and graphed by prevalence. The prevalence of this mutation in non-Asians was graphed for comparison. The majority of mutations had a higher prevalence among Asian patients compared to non-Asian patients (shown in Fig. 3).

Single-nucleotide polymorphism mutations in *TP53*, *TTN*, *OBSCN*, *MUC5B*, and *CSMD1* genes were statistically associated with worse overall survival in Asian HCC patients than non-Asian HCC patients, with a p value <0.05 considered to be statistically significant. Hemicentin 1 (*HMCN1*), a significant gene mutation within the Asian population, had a trend toward worse survival than non-Asians, but the difference was not statistically significant (shown in Fig. 4).

Gene Mutation Effects on the Survival Rate in Deceased Non-Asian Patients

We further studied if identified mutations in *TP53*, *TTN*, *OBSCN*, *MUC5B*, *CSMD1*, and *HMCN1* predict

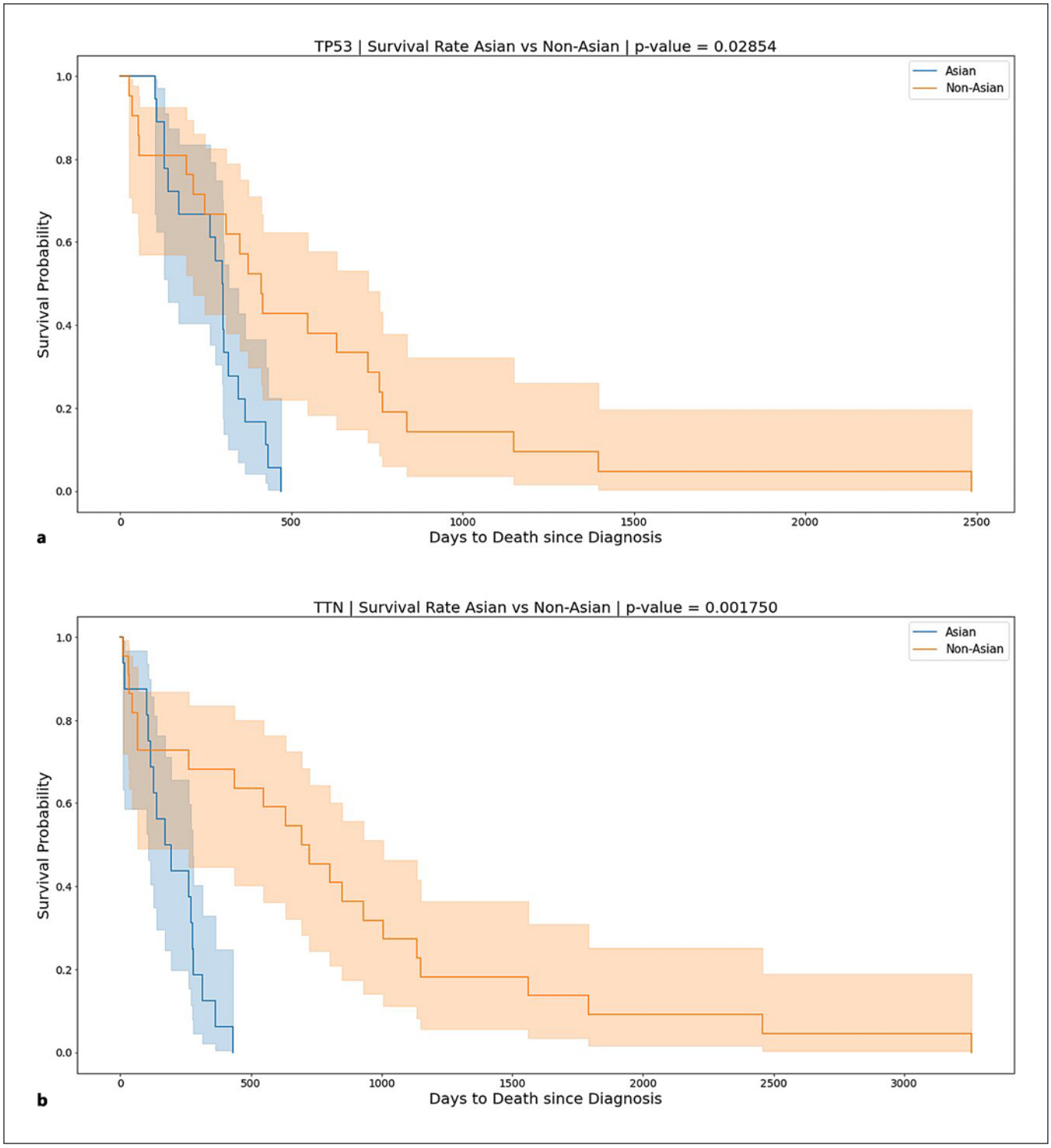
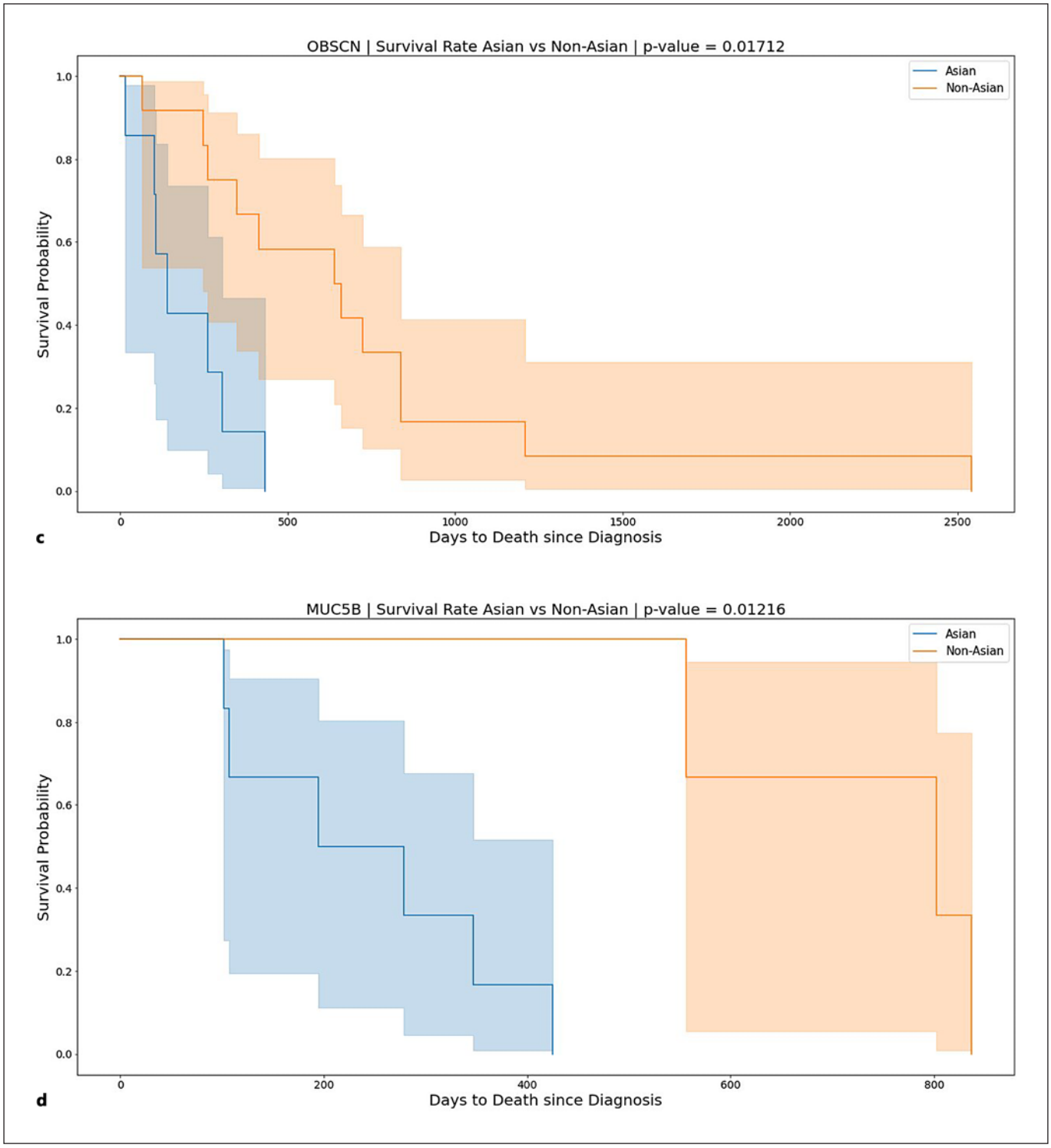
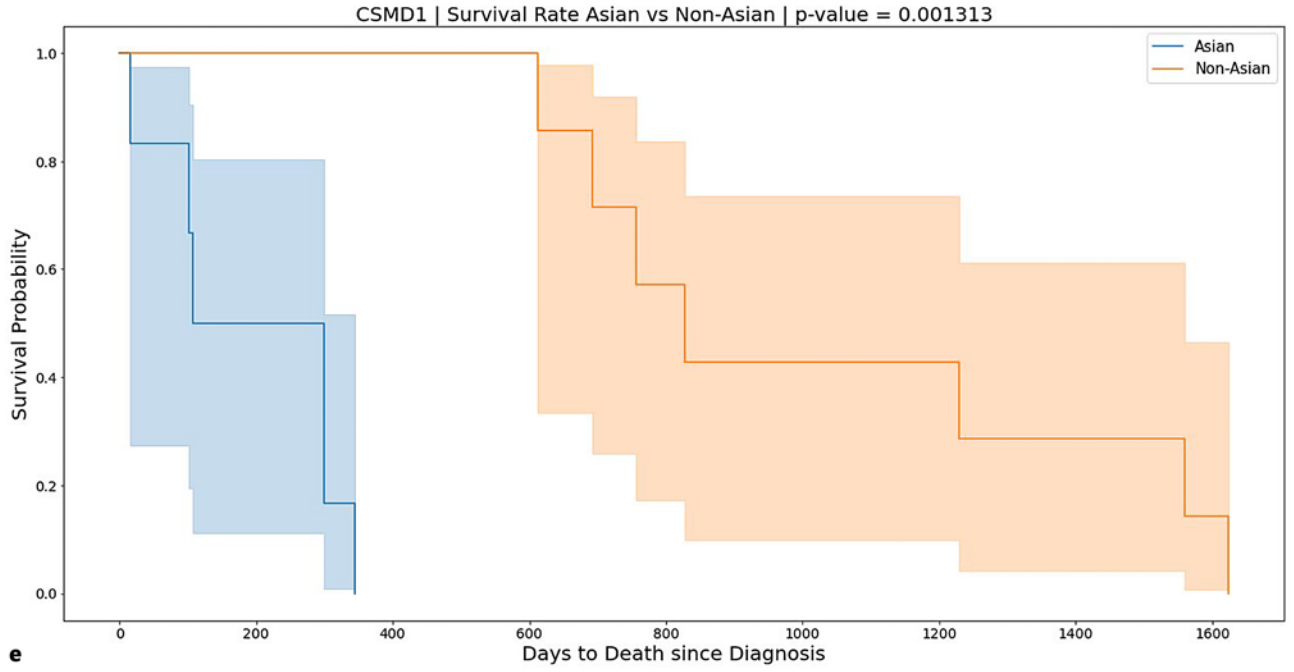


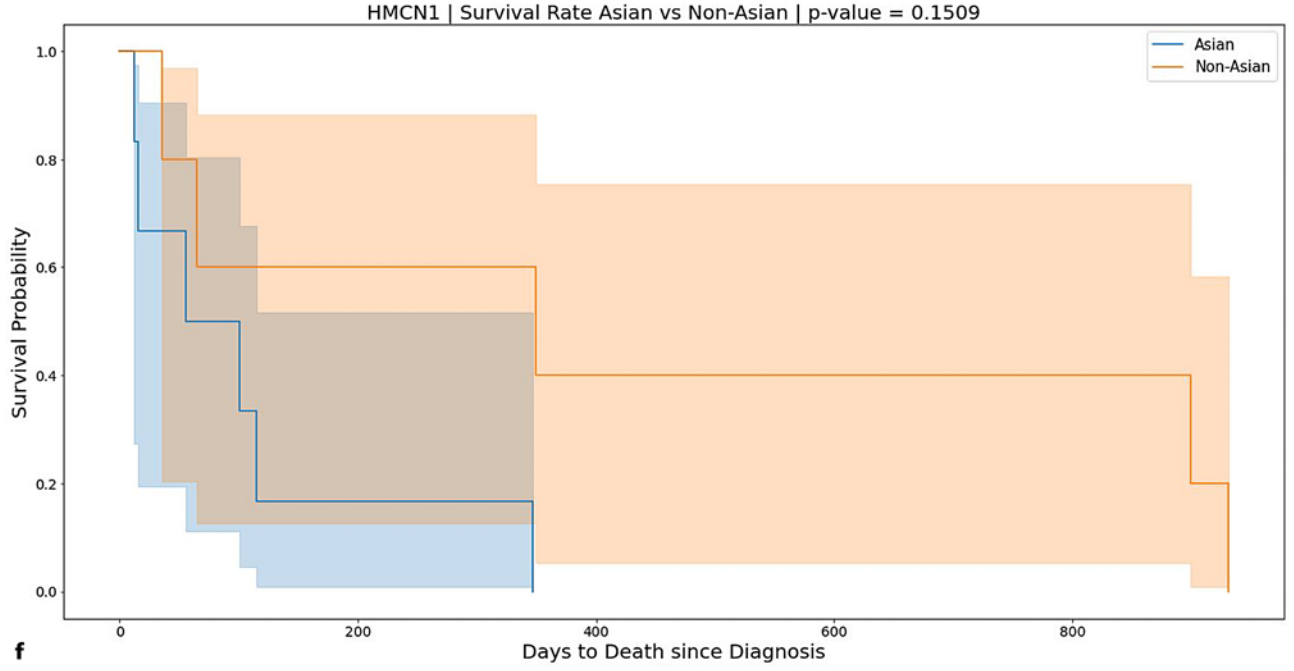
Fig. 4. Survival rates of Asian versus non-Asian HCC patients with mutations of *TP53* (**a**) *TTN* (**b**) *OBSCN* (**c**) *MUC5B* (**d**) *CSMD1* (**e**) *HMCN1* (**f**).

(Figure continued on next pages.)


OBSCN | Survival Rate Asian vs Non-Asian | p-value = 0.01712
Asian
Non-Asian
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
Days to Death since Diagnosis
c
MUC5B | Survival Rate Asian vs Non-Asian | p-value = 0.01216
Asian
Non-Asian
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
Days to Death since Diagnosis
d

4


CSMD1 | Survival Rate Asian vs Non-Asian | p-value = 0.001313
Asian
Non-Asian
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
1200
1400
1600
Days to Death since Diagnosis
e


HMCN1 | Survival Rate Asian vs Non-Asian | p-value = 0.1509
Asian
Non-Asian
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
Days to Death since Diagnosis
f

4

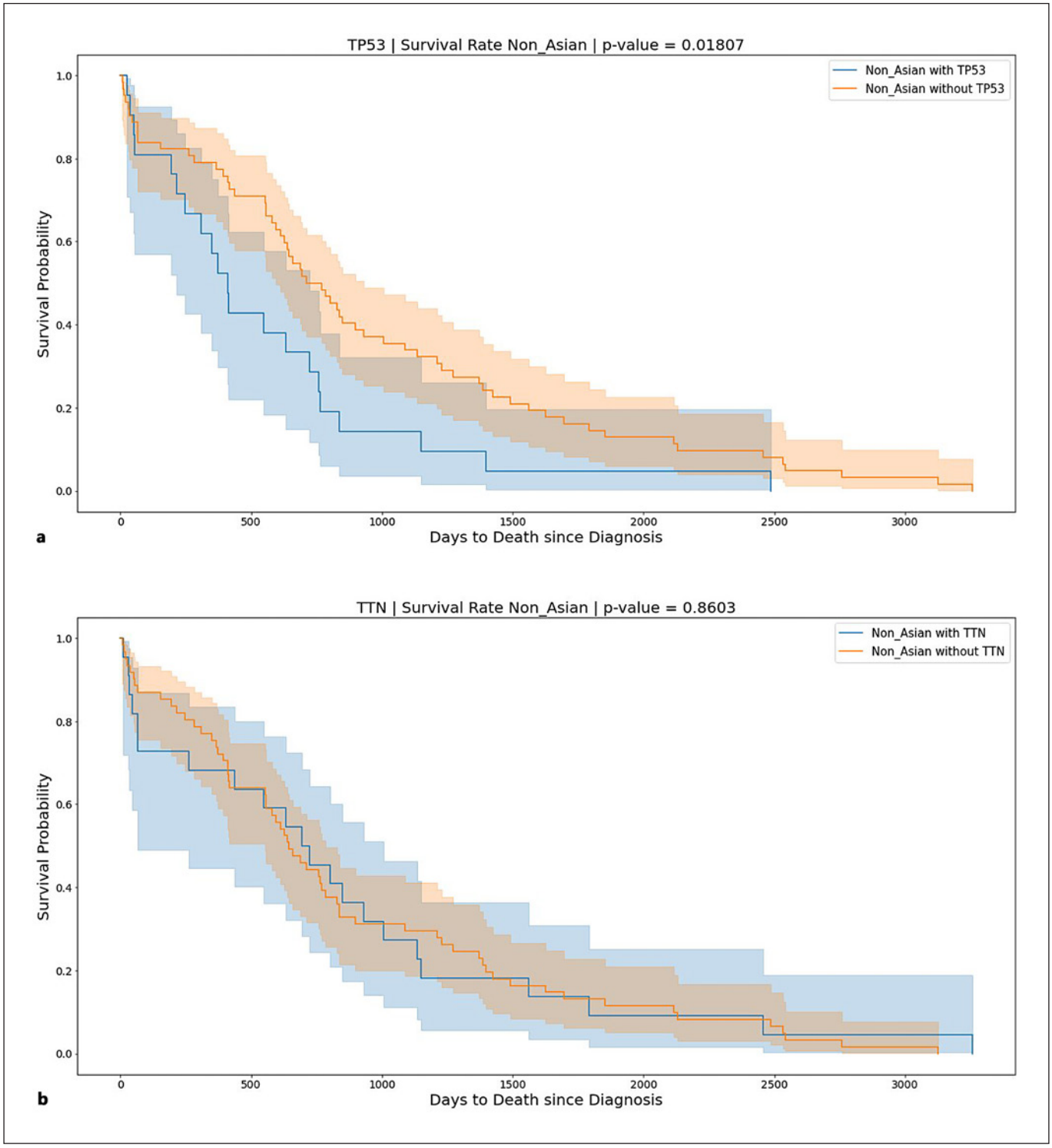
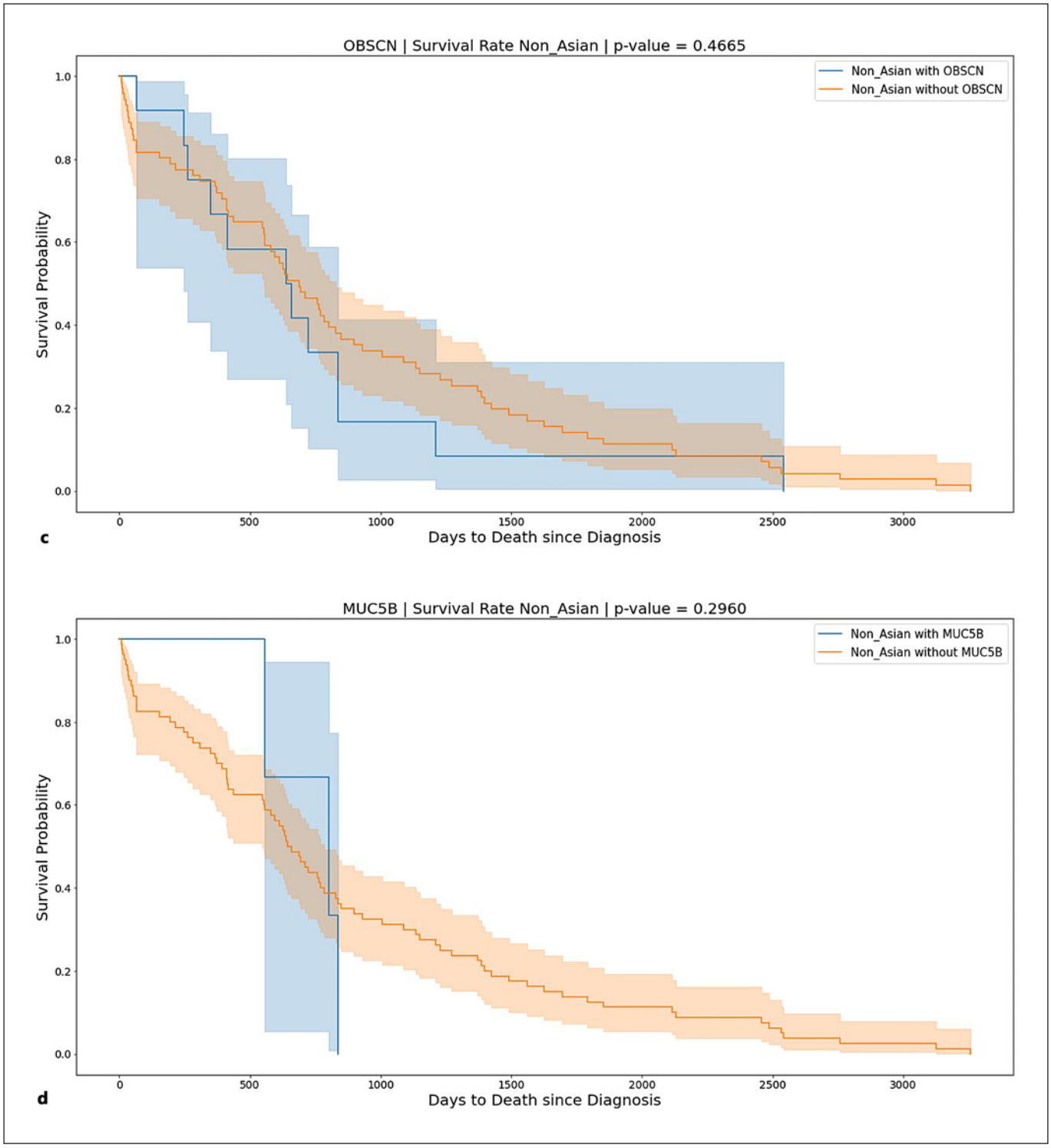
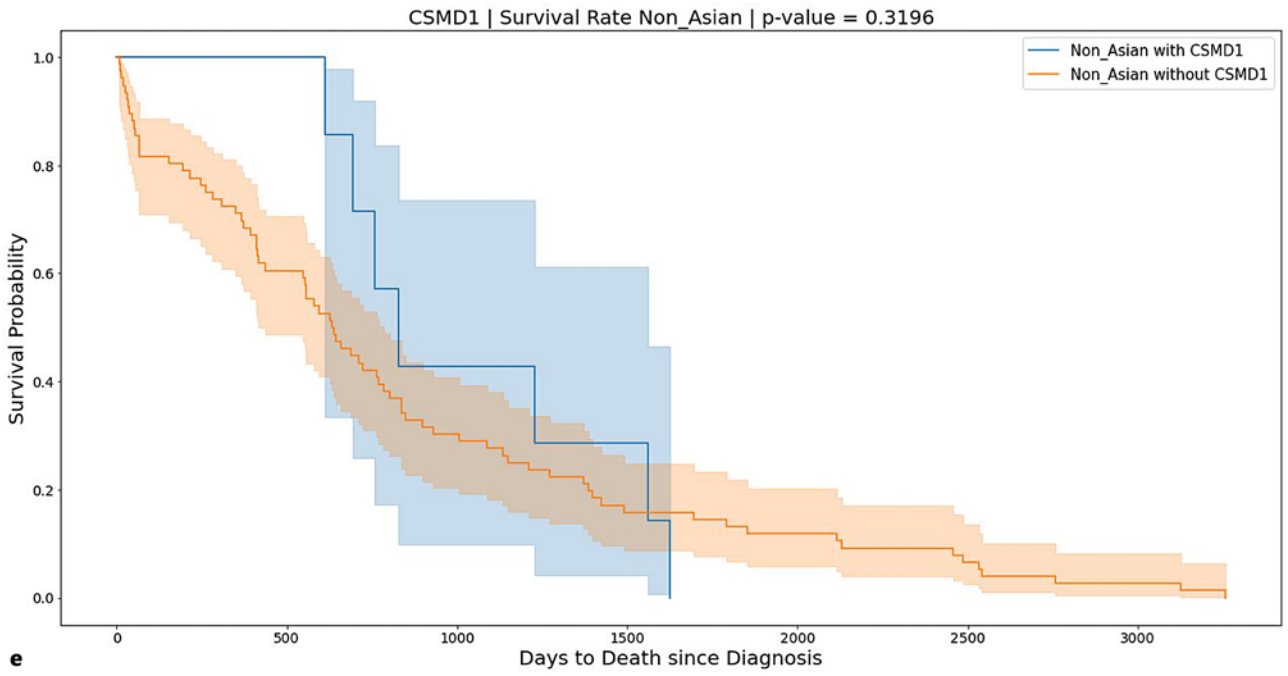


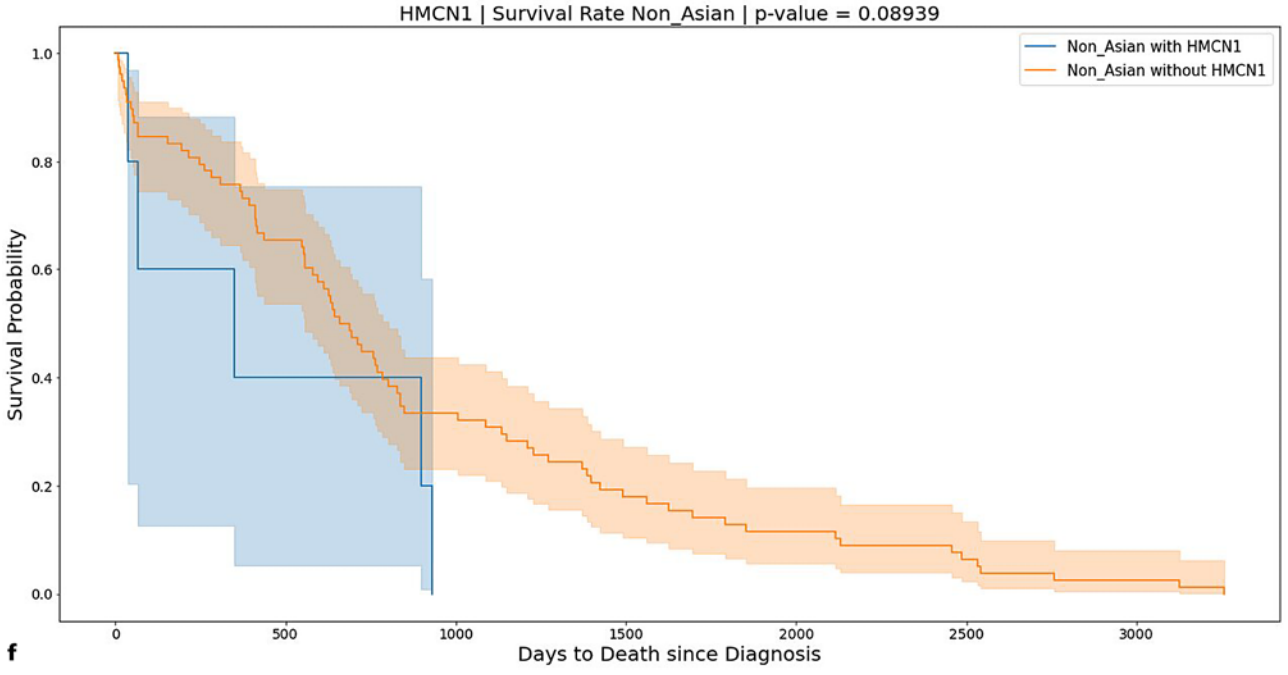
Fig. 5. Survival rates of non-Asian HCC patients with and without mutations of *TP53* (**a**) *TTN* (**b**) *OBSCN* (**c**) *MUC5B* (**d**) *CSMD1* (**e**) *HMCN1* (**f**).

(Figure continued on next pages.)


OBSCN | Survival Rate Non_Asian | p-value = 0.4665
Non_Asian with OBSCN
Non_Asian without OBSCN
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000
Days to Death since Diagnosis
c
MUC5B | Survival Rate Non_Asian | p-value = 0.2960
Non_Asian with MUC5B
Non_Asian without MUC5B
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000
Days to Death since Diagnosis
d

5


CSMD1 | Survival Rate Non_Asian | p-value = 0.3196
Non_Asian with CSMD1
Non_Asian without CSMD1
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000
Days to Death since Diagnosis
e


HMCN1 | Survival Rate Non_Asian | p-value = 0.08939
Non_Asian with HMCN1
Non_Asian without HMCN1
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000
Days to Death since Diagnosis
f

5

Table 3. Significant genes in HCC survival based on TCGA data

Group 1	Sample size	Days to death, mean	Group 2	Sample size	Days to death, mean	*p* value
Asian versus non-Asian						
Asian	44	325	Non-Asian	83	866	<0.000001
Mutated gene comparison						
Asian versus non-Asian						
Asian with *TP53*	18	270	Non-Asian with *TP53*	21	570	0.029
Asian with *TTN*	16	199	Non-Asian with *TTN*	22	840	0.002
Asian with *OBSCN*	7	195	Non-Asian with *OBSCN*	12	732	0.017
Asian with *MUC5B*	6	242	Non-Asian with *MUC5B*	3	731	0.012
Asian with *CSMD1*	6	194	Non-Asian with *CSMD1*	7	1,043	0.001
Asian with *HMCN1*	6	107	Non-Asian with *HMCN1*	5	456	0.151
Mutated gene comparison among non-Asians						
Non-Asian with *TP53*	21	570	Non-Asian without *TP53*	62	967	0.018
Non-Asian with *TTN*	22	840	Non-Asian without *TTN*	61	876	0.860
Non-Asian with *OBSCN*	12	732	Non-Asian without *OBSCN*	71	889	0.467
Non-Asian with *MUC5B*	3	732	Non-Asian without *MUC5B*	80	872	0.296
Non-Asian with *CSMD1*	7	1,043	Non-Asian without *CSMD1*	76	850	0.320
Non-Asian with *HMCN1*	5	456	Non-Asian without *HMCN1*	78	893	0.089
Mutated gene comparison among Asians						
Asian with *TP53*	18	271	Asian without *TP53*	26	363	0.301
Asian with *TTN*	16	199	Asian without *TTN*	28	397	0.019
Asian with *OBSCN*	7	195	Asian without *OBSCN*	37	350	0.065
Asian with *MUC5B*	6	243	Asian without *MUC5B*	38	338	0.239
Asian with *CSMD1*	6	195	Asian without *CSMD1*	38	346	0.074
Asian with *HMCN1*	6	108	Asian without *HMCN1*	38	359	0.003

overall survival within Asian and non-Asian populations separately. The results in Figure 5 show that mutations in *TTN*, *OBSCN*, *MUC5B*, *CSMD1*, and *HMCN1* genes were not significant in the non-Asian sample group. In contrast, the mutation in the *TP53* gene was statistically significantly associated with worse mortality among non-Asians.

We then studied if the identified six gene mutations were associated with a worse outcome within the Asian patient cohort. Mutations in TTN led to significantly lower survival rates among Asian patients (shown in Fig. 6a). Another gene mutation, *HMCN1*, was also was linked to a significantly lower survival rate within the Asian HCC patient cohort (shown in Fig. 6b).

Statistical Analysis

All calculated p values <0.05 were considered statistically significant in this study. Table 3 shows a summary of the results.

Discussion

This study hypothesizes that lower survivability in Asian HCC patients than non-Asian HCC patients may be linked to somatic gene mutation signatures rather than epigenetic differences.

To begin, the initial clinical and mutation data needed to be filtered. Only HCC patient data were analyzed in this study because the sample size of ICC patients was too small to produce significant results. We assumed that the results are still relevant as HCC accounts for 90% of liver cancer [16]. Furthermore, the survival rates of HCC patients were significantly higher than in published literature; this indicates that there may have been little follow-up with living patients, with a large majority of patients incorrectly marked as alive in the database many years later. This study only analyzed deceased HCC patient data in order to prevent skewed survival curves.

In the patient group of deceased Asian and non-Asian HCC patients, the Asian patients' days to death since diagnosis (mean = 325) is statistically shorter (p value

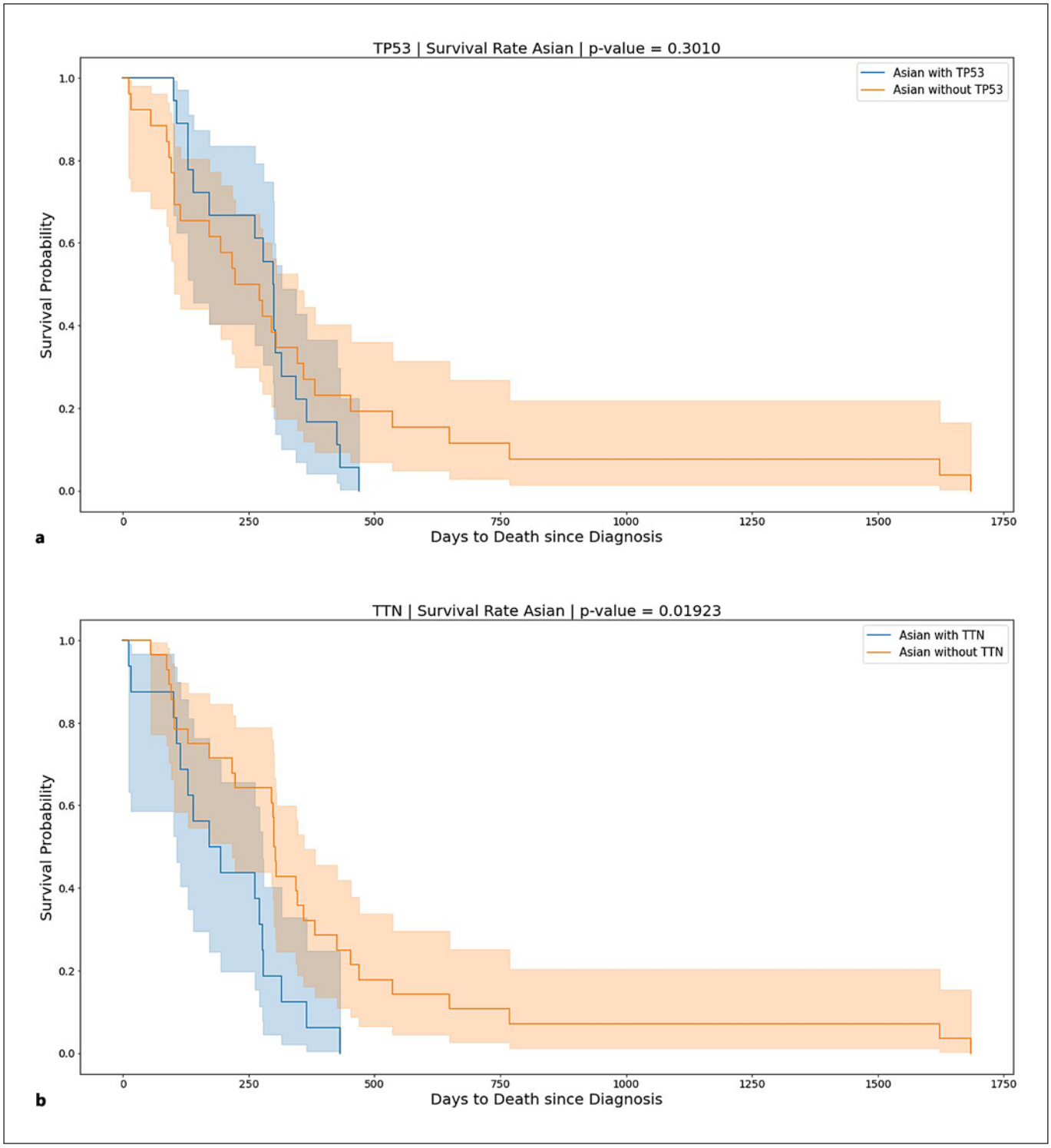
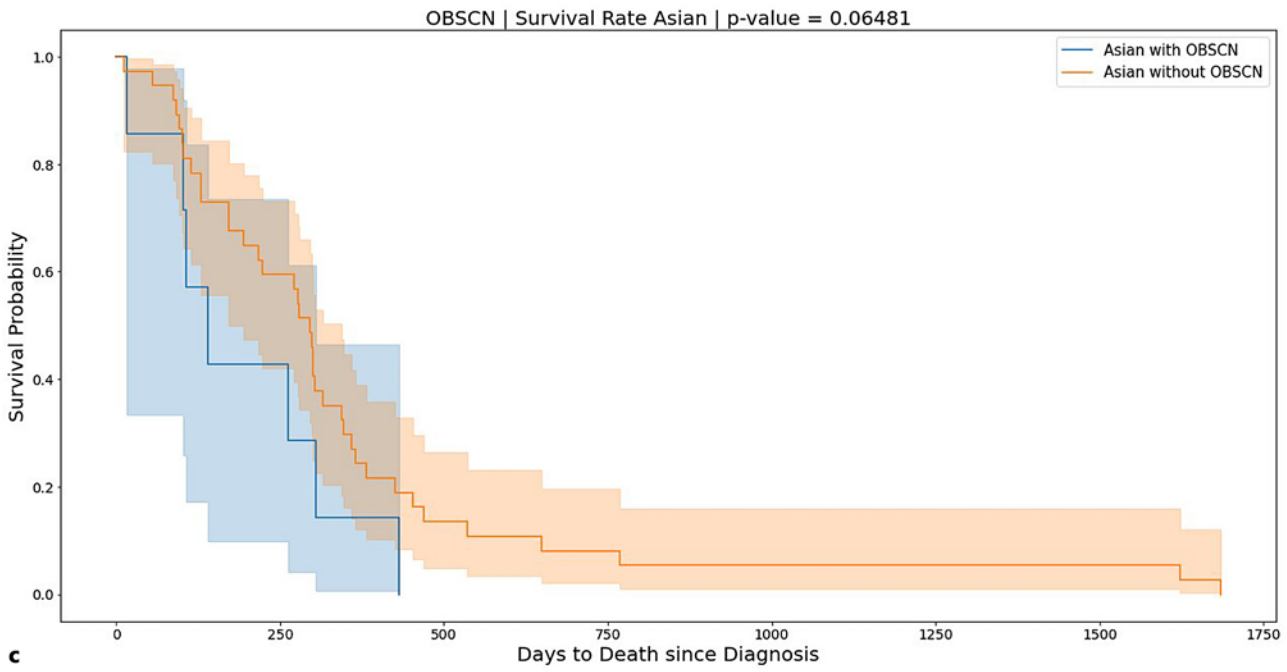
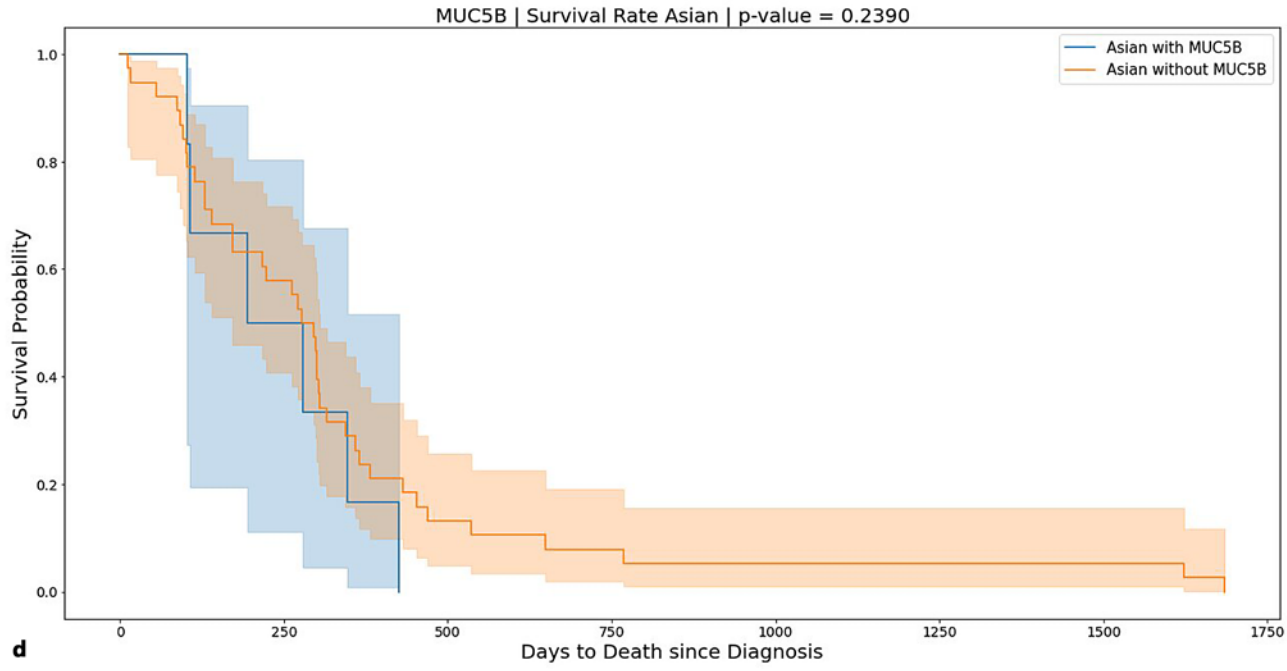


Fig. 6. Survival rates of Asian HCC patients with and without mutations of *TP53* (**a**) *TTN* (**b**) *OBSCN* (**c**) *MUC5B* (**d**) *CSMD1* (**e**) *HMCN1* (**f**).

(Figure continued on next pages.)

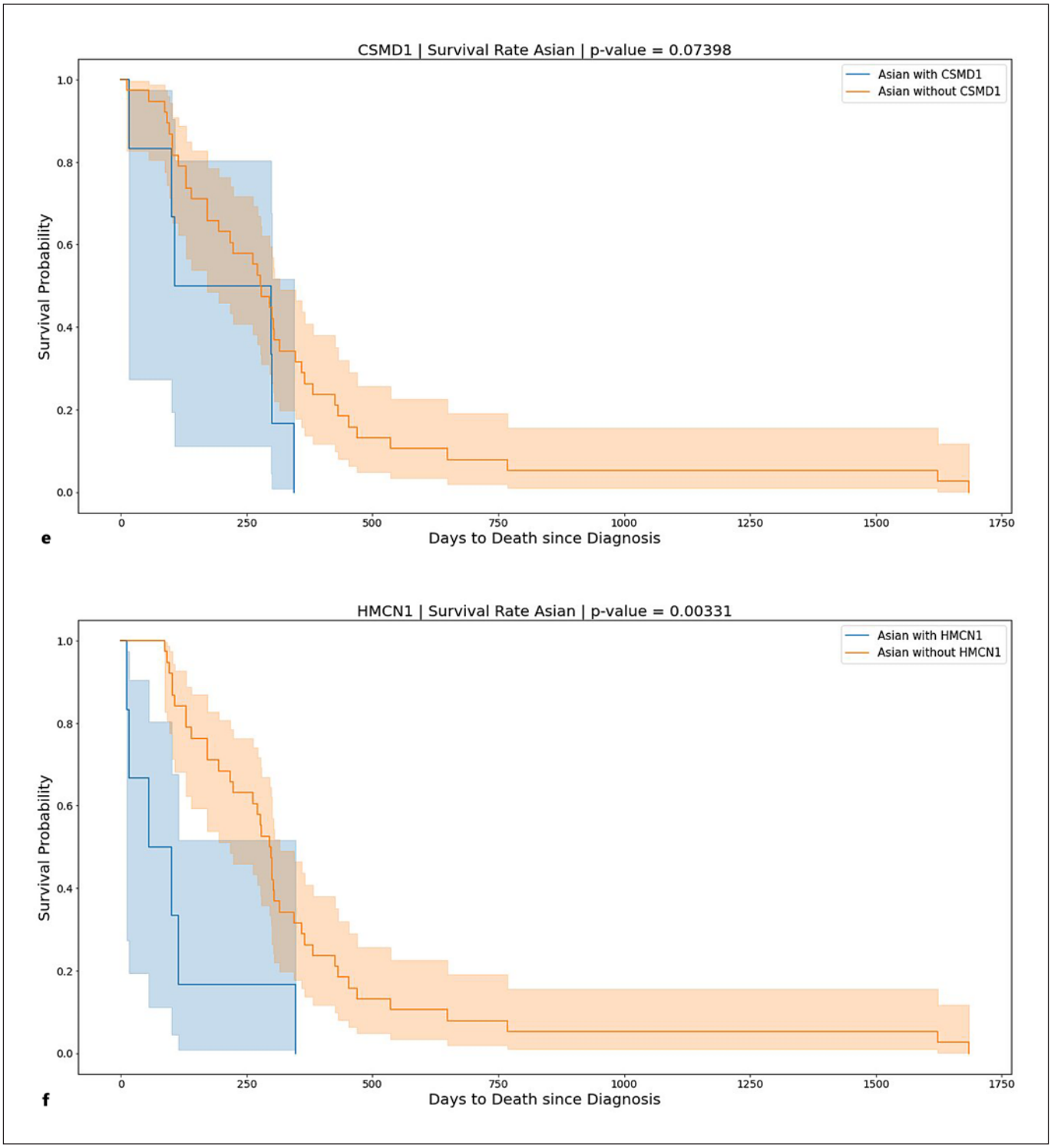

OBSCN | Survival Rate Asian | p-value = 0.06481
Asian with OBSCN
Asian without OBSCN
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
250
500
750
1000
1250
1500
1750
Days to Death since Diagnosis

c


MUC5B | Survival Rate Asian | p-value = 0.2390
Asian with MUC5B
Asian without MUC5B
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
250
500
750
1000
1250
1500
1750
Days to Death since Diagnosis

d

6


CSMD1 | Survival Rate Asian | p-value = 0.07398
Asian with CSMD1
Asian without CSMD1
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
250
500
750
1000
1250
1500
1750
Days to Death since Diagnosis
e
HMCN1 | Survival Rate Asian | p-value = 0.00331
Asian with HMCN1
Asian without HMCN1
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
0
250
500
750
1000
1250
1500
1750
Days to Death since Diagnosis
f

6

<0.000001) than non-Asian patients (mean = 866). Epigenetic factors, such as diet, rates of chronic liver diseases, and access to healthcare may play a significant role in the unfavorable prognosis for Asian patients. However, distinct somatic mutational profiles may contribute to the outcomes as well.

After identifying the 24 most frequently mutated genes in deceased Asian HCC patients, the survival rates of deceased Asian HCC patients with these mutations were compared with those of deceased non-Asian HCC patients with these gene mutations. Five genes were statistically linked to worse survival in Asians than non-Asians. These five genes are *TP53*, *TTN*, *OBSCN*, *MUC5B*, and *CSMD1*; *HMCN1* had a trend toward worse survival. Also, all identified gene mutations except *OBSCN* had a higher prevalence among Asians compared to non-Asians.

To see whether the identified genes independently determined the outcome, rather than epigenetic race-related factors, we studied the association between identified gene mutations and outcomes within Asian and non-Asian patient cohorts separately. In the non-Asian cohort, among six genes, only the *TP53* mutation was linked to worse survival; *TP53* mutation, in general, is well known poor prognostic marker in a variety of tumor histologies. Interestingly, when we analyzed these six genes in the Asian cohort, *TP53* mutation did not have a prognostic value. However, two genes were statistically significantly linked to worse outcomes in Asian HCC patients versus Asians without these mutations. These two genes mutations are *TTN* ($p = 0.019$) and *HMCN1* ($p = 0.003$).

Among all analyzed gene mutations, *TTN* was the only mutated gene shown to be a statistically significant negative prognostic marker when comparing Asians versus non-Asians and within the Asian patient cohort separately. *TTN* encodes for Titin, a large protein found in cardiac and skeletal muscles; *TTN* mutations are known to cause cardiomyopathy and muscular dystrophy [17]. *TTN* mutations have also been correlated with increased tumor mutation burden in cancer patients with the mutation. Melanoma and lung cancer patients with *TTN* mutations have shown better responses to immunotherapies with more prolonged overall and progression-free survival. However, solid tumor patients with this mutation who did not receive immunotherapy showed poor overall and disease-free survival [18].

HMCN1 encodes for immunoglobulin in adhesive and flexible cell junctions; however, its detailed function remains unknown. Mutations in *HMCN1* have been linked to cancer cell invasion and metastasis, and high mutation levels have been found in patients with head and neck squamous cell carcinoma [19].

Multiple studies, such as the ones published by Rao et al. [8] and Li et al. [20], indicates that *TP53* is a commonly mutated gene linked to HCC and can be used as a biomarker for prevention, prognosis, and treatment. This study identifies new gene mutations that can serve as prognostic biomarkers for Asian HCC patients. In particular, *TTN* and *HMCN1's* high prevalence in Asian patients (36.4% and 13.6%, respectively) make them critical gene mutations with a potential prognostication that may correlate with outcomes of various HCC treatment regimens. More research is needed to understand *TTN* mutation's role in HCC pathogenesis and explain the mechanism of its negative association among Asian patients.

Our results are limited to the data available in TCGA. The small size of the database, outliers, limited survival data, unknown potentially confounding factors, and the lack of epigenetic data impact the analysis and limit our conclusions. We may not know the causality links between epigenetic factors of HCC carcinogenesis and identified somatic mutation evolution. Nevertheless, TCGA remains the largest validated cancer genome database supervised by National Cancer Institute and widely accepted among cancer researchers. We found that mutations in five genes (*TP53, TTN, OBSCN, MUC5B, CSMD1*) were statistically linked with increased mortality in Asians compared to non-Asians, four of which (*TTN, OBSCN, MUC5B, CSMD1*) were also more prevalent in the Asian population. Performing the analysis in the Asian patient cohort separately, mutations in *TTN* and *HMCN1* genes appear to be independent negative prognostic markers for Asians. Other gene mutations, such as *OBSCN* and *CSMD1* that showed a trend toward worse survival, may be important, but the number of patients in the TCGA dataset limits this conclusion. Future studies using multiple cancer databases could help confirm the validity and applicability of the findings in this study.

Conclusion

In summary, a list of the 24 most frequently mutated genes found in deceased Asian HCC patients was extracted from TCGA. Mutations in five of these genes (*TP53*, *TTN*, *OBSCN*, *MUC5B*, and *CSMD1*) are correlated with shorter life expectancy in Asian HCC patients compared to non-Asian HCC patients. Mutations in four of these genes (*TTN*, *OBSCN*, *MUC5B*, and *CSMD1*), as well as

HMCN1 are only significant in Asian HCC patients. Furthermore, Asian HCC patients with *TTN* and *HMCN1* mutations have shorter life expectancy than Asian HCC patients without these mutations. This study identified multiple genetic biomarkers that can aid in the recognition, surveillance, prognosis, and gene therapy of HCC. Further research is needed to understand the relationship between these gene mutations and epigenetic risk factors in HCC pathogenesis.

Statement of Ethics

This study uses data from public-use data sets and does not require IRB review.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Tane Kim conceived the project, designed the computational framework, and analyzed the data. Mykola Onyshchenko was in charge of overall planning and direction. Tane Kim and Mykola Onyshchenko wrote the manuscript with input from Danny Issa.

Data Availability Statement

This study is based upon data generated by the TCGA Research Network, which can be found at https://www.cancer.gov/tcga. All data generated or analyzed during this study can be found in figshare at https://doi.org/10.6084/m9.figshare.c.5666116.v1.

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