Cancer Cell International

RESEARCH

Open Access

Pathogenic nsSNPs of protein kinase C-eta with hepatocellular carcinoma susceptibility

Tayyaba Hussain[1], Yasmin Badshah[1*], Maria Shabbir[1], Fizzah Abid[1], Ghulam Murtaza Kamal[1], Amna Fayyaz[1], Janeen H. Trembley[2,3,4], Tayyaba Afsar[5], Fohad Mabood Husain[6] and Suhail Razak[5*]

Abstract

Background Hepatocellular carcinoma (HCC) is a global health concern. Due to late diagnosis and limited therapeutic strategies, HCC based mortality rate is exponentially increasing globally. Genetic predisposition is a non-avoidable intrinsic factor that could alter the genome sequence, ultimately leading to HCC. Protein kinase C eta (PKCη) is involved in key physiological roles, hence alteration in PKCη could aid in cancer progression. Research indicates association between non-synonymous (ns) SNPs and HCC onset. However, effect of nsSNP variants of PKCη on HCC development has not been explored yet. Hence, this study aimed to investigate the association between pathogenic nsSNPs of PKCη with HCC.

Methods Non-synonymous (missense) variants of PKCη were obtained from Ensembl genome browser. These variants were filtered out to obtain pathogenic nsSNPs of PKCη. Genotyping of nsSNPs was done through Tetra ARMS PCR. For that, blood samples of 348 HCC patients and 337 controls were collected. The clinical factors that influence HCC were studied. Relative risk (RR) and Odds Ratio (OR) with 95% confidence interval was calculated by Chi-square test and P-value < 0.05 was deemed significant.

Results Five nsSNP variants of PKCη including rs1162102190 (T/C), rs868127012 (G/T), rs750830348 (G/T), rs768619375 (T/C), and rs752329416 (T/C) were identified. The retrieved nsSNPs were frequently identified in HCC patients. However, rs752329416 T/C was significantly prevalent in patients having HCC family history. Moreover, all the variants were found in HCC patients manifesting the stage II than the advance stages of HCC.

Conclusion This study can be utilized to identify potential genetic markers for early screening of HCC. Moreover, consideration of further clinical factors, and mechanistic approach would enhance the understanding that how alteration in nsSNPs could impact the HCC onset.

Keywords nsSNPs, PRKCH, Hepatocellular Carcinoma, ARMS-PCR

*Correspondence:
Yasmin Badshah
yasmeenb_1982@yahoo.com
Suhail Razak
smarazi@ksu.edu.sa
[1]Department of Healthcare Biotechnology, Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Islamabad 44000, Pakistan
[2]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA
[3]Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA
[4]Minneapolis VA Health Care System Research Service, Minneapolis, MN, USA
[5]Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia
[6]Department of Food Science and Nutrition, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

Background

Cancer is a multifactorial and heterogeneous disease, involving genetic, cellular, and immunological dysregulation that makes its etiology unpredictable [1, 2]. HCC is a prevalent cancer type with a high recurrence rate. In 2022, 75–85% emerging cases of cancers belonged to HCC that made it ranked as the sixth prevalent cancer type in that year. High mortality rates in HCC patients are due to chemoresistance, metastasis, and the progressive nature of cancerous hepatic cells [3]. Risk factors for HCC include obesity, smoking, high BMI, viral infection, aflatoxin exposure, alcohol consumption, age, family history and a sedentary lifestyle [3, 4]. Genetic polymorphisms, particularly single nucleotide polymorphism cause deleterious impact to the normal physiology that contributes to HCC onset [5]. SNPs account for 90% of genomic variation and can bring deleterious effects by altering protein function [6]. Late diagnosis further increases HCC mortality rate as cancerous cells often remain asymptomatic [7].

The protein kinase C family, vital for cellular processes, is activated by transferring phosphate ions from ATP to serine/threonine domains [8, 9] PKCη is encoded by the PRKCH gene that is found on chromosome 14-q22-23. It is involved in drug resistance in various cancers, including HCC and breast cancer (Chida et al., 1998; He et al., 2022; Pal & Basu, 2017). PKCη is associated with cellular processes like proliferation and apoptosis and may contribute to tumorigenic activities when altered [10]. PKCη and PKC θ are reported as protagonists, while PKCι and PKCζ are recognized as inhibitors in many cancers. Genotypic frequency of pathogenic non-synonymous SNPs was not investigated in HCC patients and no significant biomarkers for early HCC diagnosis have been established in past studies [11, 12]. Hence, this study focused on finding the association of potential pathogenic non-synonymous SNPs of PKCη gene with hepatocellular carcinoma.

Methods

Sample collection

Blood samples of 348 HCC patients and 337 healthy individuals (controls) were collected from District Headquarter Hospital (DHQ) and Benazir Bhutto Hospital (BBH), which are situated in Rawalpindi, Pakistan. Prior to sample collection, patient consent and disease history information were taken. Ethical approval was granted by Institutional Review Board (IRB), Atta-ur-Rahman School of Applied Bioscience (ASAB), National University of Sciences and Technology (NUST). and was done keeping in view the Declaration of Helinski principles [13].

nsSNPs Data retrieval and primer designing

The five nsSNP variants of PKCη, including rs1162102190 (T/C), rs868127012 (G/T), rs750830348 (G/T), rs768619375 (T/C), and rs752329416 (T/C) were retrieved from Ensembl genome browser [14]. For genotyping analysis, two set of primers (two outer and two inner primers) for each SNP variant were designed through utilizing an online tool, PRIMER1 (https://primer1.soton.ac.uk/primer1.html). Primer set that fulfilled optimal parameters was selected for genotyping analysis. Furthermore, primers were validated by using in-silico tools, OligoCalc (http://biotools.nubic.northwestern.edu/OligoCalc.html) and OligoAnalyser (https://www.idtdna.com/pages/tools/oligoanalyzer).

Genotyping analysis

Genomic DNA was extracted from the blood samples of HCC patients and controls by utilizing phenol-chloroform method. Designed tetra primers were utilized to identify mutant and wild genotypes in HCC patients and healthy individuals. Tetra ARMS PCR was performed by Applied Biosystems™ Veriti™ 96-Well Thermal Cycler for amplification of genotypes specific DNA fragments. 20ul of the reaction mixture was prepared by adding 1ul each primer, 2ul DNA, 12ul of a master mix (Solis bio), and 2 ul of nuclease-free water. PCR product was examined on a 2% agarose gel using gel electrophoresis. DNA fragments of varying length were visualized by UV illuminator Gel-doc system.

Statistical analysis

Statistical association between PKCη variants and HCC risk was determined by Chi-square test. For analysis, GraphPad prism v10.3.2 software [15] was used. Multiple clinical factors were also assessed, and correlation was established with genotypic distribution. Moreover, Relative Risk (RR) and Odd Ratio (OR) were determined for each variant of PKCη with 95% confidence interval (95% CI). P value <0.05 indicated significant statistical association.

Results

PKCη variants association with HCC

Prevalence of 5 pathogenic non-synonymous variants of PKCη was explored in HCC patients and healthy individuals (Supplementary File S Table S1). The prevalence of these nsSNPs was compared in HCC patients and controls by studying the band lengths obtained through gel electrophoresis (Supplementary File S figure S1). It was revealed that variant's rs1162102190, wildtype genotype TT was strongly prevalent in 69.5% of HCC patients with odd ratio (OR) of 4.023 and relative risk (RR) of 2.013 (Fig. 1a). However, homozygous wildtype genotype GG of variant rs868127012 was found in 71.26% of

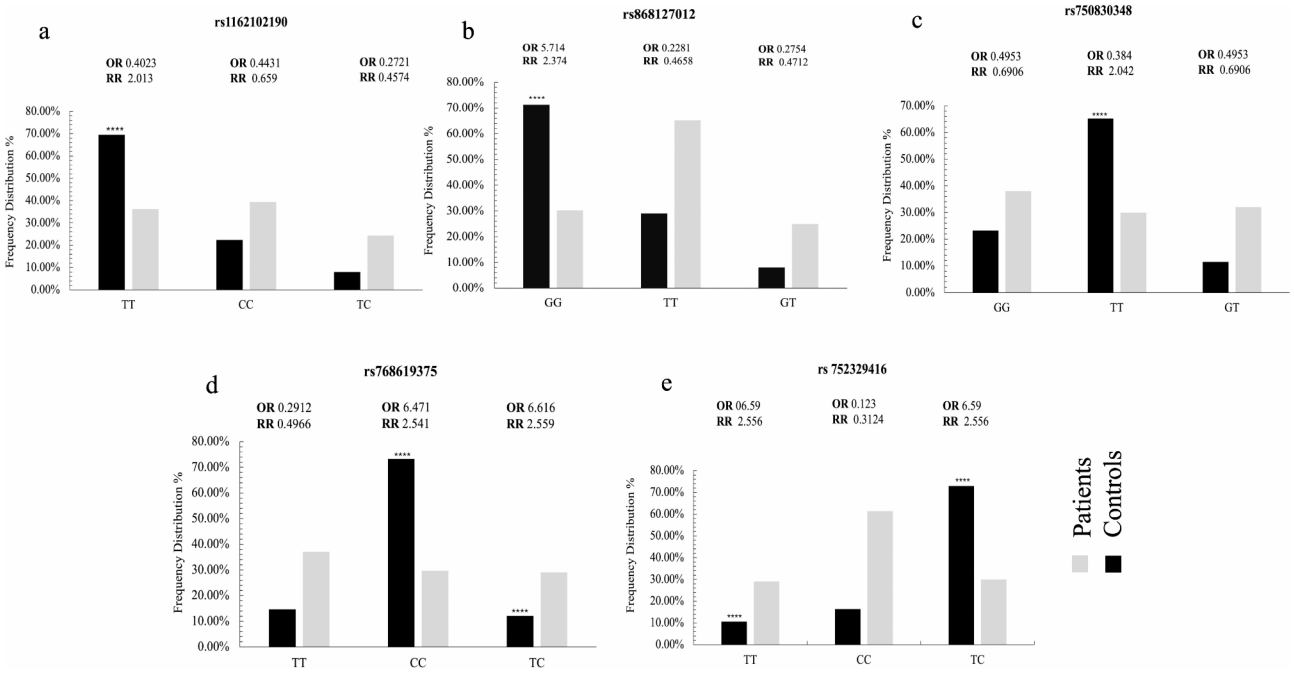

Fig. 1 Genotype frequency distribution of pathogenic PKCη nsSNP variants **(a)** rs1162102190 T/C **(b)** rs868127012 G/T **(c)** rs750830348 G/T **d)** rs768619375 T/C **(e)** rs752329416 T/C in HCC patients and healthy individuals. Chi-square test with P value < 0.05 was utilized to assess statistical significance

HCC patients with OR of 5.74 and RR of 2.37 (Fig. 1b). Homozygous mutant type genotype TT of another non-synonymous variant rs750830348 was found in 65.23% of HCC patients, with OR of 4.34 and RR of 2.04 (Fig. 1c) that indicates a higher percentage and more risk of HCC in patients with this genotype. Variant rs768619375 with homozygous mutant genotype CC was also identified in 73.27% of HCC patients with OR of 6.47 and RR of 2.54, suggesting higher risk of HCC onset in CC genotype carrying individuals (Fig. 1d). Variant rs752329416 with heterozygous TC genotype was also prevalent in 72.98% of HCC patients with OR of 6.59 and RR of 2.55 (Fig. 1e).

PKCη association with HCC associated clinical factors

It was revealed that wildtype genotype TT of non-synonymous variant rs752329416 T/C was frequently identified in hereditary cases of HCC as compared to non-hereditary cases, with OR of 6.11 and RR of 1.62. However, other variants including rs1162102190 T/C, rs868127012 G/T, rs750830348 G/T, rs768619375 T/C were not found to be significantly associated with both the categories (Table 1). Chi-square test with P value < 0.05 was utilized to assess statistical significance.

Moreover, consideration of further clinical factors, and mechanistic approach would enhance the understanding that how alteration in nsSNPs could impact the HCC onset.

The frequency of non-synonymous variants of PKCη were identified in HCC patients exhibiting fatty liver condition and non-fatty liver condition, meanwhile, no variants of PKCη (rs1162102190 T/C, rs868127012 G/T, rs750830348 G/T, rs768619375 T/C, and rs752329416 T/C) was significantly associated with fatty liver and non-fatty liver condition when studied in HCC patients Chi-square test with P value < 0.05 was utilized to assess statistical significance (Table 2).

The genotypic frequency of non-synonymous variants of PKCη was also identified in HCC patients that have undergone treatment or have not received treatment before. Moreover, none of the variants found were significantly relevant to either group (Table 3). Statistical analysis suggested the random distribution of variant genotypes in the treated and non-treated HCC patients. Chi-square test with P value < 0.05 was utilized to assess statistical significance.

The genotypic frequency of variants of PKCη was studied in HCC patients who were at different cancer stages (I-IV) (Supplementary File S Table S1). It was revealed that homozygous genotypes TT, GG of rs1162102190 T/C and rs868127012 G/T, respectively were more prevalent in HCC patients, manifesting stage II and stage III of cancer. Although, homozygous mutant genotypes TT and CC of variants rs750830348 and rs768619375 respectively were identified in HCC patients exhibiting stage II and stage III and heterozygous genotype TC of rs752329416 was more prevalent in HCC patients with stage II and Stage III (Fig. 2). Chi-square test with P value < 0.05 was utilized to assess statistical significance. Tukey's multiple comparisons test and two way ANOVA was performed to identify the statistical differences in

Table 1 Genotype frequency distribution of pathogenic PKCη nsSNP variants, rs1162102190 T/C, rs868127012 G/T, rs750830348 G/T, rs768619375 T/C and rs752329416 T/C in patients with hereditary and non-hereditary history of HCC

Variants	Genotypes	Family History *n* = 202	No Family History *n* = 146	OR	95% CI Odds Ratio	Relative Risk	95% CI Relative risk	*P* value
		n (%)	*n* (%)					
rs1162102190 T/C	**TT**	70.79%	67.12%	1.187	0.7571 to 1.891	1.076	0.8894 to 1.330	0.4818 NS
	CC	21.29%	24.14%	0.8500	0.5149 to 1.426	0.9327	0.7325 to 1.146	0.6022 NS
	TC	7.92%	8.22%	0.9606	0.4358 to 2.108	0.9831	0.6657 to 1.293	> 0.9999 NS
rs868127012 G/T	**GG**	73.27%	23%	1.107	0.6862 to 1.764	1.044	0.8610–1.298	0.0703 NS
	TT	43%	70%	0.5053	0.2512 to 1.027	0.7173	0.4644 to 1.006	0.0703 NS
	GT	30%	06%	1.349	0.0.7603 to 2.342	1.126	0.8878 to 1.366	0.3212 NS
rs750830348 G/T	**GG**	27%	29%	0.9989	0.6129 to 1.668	0.9995	0.7956 to 1.216	0.9999 NS
	TT	33%	70%	1.148	0.7347 to 1.783	1.060	0.8814 to 1.297	0.5689 NS
	GT	90%	01%	0.7345	0.3835 to 1.422	0.8707	0.6080 to 1.147	0.3922 NS
rs768619375 T/C	**TT**	86%	81%	0.7427	0.4163 to 1.339	0.8761	0.6477 to 1.118	0.3684 NS
	CC	25%	86%	1.311	0.8122 to 2.104	1.125	0.9198 to 1.414	0.2733 NS
	TC	89%	33%	0.8691	0.4570 to 1.691	0.9411	0.6735 to 1.214	0.7345 NS
rs752329416 T/C	**TT**	82%	42%	6.116	2.475 to 14.70	1.624	1.347 to 1.867	< 0.0001 ****
	CC	84%	86%	0.7594	0.4319 to 1.307	0.8856	0.6680 to 1.117	0.3915 NS
	TC	34%	71%	0.5982	0.3717 to 0.9555	0.8171	0.6877 to 0.9860	0.0424 *

* NS = Non-Significant

the frequency distribution of genotypes across different stages.

Discussion

HCC is a rising concern globally [5]. Diverse factors are responsible for HCC development and progression, including, genetic polymorphism that could potentially impact liver cancer progression [16]. The presence of SNPs, mainly non-synonymous, can modify the protein structure that can lead to improper folding and altered post translational modifications. Moreover, the protein function is directly impacted because of the variations. Also, the phenotype of the cell is changed. To add to this, the change in the protein structure and function directly affects the interaction of a protein with other proteins in the biochemical pathways that consequently causes the triggering on of oncogenes and switching off of tumor suppressor genes that makes the cancerous cells to grow uncontrollably [17, 18].

PKCη is expressed in epithelial cells and its abnormal expression is potentially associated with various cancers [19]. Defective PRKCH and its isoforms are reportedly involved in adenocarcinoma tumors and the promotion of the tumorigenic environment [20]. Few studies have been conducted regarding variants of PKCη. In one latest study, the variant rs3783782 of PKCη was found to be a potential risk factor of rheumatoid arthritis in the population of China [21].

Moreover, each disease is associated with SNP involving two alleles. One allele may be pathogenic and risk-associated while the other one may be tolerant and bring out a protective effect against the disease [22]. The SNP variations give rise to different genotypes. If the two alleles of a gene get mutated with the same SNP, the gene is said to be homozygous. Earlier studies have indicated homozygous genotypes or loss of heterozygosity to increase the cancer risk if the SNP is deleterious [23, 24]. On the other hand, heterozygous genotypes that involve a mutant allele and a normal allele may also put one at a

Table 2 Genotype frequency distribution of pathogenic PKCη nsSNP variants rs1162102190 T/C, rs868127012 G/T, rs750830348 G/T, rs768619375 T/C and rs752329416 T/C in HCC patients with fatty and non-fatty liver condition

Variants	Genotypes	Frequency Distribution		OR	95% CI Odds Ratio	RR	95% CI Relative risk	*P*-Value
		Fatty liver *n* = 237	Non fatty liver *n* = 111					
		n (%)	*n* (%)					
rs1162102190 T/C	**TT**	71.31%	64.86%	1.346	0.8196 to 2.161	1.103	0.9445 to 1.318	0.2620 NS
	CC	21.10%	25.23%	0.7926	0.4659 to 1.361	0.9255	0.7553 to 1.094	0.4096 NS
	TC	7.93%	9.91%	0.7829	0.3612 to 1.744	0.9177	0.6459 to 1.164	0.5407 NS
rs868127012 G/T	**GG**	71.73%	70.27%	1.073	0.6495 to 1.763	1.023	0.8794 to 1.218	0.8001 NS
	TT	9.70%	8.11%	1.218	0.5711 to 2.798	1.061	0.7998 to 1.276	0.6952 NS
	GT	18.57%	21.62%	0.8264	0.4787 to 1.452	0.9387	0.7570 to 1.115	0.5621 NS
rs750830348 G/T	**GG**	24.89%	22.52%	1.140	0.6720 to 1.912	1.042	0.8711 to 1.210	0.6879 NS
	TT	64.98%	66.67%	0.9277	0.5795 to 1.474	0.9765	0.8454 to 1.144	0.8093 NS
	GT	10.13%	10.81%	0.9296	0.4536 to 1.903	0.9765	0.7311 to 1.193	0.8516 NS
rs768619375 T/C	**TT**	13.92%	16.22%	0.8358	0.4457 to 1.534	0.9420	0.7347 to 1.136	0.6262 NS
	CC	73.42%	72.97%	1.023	0.6229 to 1.719	1.007	0.8651 to 1.203	> 0.9999 NS
	TC	12.66%	10.81%	1.196	0.5842 to 2.368	1.056	0.8257 to 1.256	0.7252 NS
rs752329416 T/C	**TT**	11.39%	13.51%	0.8229	0.4178 to 1.585	0.9367	0.7101 to 1.145	0.5982 NS
	CC	17.72%	18.02%	0.9800	0.5406 to 1.768	0.9935	0.8019 to 1.175	> 0.9999 NS
	TC	70.89%	68.47%	1.121	0.6903 to 1.821	1.038	0.8920 to 1.234	0.7065 NS

* NS = Non-Significant

high risk of developing cancer, if the mutant allele dominates the effect of normal allele [25]. These homozygous and heterozygous genotypes are essential in understanding the course of the disease, and how the disease responds to the treatment.

In the current study, 69.5% of the HCC patients were found to carry the wildtype TT genotype belonging to PKCη variant rs1162102190. Another wildtype genotype GG of the variant rs868127012 was found prevalent in 71.26% of HCC patients in comparison to the controls. Conversely, the mutant homozygous genotype TT and heterozygous genotype GT showed a protective effect. Moreover, the homozygous wildtype genotype GG and heterozygous mutant genotype GT of variant rs750830348 were not found to be significantly associated with HCC. However, the homozygous mutant genotype, TT of variant rs750830348 was more prevalent in 65.23% HCC patients, suggesting significant association of TT genotype with HCC risk. Moreover, the HCC onset was almost twice likely in patients carrying this genotype. Wildtype homozygous genotype TT of variant rs768619375 was also not correlated with HCC, while mutant homozygous genotype CC frequency was significantly more prevalent in 73.27% of HCC patients. Also, frequency of homozygous wildtype genotype TT of rs752329416 variant was significantly higher in healthy individuals, however, the mutant heterozygous genotype TC was identified in 72.98% HCC patients that eventually enhanced the HCC risk to about 2.55 times.

This is primarily the first study that has looked upon the genotypes of PKCη with reference to breast cancer. Other isoforms of PKC have been analyzed with respect to different cancer types. The results of this study correlate with past studies. In a study, the wildtype genotype AA corresponding to PKC γ nsSNP rs1331262028 was found to be more prevalent in HCC patients in comparison to controls [26]. In another study centered on HCC, mutant genotype AA belonging to nsSNP,

Table 3 Genotype frequency distribution of pathogenic PKCη nsSNP variants, rs1162102190 T/C, rs868127012 G/T, rs750830348 G/T, rs768619375 T/C and rs752329416 T/C in treated and non-treated HCC patients

Variants	Genotype	Treated *n* = 218	Non-Treated *n* = 130	OR	95% CI Odds Ratio	Relative Risk	95% CI Relative risk	*P* value
		n (%)	*n* (%)					
rs1162102190T/C	**TT**	69.72%	68.46%	1.061	0.6617 to 1.714	1.023	0.8634 to 1.235	0.8113 NS
	CC	21.10%	23.85%	0.8541	0.5107 to 1.420	0.9413	0.7529 to 1.135	0.5941 NS
	TC	9.17%	7.69%	1.212	0.5674 to 2.577	1.071	0.7759 to 1.329	0.6969 NS
rs868127012 G/T	**GG**	71.10%	76.92%	0.7381	0.4545 to 1.223	0.8973	0.7629 to 1.079	0.2610 NS
	TT	8.25%	9.23%	0.8850	0.4218 to 1.899	0.9540	0.6671 to 1.223	0.8439 NS
	GT	20.64%	13.85%	1.618	0.8885 to 2.920	1.177	0.9589 to 1.388	0.1112 NS
rs750830348 G/T	**GG**	24.77%	20.77%	1.256	0.7516 to 2.142	1.085	0.8909 to 1.284	0.4329 NS
	TT	65.14%	63.85%	1.058	0.6646 to 1.659	1.021	0.8659 to 1.222	0.8176 NS
	GT	10.09%	15.38	0.6173	0.3167 to 1.153	0.8178	0.5835 to 1.059	0.1734 NS
rs768619375 T/C	**TT**	14.22%	15.38%	0.9118	0.4953 to 1.703	0.9654	0.7385 to 1.188	0.7567 NS
	CC	74.77%	70.77%	1.224	0.7623 to 1.989	1.081	0.9031 to 1.329	0.4531 NS
	TC	11.01%	13.85%	0.7698	0.3978 to 1.478	0.9013	0.6590 to 1.141	0.4968 NS
rs752329416 T/C	**TT**	14.22%	19.23%	0.6963	0.3967 to 1.256	0.8644	0.6545 to 1.079	0.2303
	CC	14.22%	20.00%	0.6138	0.3458 to 1.105	0.8174	0.6101 to 1.033	0.1281
	TC	72.48%	60.77%	1.7	1.059 to 2.722	1.233	1.027 to 1.515	0.0246 *

* NS = Non-Significant

rs386134171 had higher frequency in patients than healthy individuals [27]. Genotype GG of PKCε variant rs1553369874 also exhibited an association with cervical cancer patients [28]. Another study reported that the TPMT gene exhibited mutant homozygous genotype CC of variant rs1142345, significantly associated with a higher mortality rate in treating Acute Lymphoblastic Leukemia patients [29]. These findings are supported by the fact that in PKCε, genotypes AA and CC of variant rs1553369874 and rs1345511001 respectively exhibited protective role, and no significant disease association was observed [28]. In gene MSMB, wildtype genotype and variant rs10993994 heterozygous genotype TC were significantly associated with prostate cancer patients [30].

Having the family history of HCC is considered as a risk factor that can surge HCC development [31]. This study explored the genotypic distribution of non-synonymous variants of PKCη in HCC patients with hereditary and non-hereditary history of HCC. Variant rs752329416 expressing TT genotype frequency was found significantly higher in HCC patients with family history of HCC that suggested SNP variant could be inherited that could likely increase the risk of developing HCC. Studies also point towards the germline genotypes to increase HCC risk [32]. The deleterious mutations and SNPs are causative agent behind cancer progression [33]. Apart from family history, fatty liver condition is also one of the major risk factors of HCC [34]. Genotyping of genes with SNPs or mutations increases the likelihood of HCC development in patients with fatty liver condition [35]. However, in this study, no association was established between five pathogenic non-synonymous SNP variants and fatty liver and non-fatty liver condition in HCC patients, indicating the need for wide research on these nsSNPs in a large cohort. Moreover, therapeutic outcomes for HCC vary based on disease severity and nature [36]. The past study specify towards the genotypes to influence treatment responses [37]. Conversely, when studying the genotypic distribution in treated and non-treated HCC patients, variant rs752329416 (T/C) showed the significant value when comparing five pathogenic PKCη nsSNPs data.

Moreover, cancer progression is assessed by the staging system. Each stage provides estimation of cancer severity

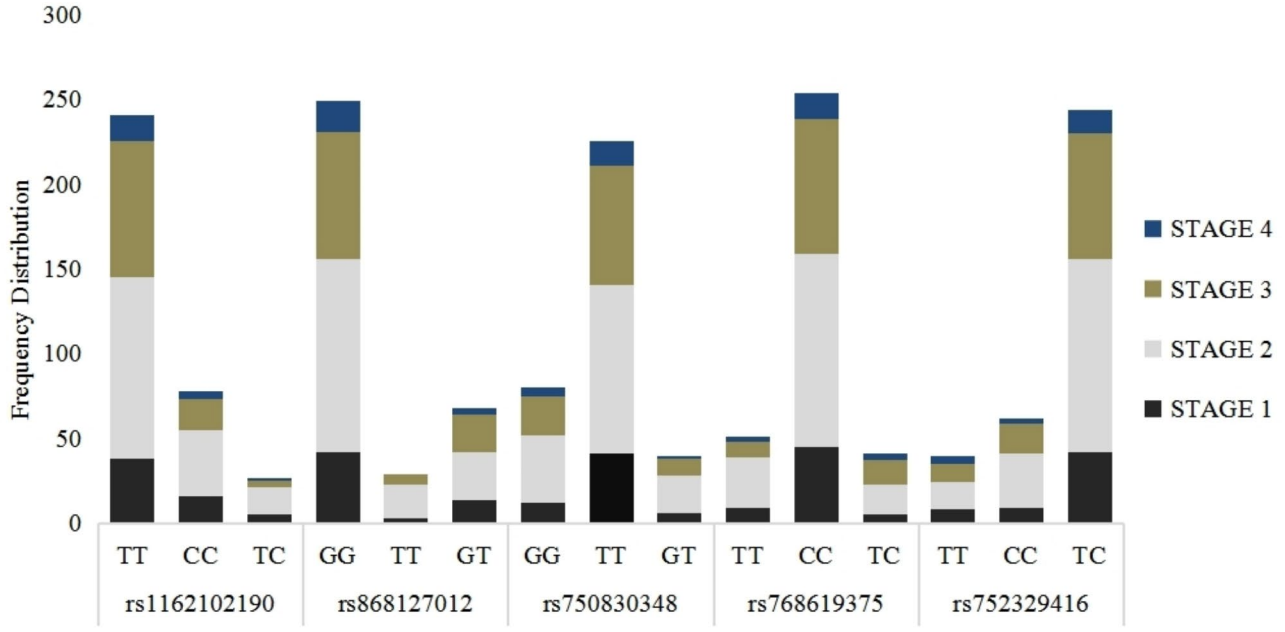

Fig. 2 Genotype frequency distribution of pathogenic PKCη nsSNP variants **(a)** rs1162102190 T/C **(b)** rs868127012 G/T **(c)** rs750830348 G/T **d)** rs768619375 T/C **(e)** rs752329416 T/C in HCC patients with early and advance stages (Stage I-IV)

and extent of tumour progression. In this study, homozygous genotypes TT and GG of variants of rs1162102190 T/C and rs868127012 G/T respectively, homozygous mutant genotypes TT and CC of rs750830348 and rs768619375 respectively, and heterozygous genotype TC were more prevalent in HCC patients who were at stage II and stage III. Previous study also highlights the role of genotypes rs2010963 and rs4604006 related to VEGF to influence patients' survival rate at different cancer stages [37].

Conclusion

In this study, PKCη variants rs1162102190 T/C, rs868127012 G/T, rs750830348 G/T, rs768619375 T/C, and rs752329416 T/C showed significant association with HCC development and progression in Pakistani population. Moreover, genotype TT of variant rs752329416 was identified in those HCC patients having familial HCC condition. Conversely, genotypic variants were not found different in HCC patients with fatty or non-fatty liver condition and only one nsSNP rs752329416 T/C appeared as significant ($P=0.02$) when comparing data of individuals who have undergone or not received prior treatment. Although, identified pathogenic non-synonymous SNP variants of PKCη were found to impact HCC development, but large data set, including individuals with multiple demographic background could help in understanding HCC pathogenesis. Moreover, consideration of further clinical factors, and mechanistic approach would enhance the understanding that how alteration in nsSNPs could impact the HCC onset.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12935-024-03536-6.

Supplementary Material 1

Acknowledgements

The authors extend their appreciation to the Researchers Supporting project number (RSPD2024R729), King Saud University, Riyadh Saudi Arabia for funding this project.

Author contributions

TH, MS, YB, AF, FA, JHT, SR, GMK, FMH, and TA designed the study, conceived the study and analyzed the results. TH, MS, YB, AF, FA, JHT, and SR conceived an initial part of the study, performed the experiment, and helped in compiling the results. MS and YB experimented. JHT, SR, GMK, FMH, and TA helped in writing the results. SR, TA, and GMK wrote the paper with input from all other authors TH, MS, YB, AF, FA, JHT, SR, GMK, FMH, and TA made a substantial contribution in the interpretation of data and revising the manuscript for intellectual content. NMA performed bioinformatics. All authors read and approved the final manuscript.

Funding

The authors extend their appreciation to the Researchers Supporting project number (RSPD2024R729), King Saud University, Riyadh Saudi Arabia for funding this project. The funding body has no role in designing the study.

Data availability

All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate
The experimental protocol for the use of human blood samples was approved (Ref: No: IRB No. 04-2019-03/06) by the ethical committee of Combined Military Hospital and ASAB, NUST. Informed consent was obtained from all subjects and/or their legal guardian(s).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 5 September 2024 / Accepted: 15 October 2024
Published online: 24 October 2024

References

1. Abdelatty A, Sun Q, Hu J, Wu F, Wei G, Xu H, Zhou G, Wang X, Xia H, Lan LJFC et al. Pan-cancer study on protein kinase C family as a potential biomarker for the tumors immune landscape and the response to immunotherapy. 2022, 9:798319.
2. Xu X, Peng Q, Jiang X, Tan S, Yang Y, Yang W, Han Y, Chen Y, Oyang L, Lin JJE et al. Metabolic reprogramming and epigenetic modifications in cancer: from the impacts and mechanisms to the treatment potential. 2023, 55(7):1357–70.
3. ME JF, Siegel RL, Isabelle Soerjomataram M, Ahmedin Jemal D. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. 2024.
4. Quiroz Reyes AG, Lozano Sepulveda SA, Martinez-Acuña N, Islas JF, Gonzalez PD, Heredia Torres TG, Perez JR. Garza Treviño ENJTiCR, Treatment: Cancer stem cell and hepatic stellate cells in hepatocellular carcinoma. 2023, 22:15330338231163677.
5. Shabbir M, Badshah Y, Khan K, Trembley JH, Rizwan A, Faraz F, Shah SA, Farooqi M, Ashraf NM, Afsar T. Association of CTLA-4 and IL-4 polymorphisms in viral induced liver cancer. BMC Cancer. 2022;22(1):518.
6. Talseth-Palmer BA, Scott RJ. Genetic variation and its role in malignancy. Int J Biomedical Science: IJBS. 2011;7(3):158.
7. Chakraborty E, Sarkar D. Emerging therapies for hepatocellular carcinoma (HCC). Cancers. 2022;14(11):2798.
8. Poole AW, Pula G, Hers I, Crosby D, Jones ML. PKC-interacting proteins: from function to pharmacology. Trends Pharmacol Sci. 2004;25(10):528–35.
9. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J Biol Chem. 1982;257(13):7847–51.
10. Basu A. The potential of protein kinase C as a target for anticancer treatment. Pharmacol Ther. 1993;59(3):257–80.
11. Motegi A, Sakurai S, Nakayama H, Sano T, Oyama T, Nakajima, TJPi. PKC theta, a novel immunohistochemical marker for gastrointestinal stromal tumors (GIST), especially useful for identifying KIT-negative tumors. 2005, 55(3):106–12.
12. Wang C, Jin M-S, Zou Y-B, Gao J-N, Li X-B, Peng F, Wang H-Y, Wu Z-D, Wang Y-P. Duan X-MJSJoG: diagnostic significance of DOG-1 and PKC-θ expression and c-Kit/PDGFRA mutations in gastrointestinal stromal tumours. 2013, 48(9):1055–65.
13. Rivera SC, Aiyegbusi OL, Ives J, Draper H, Mercieca-Bebber R, Ells C, Hunn A, Scott JA, Fernandez CV, Dickens AP. Ethical considerations for the inclusion of patient-reported outcomes in clinical research: the PRO ethics guidelines. JAMA Oncol. 2022;327(19):1910–9.
14. Howe KL, Achuthan P, Allen J, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, Azov AG, Bennett R, Bhai JJN. Ensembl 2021. 2021, 49(D1):D884-D891.
15. Mavrevski R, Traykov M, Trenchev I, Trencheva MJWTSC. Approaches to modeling of biological experimental data with GraphPad prism software. 2018, 13(1):242–7.
16. Wang H, Cao H, Xu Z, Wang D, Zeng Y. SNP rs2596542G > A in MICA is associated with risk of hepatocellular carcinoma: a meta-analysis. Biosci Rep. 2019;39(5):BSR20181400.
17. Yates CM, Sternberg MJ. The effects of non-synonymous single nucleotide polymorphisms (nsSNPs) on protein–protein interactions. J Mol Biol. 2013;425(21):3949–63.
18. Nishi H, Tyagi M, Teng S, Shoemaker BA, Hashimoto K, Alexov E, Wuchty S, Panchenko AR. Cancer missense mutations alter binding properties of proteins and their interaction networks. PLoS ONE. 2013;8(6):e66273.
19. Pal D, Outram SP, Basu A. Novel regulation of protein kinase C-η. Biochem Biophys Res Commun. 2012;425(4):836–41.
20. Aquino A, Bianchi N, Terrazzan A, Franzese O. Protein kinase C at the crossroad of mutations, cancer, targeted therapy and immune response. Biology. 2023;12(8):1047.
21. Zhuang Y, Di Y, Huang L, Zhu J. PRKCH polymorphism is associated with rheumatoid arthritis in a Chinese population. Biosci Trends. 2019;13(6):556–61.
22. Tan HJE. On the protective effects of gene SNPs against human cancer. 2018, 33:4–5.
23. Thomsen H, Chen B, Figlioli G, Elisei R, Romei C, Cipollini M, Cristaudo A, Bambi F, Hoffmann P, Herms SJBC. Runs of homozygosity and inbreeding in thyroid cancer. 2016, 16:1–11.
24. Nichols CA, Gibson WJ, Brown MS, Kosmicki JA, Busanovich JP, Wei H, Urbanski LM, Curimjee N, Berger AC. Gao GFJNc: Loss of heterozygosity of essential genes represents a widespread class of potential cancer vulnerabilities. 2020, 11(1):2517.
25. Miller DB, Piccolo SRJFG. Compound heterozygous variants in pediatric cancers: a systematic review. 2020, 11:493.
26. Abid F, Iqbal T, Khan K, Badshah Y, Trembley JH, Ashraf NM, Shabbir M, Afsar T, Almajwal A, Razak S, Analyzing PKC. Gamma (+ 19,506 A/G) polymorphism as a promising genetic marker for HCV-induced hepatocellular carcinoma. Biomark Res. 2022;10(1):87.
27. Abid F, Khan K, Badshah Y, Ashraf NM, Shabbir M, Hamid A, Afsar T, Almajwal A, Razak SJCCI. Non-synonymous SNPs variants of PRKCG and its association with oncogenes predispose to hepatocellular carcinoma. 2023, 23(1):123.
28. Zafar S, Khan K, Badshah Y, Shahid K, Trembley JH, Hafeez A, Ashraf NM, Arslan H, Shabbir M, Afsar T. Exploring the prognostic significance of PKCε variants in cervical cancer. BMC Cancer. 2023;23(1):819.
29. Cardoso de Carvalho D, Pereira Colares Leitão L, Mello Junior FAR, Vieira Wanderley A, Souza TPd A, Rodrigues Fernandes M, Santos S, Salim Khayat A. Association between the TPMT* 3 C (rs1142345) polymorphism and the risk of death in the treatment of acute lymphoblastic leukemia in children from the Brazilian Amazon Region. *Genes* 2020, 11(10):1132.
30. Shahkar G, Hashemi M, Eskandari E, Ziaee SAM, Basiri A, Narouie B, Bahari G. The rs10993994 functional polymorphism in the MSMB gene promoter increase the risk of prostate cancer in an Iranian population. Meta Gene. 2017;14:100–4.
31. McGlynn KA, Petrick JL, El-Serag HBJH. Epidemiology of hepatocellular carcinoma. 2021, 73:4–13.
32. Kubota N, Fujiwara N, Hoshida YJJCM. Clinical and molecular prediction of hepatocellular carcinoma risk. 2020, 9(12):3843.
33. Müller M, Bird TG, Nault J-CJJ. The landscape of gene mutations in cirrhosis and hepatocellular carcinoma. 2020, 72(5):990–1002.
34. Suresh D, Srinivas AN, Kumar DPJFO. Etiology of hepatocellular carcinoma: special focus on fatty liver disease. 2020, 10:601710.
35. Liu Y-L, Patman G, Leathart J, Piguet A-C, Burt A, Dufour J-F, Day C, Daly A, Reeves H. Anstee QJJoh: carriage of the PNPLA3 rs738409 C > G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. 2014, 61(1):75–81.
36. Yang C, Zhang H, Zhang L, Zhu AX, Bernards R, Qin W. Wang CJNrG, hepatology: evolving therapeutic landscape of advanced hepatocellular carcinoma. 2023, 20(4):203–22.
37. Scartozzi M, Faloppi L, Svegliati Baroni G, Loretelli C, Piscaglia F, Iavarone M, Toniutto P, Fava G, De Minicis S. Mandolesi AJIjoc: VEGF and VEGFR genotyping in the prediction of clinical outcome for HCC patients receiving sorafenib: the ALICE-1 study. 2014, 135(5):1247–56.

Publisher's note