Detection of SMARCB1 gene mutations in Egyptian hepatocellular carcinoma patients using targeted next-generation sequencing: A pilot study

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ABSTRACT

Background and objectives: Hepatocellular carcinoma (HCC) is the second most common type of cancer and has a significant mortality rate due to late identification. Although while early-stage cancer is becoming more curable, advanced forms of the disease have a poor prognosis because to a high probability of return. Understanding HCC's pathogenic process and its related genetic alterations is now crucial for effective treatment. This study aimed to detect the SMARCB1 mutations in HCC Egyptian patients using next-generation sequencing (NGS), and their relationships with clinicopathological traits were investigated.
Methods: This cross-sectional study involved 21 HCC Egyptian participants. Using NGS panel (AmpliSeq) containing the SMARCB1 gene.
Results: Among the HCC patients, the viral infections, hypertension and family history were reported as a risk factors for HCC with a significant association. As well as the clinicopathological features (ascites, portal vein invasion, metastasis and Child PUGH class) showed a significant association with HCC. The findings showed that the SMARCB1 gene had 31 single nucleotide variations (SNV), with an incidence of 86%. The alterations were distributed out in between deleterious, undefinable importance, and tolerated deviations, with missense variations (54.9%) accounting for the majority of the modifications. The clinicopathological characteristics and the SMARCB1 gene mutations did not significantly correlate.
Conclusion: This study concluded that the identification of various genetic variations in the SMARCB1 gene can aid in diagnosing and prognosis of HCC.

Keywords: *Hepatocellular carcinoma (HCC), next generation sequencing (NGS), SMARCB1 gene, genetic alternations.*

1. INTRODUCTION

The fourth leading cause of cancer-related deaths worldwide and the fifth most prevalent cancer overall is hepatocellular carcinoma (HCC) (Yang *et al*., 2019). HCC accounts for 7% of all malignancies with 854,000 new cases and 810,000 deaths every year. Approximately, 90% of primary liver tumors are caused by HCC diagnosis

(Akinyemiju *et al*., 2017). Hepatitis B and C viruses (HBV and HCV), liver cirrhosis and excessive alcohol use are the main environmental and genetic risk factors for HCC (NASH). Diabetes, tobacco usage, and genetic disorders are a few more (McGlynn *et al*., 2021). Usually HCC patients have already reached an advanced stage when they are diagnosed. Due to the complex molecular pathways and cellular heterogeneity of HCC, traditional clinical indicators such alpha-fetoprotein (AFP), TNM staging system, and vascular invasion have a poor capacity for predicting the prognosis of HCC. Thus, new and more precise techniques are needed to comprehend HCC development principles in order to facilitate early identification, forecast the prognosis, and guide personalized treatment (Chen *et al*., 2020).

Cancer is characterized by genetic alterations that alter cell fate. Previous study showed the biological mechanism underlying these mutations reflects what occurs within the cell (Fares *et al*., 2020). It is generally believed that two to eight so-called driver gene mutations as well as multiple passenger gene mutations are required for the growth of tumors (Llovet *et al*., 2021). The investigation of cancer genomes and the identification of driver changes have been made possible by developments in next-generation sequencing (NGS). These developments have made it possible to process the HCC genome, which has allowed researchers to identify somatic mutations, structural changes, HBV integration, RNA editing, and modifications to retrotransposons (Bousali *et al*., 2021). Several studies have identified 12 genes as having recurrent mutations as important HCC-causing factors (Huang *et al*., 2018). ARID2, a tumor suppressor that is frequently mutated in HCC patients, is a member of the SWI/SNF-related chromatin remodeling complexes (Loesch *et al*., 2020).

SMARCB1 (SNF5, BAF47, and INI1) is a common subunit of SWI/SNF complexes, but SWI/SNF complexes can contain a variety of combinations, such as the mutually exclusive catalytic subunits SMARCA4 (BRG1) or SMARCA2 (BRM) and core subunits SMARCC1 (BAF155) or SMARCC2 (BAF170) (Jancewicz et al., 2019; Seong Hwi Hong *et al*., 2021). The potential tumor suppressor gene SMARCB1 is located at 22q11.2 (Dogan *et al*., 2020).Several research have examined SMARCB1's potential additional functions in malignancies, particularly HCC (Sun *et al*., 2016; Mochizuki *et al*., 2018).

Therefore, the current study's objectives include identifying SMARCB1 mutations using next-generation sequencing (NGS) in Egyptian HCC patients and examining the relationship between these mutations and clinicopathological characteristics.

2. PATIENTS AND METHODS

2.1. Study design and participants

This study included 21 HCC patients who were recruited prospectively from the inpatient and outpatient clinics of the oncology clinic at the Liver National Institute-Menoufia University in Egypt. Age, sex, history of (hepatitis, diabetes mellitus, hypertension), smoking, presence of cirrhosis according to Heidelbaugh and Bruderly **(**2006), clinical presentations, laboratory parameters, Child-Pugh class, size and number of the primary tumor. Menoufia University's Ethics Committee gave the study their approval (NLI IRB procedure 00003413, December 2020).The study did not include any other cancer patients.

2.2. Sample collection and cell free DNA extraction

Following the collection of peripheral blood samples (3 mL) in EDTA-containing tubes, plasma was separated within an hour using two centrifugation stages of 2,000 xg at

4°C for 10 minutes, followed by 16,000 xg at 4°C for 10 minutes (Jeong *et al*., 2019). Immediately after collection, plasma samples were aliquoted and kept at -80°C for up to nine months. According to the manufacturer's recommendations, circulating cell free DNA was extracted from plasma using the QIAamp® DSP Virus spin kit Version 1 (QIAGEN, Hilden, Germany).

2.3. Next generation sequencing

The genomic DNA was extracted and purified using the Gene JET purification kit (K0721) from Thermo scientific. They contained the Ion AmpliSeqTM HiFi Master Mix and the Ion AmpliSeqTM customised NGS panel covering the SMARCB1 gene (version 2; Thermo Fisher Scientific, Inc.) (Ion AmpliSeqTM Library kit 2.0, Thermo Fisher Scientific, Inc). The library was subsequently quantified in accordance with the instructions using the ion library TaqMan® Quantitation Kit from Thermo Fisher Scientific, Inc. The templates were enhanced and equipped using the Ion OneTouchTM2 system (Life Technologies). Thermo Fisher Scientific, Inc.'s Ionsphere quality control kit was used to make sure that between 10% and 30% of ISPs produced were template-positive. The template ISPs were loaded onto Ion 316TM chips upon enrichment, and then sequenced in accordance with the manufacturer's instructions using the IonPGMTM Sequencing Hi-Q view kit v2 and PGMTM (Life Technologies) (Morishita *et al*., 2018).

2.4. Bioinformatics data analysis

Thermo Fisher's Ion reporter server 5.10 was used to analyze normal and tumor samples using the default plugin parameters for the ion ampliseq custom panel technique. Using Torrent Suite, data was compared to Human Genome Version 19 (hg19) (version 3.6.2; Thermo Fisher Scientific, Inc.). The Coverage Analysis plug-in was used (version 3.6; Thermo Fisher Scientific, Inc.). Quality >20, average base coverage >500x reads, allele frequency >10%, and general uniformity >80% were the cutoffs. A plug-in called Variant Caller discovered mutations (version 3.6; Thermo Fisher Scientific, Inc.). The Integrated Genome Viewer (IGV) from the Broad Institute (www.broadinstitute.org) was used to verify each mutation (Helga *et al*., 2013).

2.5. Statistical analysis

Data for categorical variables were expressed as frequencies and percentages, and data for continuous variables were expressed as mean Standard Deviation or median (IQR). The risk associated with each group was determined using the odds ratio (OR) and 95% confidence intervals (CI). The threshold for statistical significance was set at $P<0.05$. SPSS version 28 was used for the statistical analysis (Chicago, IL, USA).

3. RESULTS

3.1. Demographic and clinical characteristics

The study population comprised of 18 (85.7%) males and 3 (14.3%) females. Of these, 13 (61.9%) were <60 years old and 8 (38.1%) were ≥60 years old, with the mean age (62.19 ±8.85) and median (63) years. The serum level of AFP in HCC patients ranged from 4.9 to 42443 ng/ml, with the mean (2417.07± 9230.79). A total of 19 patients had HCV and 1 patient had HBV, 13 (61.9%) had bilharzia antibodies. Over 47.6% of HCC patients had co-morbidities, diabetes (33.3%) and hypertension (14.3%) were among the common co-morbid conditions. The full patient characteristics and risk factors are described in Table 1.

3.2. Clinicopathological features

Table 2 lists the clinicopathological features of the patients with HCC. Four cases had ascites, in which 3 were minimal and 1

moderate, HCC had PVI in 3 cases (14.3%) among the frequently detected radiological imaging features. Three patients had metastasis in lymph node and lung (14.3% for each). The majority of patients (76.2%) Child PUGH class A, 957.1%) had multiple tumors, and the tumors' size in (81.0%) of the patients was >5 cm. The Barcelona Clinic Liver Cancer (BCLC) staging system's classification of the HCC stages. Over (33.35%) of patients had primary (BCLC-A) and of advanced (BCLC-C) for each, followed by 23.8% had (BCLC-B) and 9.5% had a terminal (BCLC-D) stage HCC.

3.3. Mutation Identification of SMARCB1 gene

In all 21 patients underwent SMARCB1 gene sequencing, mutations were identified in 15 patients. Thirty six somatic mutations were detected, of these 86 % (31/36) were single nucleotide variants (SNVs), 2.8% (1/36) were copy number variants (CNVs), 5.6% (2/36) were multi-nucleotide variant (MNVs) and 5.6% (2/36) were insertions/deletion variants (INDELs). Among SNVs, 54.9% (17/31) were missense mutations, 16.1% (5/31) were nonsense mutations, and 29% (9/31) were intron variants (Table 3). Summary of mutations were represented in Figure 1.

3.4. Correlation Analyses between SMARCB1 Gene Mutation and Clinical Characteristics

Regarding the association between the SMARCB1 gene mutation and clinical characteristics represented in Table 4. The mean age of patients with mutant and wild type were (62.4±8.68 and 61.66±9.27 respectively), with insignificant difference (P=0.864). Moreover, the mean value of AFP among patients with mutant type was 3422.12±10878.99 and in wild type patients was 21.34± 14.2, but this variation is not significant (P=0.460). No significant statistical differences were observed between wild and mutant type. Moreover, the clinicopathological features association with mutations are represented in Table 5.

4. DISCUSSION

Precision medicine's primary goal when treating cancer patients is to modify clinical management in accordance with targeted molecular profiling. More and more cancer patients are having their somatic mutations identified through next-generation sequencing, and this knowledge can help guide therapy choices (Kassem *et al*., 2018). Here, we described the SMARCB1 gene mutations in Egyptian patients with HCC and examined their relationships to clinicopathological characteristics and prognosis. In healthy cells, SMARCB1 is a well-known tumor suppressor, but when it is suppressed, it becomes extremely tumorigenic. In many cancers, miRNA regulation, gene mutation, and/or gene deletion are the mechanisms causing weak SMARCB1 expression (Kalimuthu and Chetty, 2016). As a potential therapeutic target for HCC and an important prognostic indicator, SMARCB1 may contribute to the suppression of the disease (Hu *et al*., 2020).

Risk factors for HCC include male gender, smoking, obesity, diabetes, and co-infection with either HBV or HIV (Samant *et al*., 2021). It is commonly accepted that male patients have a much higher incidence of HCC than female patients (McGlynn *et al*., 2021). In addition, the most recent study discovered that 85.7% of HCC patients are male. Yet, the analysis of the impact of sex differences on disease outcomes showed up with conflicting results (Braunwarth *et al*., 2020; Rich *et al*., 2020). HCC is growing increasingly and more prevalent among elderly people worldwide (Arora *et al*., 2020). The recent study found that 61.9% of participants with HCC are 60 years of age or older. Hence, a number of factors could be

linked to the age difference (Mak and Kramvis, 2021).

HCC is a complicated disease with several risk and cofactors. Virus infections, smoking, and consuming alcohol are risk factors for HCC (Llovet *et al*., 2021). There was no statistically significant association between smoking and HCC because there were only 9.5% of smokers in the research sample. There is growing evidence that having a family history of liver cancer considerably raises the risk of HCC with an aggressive character. In this study 19.0% of the HCC patients reported a positive family history. This discovery supports that made by Turati *et al*., (2012), who discovered a link between a family history of liver cancer and an increased chance of getting HCC. Patients with diabetes mellitus, whose incidence is steadily rising globally, are two to three times more likely to develop HCC (Li *et al*., 2017). In the current study 33.3% of patients had DM, which was not correlated significantly. Primary hypertension and HCC mortality have been associated, however the reasons for this association are not entirely understood (Lopez-Lopez *et al*., 2020). In this study 14.3% of the 21 HCC patients also had hypertension, and this association was significant (P = 0.01).

In this study, bilharzia antibodies were present in 61.9% of the 21 HCC patients. Our findings concur with those of Ramadan *et al*. (2021), who found that 67.7% of Egyptian HCC patients had schistosoma antibodies. Bilharzia was reported in this analysis as a risk factor for HCC (OR=1.625, 95% CI 0.558-4.73). According to the results of the current study, HCV was the main contributing factor to the development of HCC, and it is still the main contributing factor now (Brozzetti *et al*., 2021). There is a 15–20-fold greater risk of developing HCC compared to those without chronic HCV infection (Axley *et al*., 2017). In this study, anti-HCV antibodies were present in 90.5% of HCC patients. A 9.5-fold greater risk of HCC was reported in the current analysis when HCV infection was present. In contrast, HBV was detected in 4.7% of individuals. Although the ideal AFP cutoff value is up for debate, the serum AFP level is still a critical diagnostic marker for the detection of HCC. There may be a rise in serum AFP due to cirrhosis, chronic liver disease, and various malignancies (Liu *et al*., 2014; Reim *et al*., 2017). In the current study, the mean serum AFP level was 2417.07± 9230.79, the median was 42 ng/dL. According to Zhang *et al*. (2020), a blood AFP level of 400 ng/dL provides the highest sensitivity and specificity for detecting HCC.

Ascites and HCC patients were shown to be statistically significantly correlated in this study (P=0.023). The results of Hsu *et al*., (2012), who discovered that 23% of patients had ascites at the time of diagnosis, are consistent with this observation. Studies have shown that the prevalence of portal vein invasion (PVI), which is found in 30% to 62% of cases with advanced HCC, varies; it is undoubtedly underreported (Shehta *et al*., 2021). A positive PVI that was significantly associated with HCC was present in 14.3% of patients in the current study (P = 0.01). Local lymph nodes and the lung are the most prevalent extrahepatic HCC metastatic sites in terms of frequencies (Becker *et al*., 2014). The metastatic sites in this study were the lymph nodes and the lungs (14.3% for each). For many years, the most widely used method to assess liver function and gauge the efficacy of treatments was the traditional Child-Pugh grading system (Zhao *et al*., 2020). Child's A predominated in 21 HCC patients, followed by Child's B with 14.3% and Child's C with 9.5%. In these patients, 42.9% had multiple lesions while 57.1% had just single. According to BCLC staging, stages A and C were more common (33.35% for each). The study population's CT scan results showed that big tumors measuring

more than 5 cm in diameter were seen in 81.0% of the 21 cases of HCC found by CT scan. Stages A and C (33.35% for each) were more common, according to the BCLC staging.

In the present study, we used targeted sequencing to evaluate a group of HCC Egyptian patients for genetic variations of the SMARCB1 gene. There were some newly discovered and previously characterized genetic variations that might or might not have biological importance. SMARCB1 is one of the most potent tumor suppressors that is frequently altered in malignancies (Shain and Pollack, 2013; Langer *et al*., 2019). Our findings are the earliest investigation of the SMARCB1 in the genetic epidemiology of HCC Egyptian patients. Also, a limited data about the association between HCC and SMARCB1 gene mutations were reported. According to our current results, a SMARCB1 mutation could be present in as many as 71.4% of HCC cases. The inability to distinguish between benign and malignant tumors when SMARCB1 mutations are present may make it challenging to determine the risk of HCC. This study identified 54.9% were missense mutations and 16.1% were nonsense mutations. In the present study, through data mining analyses, we visualized the prognostic landscape of SMARCB1 mutation in HCC.

The association between the SMARCB1 mutations and clinicopathologic features showed a non-significant association with all studied variables. Our result suggests that the male predominance in the SMARCB1 mutation rate is a reflection of a higher frequency of HCC in males. Our study of smoking's different aspects suggests that smoking is a predictor of a decreased SMARCB1 mutation rate. No significant relationship between a family history and SMARCB1 mutation in HCC was found in this study. Our findings show that HCV infection is present in every case of BRAF mutation, proving that HCV causes SMARCB1 mutation in HCC patients. This study findings show that HCV infection may induces SMARCB1 mutation in patients with HCC. Moreover, the mean value of AFP among patients with mutant type was 3422.12±10878.99 and in wild type patients was 21.34± 14.2, but this variation is not significant (P=0.460).

5. CONCLUSION

At present, it is important for clinicians to facilitate genetic testing as use of NGS led to the discovery of a number of unique gene mutations in HCC, including both confirmed and disproven mutations. The origin and progression of HCC are best understood because to these findings, which offer new view. Larger patient cohorts are required in order to fully comprehend SMARCB1 genetic alterations and their impact on the development of HCC. To fully explain genetic alterations in HCC, additional studies are needed, including whole exome sequencing.

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Table 1: HCC patient characteristics and risk factors

Variables		HCC (n = 21)		*P*-value
		No.	**%**	
Smoking	**Yes**	2	9.5%	0.213
	No	14	66.7%	
	Ex. smoker	5	23.8%	
Bilharzia	**Yes**	13	61.9%	0.373
	No	8	38.1%	
Hepatic encephalopathy	**Yes**	0	0.0%	0.01*
	No	21	100.0%	
Family history	**Yes**	4	19.0%	0.023*
	No	17	81.0%	
Viral infection	**HCV**	19	90.5%	0.005*
	HBV	1	4.75%	0.005*
	NBNC	1	4.75%	0.005*
Comorbidities	**DM**	7	33.3%	0.213
	HTN	3	14.3%	0.01*

Table 2: Clinicopathological features of HCC patients

Variables	HCC (n = 21)		*P*- value
	No.	**%**	
Ascites			
No	17	81.0%	0.023*
Minimal	3	14.3%	
Moderate	1	4.7%	
Portal Vein Invasion			
Negative	18	85.7%	0.01*
Positive	3	14.3%	
LN Metastasis			
Negative	18	85.7%	0.01*
Positive	3	14.3%	
Lung Metastasis			
Negative	18	85.7%	0.01*
Positive	3	14.3%	
Child PUGH class			
A	16	76.2%	0.05*
B	3	14.3%	
C	2	9.5%	
Single	9	42.9%	--
Multiple	12	57.1%	
Small (<3 cm)	3	14.3%	--
Medium (3 - 5 cm)	1	4.7%	
Large (>5 cm)	17	81.0%	
A	7	33.35%	

B	5	23.8%	--
C	7	33.35%	
D	2	9.5%	

Table 3: Summary of SMARCB1 gene variation in HCC detected by targeted sequencing.

Locus	Types	Variant Frequency	Amino Acid Change	Coding
chr22:24133953	CNV	1	0	0
chr22:24133980	SNV	0.04	p.Tyr44Cys	c.131A>G
chr22:24134006	SNV	0.04	p.Arg53=	c.157C>A
chr22:24134009	SNV	0.04	#N/A	#N/A
chr22:24134014	SNV	0.04	#N/A	#N/A
chr22:24134022	SNV	0.44	p.Glu58Gly ...(2)	c.173A>G ...(2)
chr22:24134049	SNV	0.04	p.Ser67Ter	c.200C>G
chr22:24143207	SNV	0.08	p.Cys147Ser	c.439T>A
chr22:24143209	SNV	0.04	p.Cys147Ter	c.441C>A
chr22:24143210	SNV	0.04	p.Ser148Pro	c.442T>C
chr22:24143216	SNV	0.16	p.Thr150Pro ...(3)	c.448A>G ...(3)
chr22:24143217	SNV	0.04	p.Thr150Ser	c.449C>G
chr22:24143219	SNV	0.04	p.Ile151Phe	c.451A>T
chr22:24143220	SNV	0.08	p.Ile151Thr	c.452T>C
chr22:24143220	SNV	0.16	p.Ile151Thr	c.452T>C
chr22:24143304	SNV	0.04	#N/A	#N/A
chr22:24143306	MNV	0.04	#N/A	#N/A
chr22:24143309	SNV	0.04	p.?	c.500+41G>A
chr22:24145511	SNV	0.12	p.His177Pro	c.530A>C
chr22:24145512	SNV	0.12	#N/A	#N/A
chr22:24145524	SNV	0.08	#N/A	#N/A
chr22:24145556	SNV	0.04	p.Asp192Gly	c.575A>G
chr22:24145594	SNV	0.12	#N/A	#N/A
chr22:24145595	SNV	0.12	#N/A	#N/A
chr22:24145597	SNV	0.04	#N/A	#N/A
chr22:24176306	SNV	0.04	p.?, p.?	c.1119-22G>C, c.*708C>G
chr22:24176309	MNV	0.04	p.?, p.?	c.1119-19_1119-18delinsAG, c.*705AG>CT
chr22:24176319	SNV	0.04	#N/A	#N/A
chr22:24176321	SNV	0.04	p.?, p.?	c.1119-7C>G, c.*693G>C
chr22:24176322	INDEL	0.04	#N/A	#N/A
chr22:24176323	SNV	0.08	#N/A	#N/A
chr22:24176335	INDEL	0.04	p.Arg376MetfsTer69, p.? ...(2)	c.1126_1127insT, c.*679T>AT ...(2)
chr22:24176337	MNV	0.04	p.[Arg376Ser;Arg377Ser], p.Arg377LeufsTer68, p.?, p.?	c.1128_1130delGCGinsTTC, c.1129_1130insT, c.*676G>AG, c.*677CGC>GAA
chr22:24176342	SNV	0.04	p.Leu378His, p.?	c.1133T>A, c.*672A>T
chr22:24176344	SNV	0.04	p.Ala379Pro, p.?	c.1135G>C, c.*670C>G
chr22:24176345	SNV	0.04	p.Ala379Val, p.?	c.1136C>T, c.*669G>A

Table 4: Comparison between wild and mutant type of SMARCB1 gene with clinical characteristics.

Variables		Mutant type (n=15)		Wild type (n=6)		OR (95% CI)	*P*-value
		No.	%	No.	%		
Sex	**Male**	12	80%	6	100%	0.80 (0.205-3.125)	0.748
	Female	3	20%	0	0%		
Smoking	**Yes**	1	6.7%	1	16.7%	0.727 (0.094-5.633)	0.760
	No	11	73.3%	4	66.6%		
	Ex. smoker	3	20%	1	16.7%		
Bilharzia	**Yes**	9	60%	4	66.6%	0.90 (0.1986-4.079)	0.891
	No	6	40%	2	33.4%		
Hepatic encephalopathy	**Yes**	0	0.0%	0	0%	1.00 (0.262-3.815)	1.00
	No	15	100.0%	6	100%		
Family history	**Yes**	2	13.3%	2	33.4%	1.30 (0.299-5.637)	0.726
	No	13	86.7%	4	66.6%		
Viral infection	**HCV**	15	100%	4	66.6%	1.50 (0.351-6.418)	0.584
	HBV	1	6.7%	0	0%	1.26 (0.045-35.118)	0.892
	NBNC	0	0%	2	33.4%	0.08 (0.003- 1.99)	0.126
Comorbidities	**DM**	4	26.7%	3	50%	0.533 (0.09- 3.14)	0.487
	HTN	1	6.7%	2	33.4%	0.20 (0.015-2.64)	0.222

Table 5: Comparison between wild and mutant type of SMARCB1 gene with clinicopathological features.

Variables	Mutant type (n = 15)		Wild type (n=6)		*P*- value
	No.	**%**	**No.**	**%**	
Ascites					
No	12	80%	5	83.3%	0.955
Minimal	2	13.3%	1	16.7%	
Moderate	1	6.7%	0	0.0%	
Portal Vein Invasion					
Negative	13	86.7%	5	83.3%	0.956
Positive	2	13.3%	1	16.7%	
LN Metastasis					
Negative	13	86.7%	5	83.3%	0.956
Positive	2	13.3%	1	16.7%	
Lung Metastasis					
Negative	13	86.7%	5	83.3%	0.956
Positive	2	13.3%	1	16.7%	
Child PUGH class					
A	11	73.4%	5	83.3%	0.859
B	2	13.3%	1	16.7%	
C	2	13.3%	0	0.0%	
CT radiological findings					
Tumor number					
Single	7	46.7%	2	33.3%	0.719
Multiple	8	53.3%	4	66.7%	
Tumor Size					
Small (<3 cm)	3	20%	0	0.0%	0.658
Medium (3 - 5 cm)	1	6.7%	0	0.0%	
Large (>5 cm)	11	73.3%	6	100%	
BCLC					
A	6	40%	1	16.7%	0.459
B	3	20%	2	33.3%	
C	4	26.7%	3	50%	
D	2	13.3%	0	0.0%	

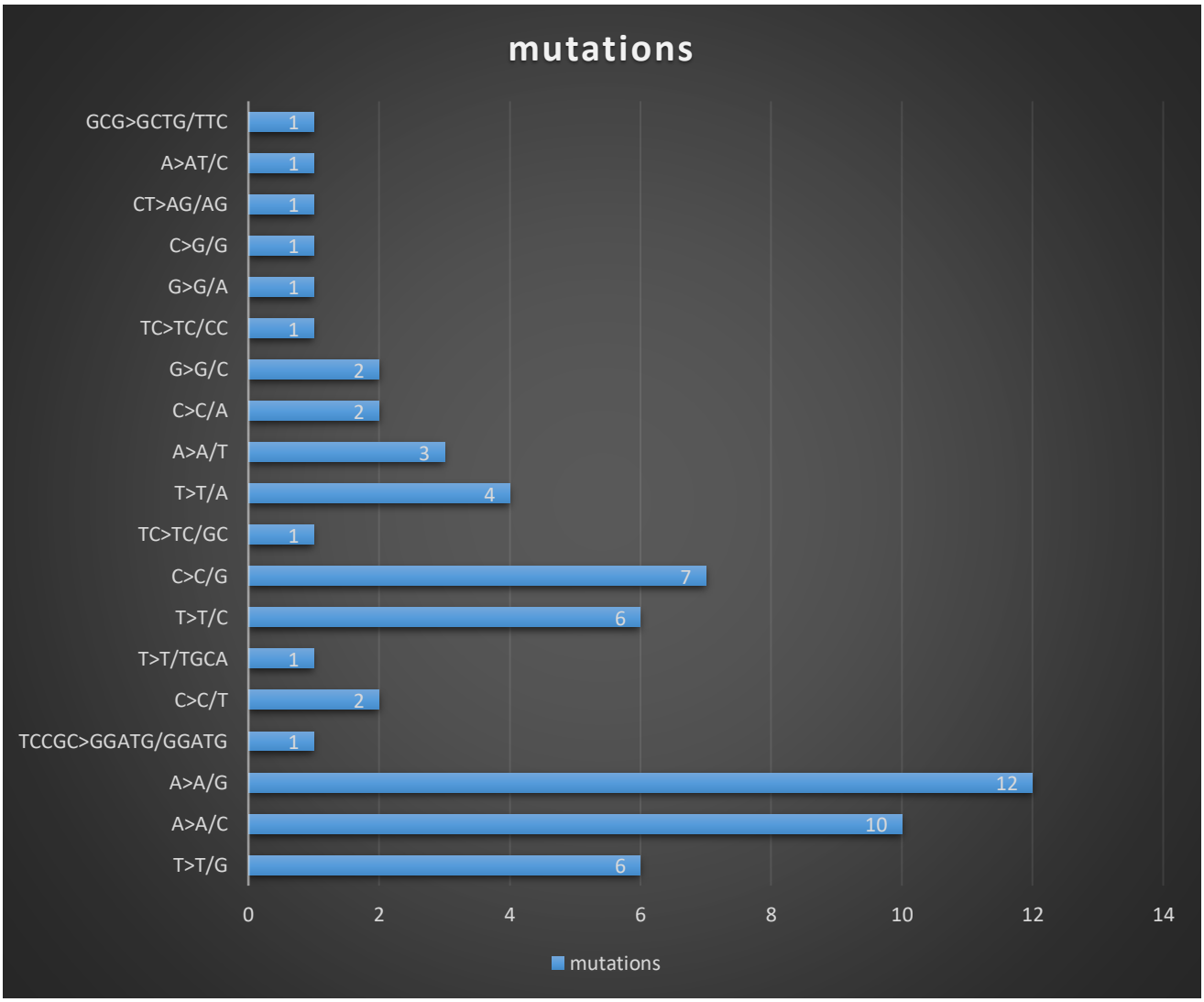

Figure 1: Summary of mutations in SMARCB1 gene among HCC patients**.**