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The significance of Exonuclease 1 K589E polymorphism on hepatocellular carcinoma susceptibility in the Turkish population: a case–control study

Süleyman Bayram · Hikmet Akkız · Aynur Bekar · Ersin Akgöllü · Selçuk Yıldırım

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Abstract Exonuclease 1 (Exo 1) is an important nuclease involved in mismatch repair system that contributes to maintain genomic stability, to modulate DNA recombination, and to mediate cell cycle arrest. A guanine (G)/adenine (A) common single nucleotide polymorphism at first position of codon 589 in Exo 1 gene determines a glutamic acid (Glu, E) to lysine (Lys, K) (K589E) aminoacidic substitution which may alter cancer risk by influencing the activity of Exo 1 protein. Exo 1 K589E polymorphism has been studied in various cancers, but its association with hepatocellular carcinoma (HCC) has yet to be investigated. To determine the association of the Exo 1 K589E polymorphism with the risk of HCC development in a Turkish population, a hospital-based case–control study was designed consisting of 224 subjects with HCC and 224 cancer-free control subjects matched for age, gender, smoking and alcohol status. The genotype frequency of the Exo 1 K589E polymorphism was determined by using a polymerase chain reaction–restriction fragment length polymorphism assay. Our data shows that the Lys/Lys genotype of the Exo 1 K589E polymorphism is associated with increased risk of HCC development in this Turkish population [odds ratio (OR) = 2.15, 95% confidence interval (CI): 1.13–4.09, $P = 0.02$]. Furthermore, according to stratified analysis, a significant association was observed between the homozygote Lys/Lys genotype and HCC risk in the subgroups of male gender (OR = 2.67, 95% CI: 1.27–5.61, $P = 0.009$) and patients with non-viral-related HCC (OR = 3.14, 95% CI: 1.09–8.99, $P = 0.03$). Because our results suggest for the first time that the Lys/Lys homozygote genotype of Exo 1 K589E polymorphism may be a genetic susceptibility factor for HCC in the Turkish population, further independent studies are required to validate our findings in a larger series, as well as in patients of different ethnic origins.

Keywords Case–control study · Exonuclease 1 · Exo 1 K589E polymorphism · Genetic susceptibility · Hepatocellular carcinoma

Abbreviations

SNP	Single nucleotide polymorphism
HCC	Hepatocellular carcinoma
CI	Confidence interval
OR	Odds ratio

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third leading cause of cancer death. Because of its high fatality rates, the incidence and mortality ratios are approximately equal [1]. It is now well established that multiple risk factors contribute to hepatocarcinogenesis, including chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections, cirrhosis, carcinogen exposure (such as aflatoxin B1), excessive alcohol drinking [2, 3]. Although many individuals exposed to these risk factors, HCC develops only in a small group of exposed people, implying that genetic factors might contribute to the carcinogenic mechanism. So the search for genetic

S. Bayram (✉)
Department of Nursing, Adıyaman School of Health, Adıyaman University, 02040 Adıyaman, Turkey
e-mail: slymnbyrm81@gmail.com

H. Akkız · A. Bekar · E. Akgöllü · S. Yıldırım
Department of Gastroenterology, Faculty of Medicine, Çukurova University, 01330 Adana, Turkey

factors that could help to select patients at higher risk and thus to modulate the indications of screening procedures is necessary [4]. Moreover, identification of predictive factors could lead to a better diagnosis and planning of new prevention strategies in these patients [5].

Human cells are exposed to constant endogenous (e.g. reactive oxygen species) and exogenous (e.g. UV-radiation) stresses that threaten DNA. In addition, DNA damage can arise from spontaneous replication errors [6]. Human DNA repair mechanisms are thought to prevent or delay genetic instability and tumorigenesis, thus acting as a barrier against cancer development [7, 8]. It is thought that individuals with defects in their DNA repair mechanisms lose their natural protection against tumorigenesis and are more susceptible to cell transformation and cancer. One of the major DNA repair pathways in human cells is the mismatch repair (MMR), which maintains genomic stability, modulates DNA recombination, and mediates cell cycle arrest [9]. The gene Exonuclease 1 (Exo 1; MIM # 606063) is a member of the MMR system, and also belongs to the RAD2 nuclease family. It locates at chromosome 1q42–q43, contains one untranslated exon followed by 13 coding exons and encodes an 846 amino acid protein [10–12]. Exo 1 can interact physically with the MMR proteins MSH2 and MLH1 in both yeast and human cells, and with MSH3 in human cells [13–17]. Exo 1 functions in DNA replication, repair, recombination, mutation avoidance and is essential for male and female meiosis [18, 19]. A guanine (G)/adenine (A) common single nucleotide polymorphism (SNP) at first position of codon 589 in exon 13 of Exo 1 gene (dbSNP ID: rs 1047840), resulting in the substitution of an glutamic acid (Glu, E) residue (GAG) by lysine (Lys, K) residue (AAG) (also designated Exo 1 K589E) in the exonic splicing enhancer (ESE), has been suggested to influence the products of Exo 1 mRNA [20]. Several case–control studies have investigated the association between Exo 1 K589E polymorphism and cancer risk including lung cancer [20, 21], non-small cell lung cancer [22], breast cancer [23], gastric cancer [24], oral cancer [25], and glioma [26].

Sequence variants in DNA repair genes also are thought to modulate DNA repair capacity and consequently may be associated with altered cancer risk [27]. Since SNP is the most frequent and subtle genetic variation in the human genome and has great potential for application to association studies of complex disease [28]. We hypothesized that K589E polymorphism in Exo 1 gene may act as a genetic modifier in individual susceptibility to HCC. According to our recent knowledge, no research has been conducted to evaluate Exo 1 K589E polymorphism and risk of HCC development. To test the hypothesis that the polymorphism of Exo 1 K589E is associated with risk of developing HCC, we performed genotyping analysis using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in a hospital-based case–control study of 224 HCC patients and 224 age, gender, smoking and alcohol consumption matched cancer-free controls in Turkish population.

Materials and methods

Study population

The study population and subject characteristics were previously described elsewhere [29, 30]. This is an ongoing molecular epidemiologic study of HCC conducted in Adana, Turkey and the subject recruitment was approved by the Committee for Ethics of Medical Experiment on Human Subjects, Faculty of Medicine, Çukurova University. Briefly, all subjects were genetically unrelated Turkish and were from Çukurova and the surrounding regions of southern Turkey. Submission of the individuals to the study was conditioned by an obtained written informed consent form regarding the use of their blood samples for research studies. The study proceeded in agreement with the Helsinki declaration approved on the World Medical Association meeting in Edinburgh. Blood samples were collected from 224 consecutive patients with HCC seen in the department of gastroenterology and general surgery between September 2005 and June 2011. During the same time, 224 unrelated community residents with no evidence of hepatocellular or other cancer who entered the hospital for health check-ups were enrolled as the control group. The 224 cancer-free control subjects did not have a history of liver disease and had no serological evidence of hepatitis B or C virus infection. Each control was pair-matched by sex, age (±3 years), smoking and alcohol consumption to a patient with HCC. These characteristics allowed us the choice of a control population without any possible risk bias for HCC. The HCC diagnostic criteria was based on the guideline proposed by European Association for the Study of the Liver (EASL) [31]. We gave a diagnosis of HCC when a patient had one or more risk factors (i.e., HBV or HCV infection, or cirrhosis) and one of the following: >400 ng/ml α-fetoprotein (AFP) and at least one positive finding following examination using spiral computed tomography (CT), contrast-enhanced dynamic MRI, or hepatic angiography; or <400 ng/ml AFP and at least two findings following CT, MR, or hepatic angiography. A positive HCC finding using dynamic CT or MRI is indicative of arterial enhancement followed by venous washout in the delayed portal/venous phase. In addition, we performed histopathological diagnosis for cases that did not fulfill all of the clinical non-invasive diagnostic criteria of HCC. Cirrhosis was diagnosed with liver biopsy,

abdominal sonography, and biochemical evidence of parenchymal damage plus endoscopic esophageal or gastric varices [32]. Patients with cirrhosis were classified into three Child-Pugh grades based on their clinical status [33]. Serum HBsAg and Anti-HCV were assessed using an immunoassay (Abbott Laboratories, Abbott Park, IL, USA). Serum AFP concentration was measured by microparticle enzyme immunoassay (Abbott Laboratories, AXSYM, USA). Heavy alcohol intake was defined as a daily minimum consumption of 160 g alcohol for at least 8 years.

All subjects were interviewed using a structured questionnaire to obtain information on demographic factors and health characteristics. Technicians who performed the blood tests were blinded to the identity and disease status of participants. Peripheral blood samples taken from patients and controls, and blood specimens, including white blood cells and serum, were frozen at −20°C until analysis.

DNA extraction

A 5 ml sample of venous blood was collected from each subject into a test tube containing EDTA as anticoagulant. Genomic DNA was extracted from peripheral whole blood using High Pure PCR Template Preparation Kit (Roche Diagnostics. GmbH, Mannheim, Germany) according to the manufacturer's protocol.

Polymerase chain reaction-restriction fragment length polymorphism analysis

PCR-RFLP analysis was performed to determine the genotype of the G/A polymorphism of Exo 1 gene, as described previously [25]. The 306 base pair (bp) fragment encompassing the G to A polymorphic site in Exo 1 region was amplified using specific primers 5′-GAC ACA GAT GTA GCA CGT AA-3′ and 5′-CTG CGA CAC ATC AGA CAT AT-3′. Amplification was performed in GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Singapore). The 20 µl PCR mixture contained approximately 250 ng DNA, with 0.25 µM of both primer, 0.1 mM of each dNTP, 1× PCR buffer, 1.5 mM $MgCI_2$ and 1 U Taq polymerase (Promega, Madison, WI, USA). The following cycling conditions were used: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 10 min. As a negative control, PCR mix without DNA sample was used to ensure contamination free PCR product. After confirmation of successful PCR amplification by 1.5% agarose gel electrophoresis, each PCR product was digested overnight with 5 units *Mse*I (from an *Escherichia coli* strain that carries the *Mse*I gene from *Micrococcus* species, recognizing the sequence 5′-T$^{\downarrow}$TAA-3′) enzyme at 37°C (New England Biolabs Inc., Beverly, MA) and electrophoresed on 3% agarose gel containing 0.5 µg/ml ethidium bromide and visualized under UV illumination. PCR products with Lys at the polymorphic site were digested into two fragments, 196 and 110 bp, while those with Glu were not because of the absence of an *Mse*I restriction site. Samples yielding 196 and 110 bp fragments were scored as Lys/Lys, those with single 306 bp fragments as Glu/Glu, and 306, 196 and 110 bp as Glu/Lys. This assay was illustrated in Fig. 1. To ensure quality control, genotyping was performed without knowledge of the subjects' case/control status and a 15% random sample of cases and controls was genotyped twice by different persons; reproducibility was 100%.

Statistical analysis

The sample size was calculated using the QUANTO 1.1 program (http://hydra.usc.edu/gxe). The desired power of our study was set at 80%. Data analysis was performed using the computer software Statistical Package for Social Sciences (SPSS) for Windows (version 10.0). Differences in the distributions of demographic characteristics between the cases and controls were evaluated using the Student's *t*-test (for continuous variables) and χ^2 test (for categorical variables). The observed genotype frequencies were

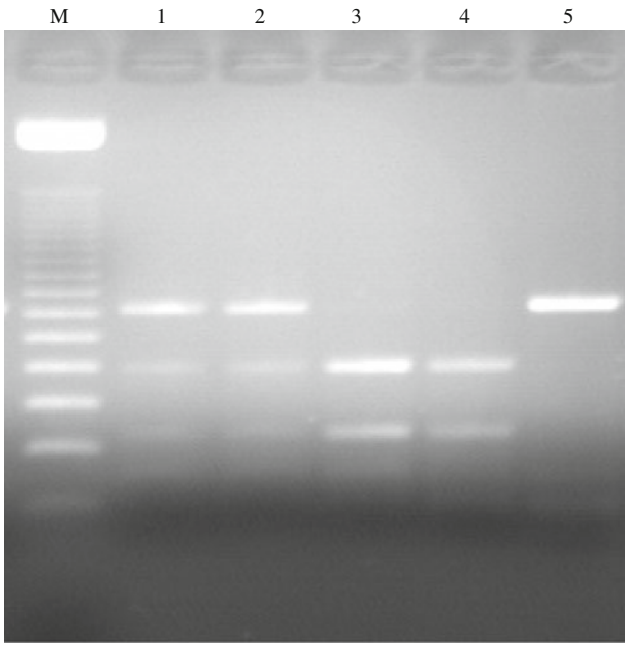

Fig. 1 Analysis of Exo 1 E589K Polymorphism. A representative agarose gel picture showing PCR-RFLP analysis of Exo 1 E589K genotypes in genomic DNAs of study subjects with restriction endonuclease enzyme MseI. *M* 50-bp DNA ladder, *lane 1 and 2* Glu/Lys heterozygous (306, 196 and 110 bp), *lane 3 and 4* Lys/Lys homozygous (196 and 110 bp), *lane 5* Glu/Glu homozygous (306 bp)

compared with expected values calculated from Hardy–Weinberg equilibrium theory ($p^2 + 2pq + q^2 = 1$; where p is the frequency of the wild-type allele and q is the frequency of the variant allele) by using a χ^2 test with degree of freedom equal to 1 among cases and controls, respectively. Pearson's χ^2 test was used to determine whether there was any significant difference in allele and genotype frequencies between patients and controls. The associations between Exo 1 K589E genotypes and the risk of HCC were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from binary logistic regression analysis. The homozygous genotype for the Glu allele of Exo 1 was used as the reference in calculating ORs and 95% CIs. Statistical modeling was performed on the relative risk of the Lys/Lys genotype or the Glu/Lys genotype against the Glu/Glu genotype independently. Furthermore, to estimate the recessive or dominant effect of Exo 1 K589E genotype on risk, statistical modeling was performed on the relative risk of the Lys/Lys genotype against the Glu/Lys + Glu/Glu genotype (recessive model) or the Glu/Lys + Lys/Lys genotype against the Glu/Glu genotype (dominant model). Probability levels less than 0.05 were used as a criterion of significance.

Results

General characteristic of the subjects

A total of 448 Turkish subjects were enrolled in our study. General characteristic of the subjects are summarized in Table 1. As expected, no significant difference was found between case patients and control subjects with regard to age and sex ($P = 0.68$ and $P = 1.00$, respectively) which implied that age and sex matched adequately. Similarly, there were no significant differences in smoking status and alcohol consumption between case and control group. In addition to these, Table 1 shows the distribution of demographic variables such as AFP, marker of hepatitis, cirrhosis and Child-Pugh grade of cases.

Table 1 Distribution of selected characteristics in patients with hepatocellular carcinoma and controls

Characteristic	Patients (%) ($n = 224$)	Controls (%) ($n = 224$)	P value[a]
Age (years), mean ± SD (range)	60.66 ± 11.17 (20–87)	60.22 ± 10.97 (20–90)	0.68
Male sex	180 (80.4%)	180 (80.4%)	NS
Smoking status			NS
Ever	107 (47.8%)	107 (47.8%)	
Never	117 (52.2%)	117 (52.2%)	
Alcohol status			NS
Drinker	64 (28.6%)	64 (28.6%)	
Non-drinker	160 (71.4%)	160 (71.4%)	
Viral infection			
HBsAg positive	133 (59.4%)	–	
Anti-HCVAb positive	54 (24.1%)	–	
Both positive	3 (1.3%)	–	
Both negative	34 (15.2%)	–	
Liver cirrhosis			
Present	185 (82.6%)	–	
Absent	39 (17.4%)	–	
Child-Pugh classification			
A	39 (21.0%)	–	
B	68 (36.8%)	–	
C	78 (42.2%)	–	
α-Fetoprotein (ng/ml)			
<100	98 (43.8%)	–	
100–400	36 (16.1%)	–	
>400	90 (40.1%)	–	

NS not significant, *n* total number of case patients or control subjects

[a] P values were derived from Pearson χ^2 test except age; Student's t-test was used for age. All P values are two-sided

Genotype frequency distribution of Exo 1 K589E polymorphism

The frequency distributions of the different genotypes for Exo 1 K589E polymorphism are shown in Table 2. The genotypic frequencies of the control ($n = 224$; $\chi^2 = 0.005$, df = 1, $P = 0.94$) were in Hardy–Weinberg equilibrium, suggesting that there was no population stratification and no sampling bias. The patients' frequencies were also in Hardy–Weinberg equilibrium ($n = 224$; $\chi^2 = 0.018$, df = 1, $P = 0.89$). The allelic frequencies of case subjects (Glu, 0.63; Lys, 0.37) were not significantly different from those of the control subjects [Glu, 0.68; Lys, 0.32 ($p = 0.12$)]. But, genotypic frequencies in the cases were not similar to that of the controls, differences being statistically significant ($\chi^2 = 7.28$, df = 2, $P = 0.03$).

Exo 1 K589E polymorphism and risk of hepatocellular carcinoma

To evaluate the risk of HCC according to the Exo 1 K589E genotype, logistic regression analysis was conducted (Table 2). Using the Glu/Glu genotype as the reference genotype, Glu/Lys genotype decreased risk but not significantly associated with the risk of HCC (OR = 0.91; 95% CI = 0.61–1.35, $P = 0.63$). Unlike that, compared with the Glu/Glu genotype, cases carrying the two Lys alleles have a 2.15-fold increase in the risk for HCC (95% CI = 1.13–4.09, $P = 0.02$). With the Glu/Glu genotype as

reference, the OR for combined Glu/Lys and Lys/Lys genotypes (dominant genetic model) together was 1.08 (95% CI = 0.74–1.56, $P = 0.70$). In the recessive genetic model, we detected that a significantly increased risk of HCC in subjects with the variant homozygote Lys/Lys of Exo 1 K589E polymorphism, when compared with homozygote Glu/Glu and heterozygote Glu/Lys carriers (OR = 2.26 95% CI = 1.22–4.16, $P = 0.009$).

Stratified analyses

To observe whether the effect of genetic variation was modified by epidemiologic factors, HCC patients and controls were stratified on the basis of various host characteristics including sex, HCC etiology (viral infection status) and age. Firstly, we compared frequency distribution of genotypes/alleles of Exo 1 K589E polymorphism in subjects after segregation on basis of gender (Tables 3, 4). Interestingly, in male patients, the risk of HCC was significantly higher with Exo 1 K589E Lys/Lys genotype compared with subjects with the Glu/Glu genotype (OR = 2.67, 95% CI = 1.27–5.61, $P = 0.009$) between HCC patients and controls (Table 3). However, there was no significant association between Exo 1 K589E polymorphism and HCC risk for female subjects. We next investigated whether HCC etiology (viral infections) influenced the effects of Exo 1 K589E genotypes on patients with HCC (Table 4). In non-viral infection-related HCC subgroup logistic regression analysis showed that subjects homozygous for the Lys/Lys genotype had a 3.14-fold increased risk of developing HCC compared those with Glu/Glu genotype (95% CI = 1.09–8.99, $P = 0.03$). However, in HBV-related and HCV-related HCC subgroups the homozygous Lys/Lys genotype had also increased risk but not statistically significant associated with the risk of HCC (OR = 1.91; 95% CI = 0.92–3.96, $P = 0.08$ and OR = 2.18; 95% CI = 0.87–5.50, $P = 0.10$, respectively) (Table 4). Age at diagnosis with HCC

Table 2 Alleles and genotypes frequency distribution of Exo 1 K589E polymorphism among cases and controls as well as the association with hepatocellular cancer risk

	Cases (%), $n = 224$	Control (%), $n = 224$	P value[a]	OR (95% CI)
Allele frequency				
Glu	284 (63.4%)	306 (68.3%)		1.00 (Reference)
Lys	164 (36.6%)	142 (31.7%)	0.12	1.24 (0.94–1.64)
General genotype				
Glu/Glu	95 (42.4%)	99 (44.2%)		1.00 (Reference)
Glu/Lys	94 (42.0%)	108 (48.2%)	0.63	0.91 (0.61–1.35)
Lys/Lys	35 (15.6%)	17 (7.6%)	**0.02**	2.15 (1.13–4.09)
Dominant genotype				
Glu/Glu	95 (42.4%)	99 (44.2%)		1.00 (Reference)
Glu/Lys + Lys/Lys	129 (57.6%)	125 (55.8%)	0.70	1.08 (0.74–1.56)
Recessive genotype				
Glu/Glu + Glu/Lys	189 (84.4%)	207 (92.4%)		1.00 (Reference)
Lys/Lys	35 (15.6%)	17 (7.6%)	**0.009**	2.26 (1.22–4.16)

[a] Data were calculated by logistic regression analysis

Bold emphasize P value < 0.05

Table 3 Comparison of frequency distribution of alleles and genotypes of Exo 1 K589E polymorphism among male and female subjects as well as the association hepatocellular cancer risk

Gender	Allele/genotype	N_{Case}[a] (%)	$N_{Control}$[b] (%)	P value[c]	OR (95% CI)
Male		180	180		
	Glu	223 (61.9%)	246 (68.3%)		1.00 (Reference)
	Lys	137 (38.1%)	114 (31.7%)	0.08	1.32 (0.98–1.80)
	Glu/Glu	73 (40.5%)	78 (43.3%)		1.00 (Reference)
	Glu/Lys	77 (42.8%)	90 (50.0%)	0.69	0.91 (0.59–1.42)
	Lys/Lys	30 (16.7%)	12 (6.7%)	**0.009**	2.67 (1.27–5.61)
Female		44	44		
	Glu	61 (40.9%)	60 (42.0%)		1.00 (Reference)
	Lys	27 (59.1%)	28 (58.0%)	0.87	0.95 (0.50–1.79)
	Glu/Glu	22 (50.0%)	21 (47.7%)		1.00 (Reference)
	Glu/Lys	17 (38.6%)	18 (40.9%)	0.82	0.90 (0.37–2.20)
	Lys/Lys	5 (11.4%)	5 (11.4%)	0.95	0.95 (0.24–3.78)

[a] Number of cases

[b] Number of controls

[c] Data were calculated by logistic regression analysis

Bold emphasize P value < 0.05

Table 4 Comparison of frequency distribution of alleles and genotypes of Exo 1 K589E polymorphism based on hepatocellular carcinoma etiology as well as the association hepatocellular cancer risk

Subgroup	Allele/genotype	N_{Case}[a] (%)	$N_{Control}$[b] (%)	P value[c,d]	OR (95% CI)
HBV-related HCC		133	224		
	Glu	172 (64.7%)	306 (68.3%)		1.00 (Reference)
	Lys	94 (35.3%)	142 (31.7%)	0.32	1.18 (0.86–1.62)
	Glu/Glu	58 (43.6%)	99 (44.2%)		1.00 (Reference)
	Glu/Lys	56 (42.1%)	108 (48.2%)	0.60	0.88 (0.56–1.40)
	Lys/Lys	19 (14.3%)	17 (7.6%)	0.08	1.91 (0.92–3.96)
HCV-related HCC		56	224		
	Glu	72 (63.2%)	306 (68.3%)		1.00 (Reference)
	Lys	42 (36.8%)	142 (31.7%)	0.30	1.26 (0.82–1.93)
	Glu/Glu	24 (42.1%)	99 (44.2%)		1.00 (Reference)
	Glu/Lys	24 (42.1%)	108 (48.2%)	0.79	0.92 (0.50–1.72)
	Lys/Lys	9 (15.8%)	17 (7.6%)	0.10	2.18 (0.87–5.50)
Non-viral-related HCC		34	224		
	Glu	40 (58.8%)	306 (68.3%)		1.00 (Reference)
	Lys	28 (41.2%)	142 (31.7%)	0.12	1.50 (0.89–2.54)
	Glu/Glu	13 (38.1%)	99 (44.2%)		1.00 (Reference)
	Glu/Lys	14 (41.2%)	108 (48.2%)	0.98	0.99 (0.44–2.20)
	Lys/Lys	7 (20.6%)	17 (7.6%)	0.03	3.14 (1.09–8.99)

[a] Number of cases

[b] Number of controls

[c] Data were calculated by logistic regression analysis

[d] Adjusted for age, sex, smoking and drinking status

(mean ± standard deviation) was not significantly different among Exo 1 K589E genotypes (Glu/Glu: 61.11 ± 11.90, Glu/Lys: 59.79 ± 10.14 and Lys/Lys: 61.77 ± 11.92, $P = 0.58$). In addition to this, we did not find any significant association between different genotypes of Exo 1 K589E polymorphism and demographical variables like liver cirrhosis, child plugh grade of cases and AFP levels (data not given).

Discussion

This molecular epidemiological study investigated whether the Exo 1 K589E polymorphism could have an impact on susceptibility to HCC. We found, for the first time, that Lys/Lys genotype of Exo 1 K589E polymorphism was associated with significantly increased risk of HCC. Moreover, stratified analysis showed that the differences between cases and controls were statistically significant in non-viral-related HCC, but not in HBV and HCV-related HCC subgroups. This observed statistical association suggesting that Exo 1 K589E polymorphism related hepatocarcinogenesis pathway might independent of any contributory roles of the oncogenic hepatitis viruses. Since limitation sample size, large studies will be needed to verify these results. The worldwide incidence of HCC is much higher in male compared with female individuals. After stratification of all subjects on gender, we observed that Lys/Lys genotype of Exo 1 K589E polymorphism gene was associated with the risk for HCC in male individuals. However, the genotype distributions did not display significant difference between cases and controls in the female cohort ($P = 0.66$). Furthermore, analysis using binary logistic regression revealed that neither Glu/Lys nor Lys/Lys genotype conferred increased risk for HCC in females, compared with Glu/Glu genotype ($P = 0.82$ and 0.95, respectively), suggesting that the risk of HCC is conferred by the Lys/Lys genotype only in male but not in female individuals. The observed gender difference may result from the interaction between the polymorphism and the sexual hormones during carcinogenesis, which has been exemplified by the case of MDM2 SNP309 [34]. Furthermore, recent study has pointed out the association between female gender and a blunted inflammatory response mediated through inhibition of MyD88-dependent interleukin-6 production [35]. On the other hand, the number of female HCC cases in our study cohort is too few (only 44 cases) to get solid conclusion. Therefore, the correlation between the Exo 1 K589E polymorphism and risk for HCC in females requires further investigation in a larger cohort in the future.

Our results in line with previous findings showing that there was an association between the Exo 1 K589E polymorphism and risk for various cancers including lung cancer [20, 21], breast cancer [23], gastric cancer [24], and oral cancer [25]. On the contrary, Zienolddiny et al. [22] have found no significant association of Exo 1 K589E polymorphism and risk of non-small cell lung cancer in Caucasian Norwegian population. Interestingly, Chang et al. [26] reported marked differences in genotype distribution of Exo 1 K589E polymorphism, Lys allele or Lys/Lys genotype being associated with an decreased risk of

glioma. A rational explanation for this cancer-dependent difference in risk conferred by the examined Exo 1 K589E polymorphism may be attributable to differences in the pathways of carcinogenesis among the various types of human cancers. These discrepancies may also be due to the differences of the ethnic variation. For instance, Glu allele frequency of Exo 1 K589E polymorphism among the different ethnicities is as follows: 0.615 in Caucasians, 0.767 in Japanese, 0.721 in Chinese and 0.535 in Africans [36]. This study found that the Glu allele frequency of Exo 1 K589E polymorphism was 0.680 among our Turkish control subjects, similar to the reported allele frequencies in Caucasians. Further investigations of Exo 1 K589E polymorphism in various types of cancer and different populations are in needed and Exo 1 K589E may be a promising biomarker for specific types of cancers. Besides, recent studies have found several polymorphisms in MMR genes (MLH1, MSH2, MSH3, MSH6, PMS1, TREX1, TP73) that are associated with human cancer [37–39]. One of these polymorphisms, MLH1 −93G>A is located in the core promoter of MLH1, 93 bases upstream of the transcription start site in a region that is required for maximal transcriptional activity [40]. Chen et al. [41] have found hMLH1-93 A allele significantly increased HBV-related HCC risk in Taiwanese population. But other common variants in MMR genes have not been studied yet worldwide, genetic association studies to investigate these polymorphisms in hepatocarcinogenesis should be done in the future.

Our results showing an association between the risk of HCC and Exo 1 K589E polymorphism may be biologically plausible because they are parallel in several ways to the laboratory and clinical findings. Exo 1 playing an essential role as both 5′–3′ and 3′–5′ nucleases and contributing to the overall stability of MMR complex [18, 42]. The MMR system corrects base–base mispairs and small insertion/deletion loops, responsible for maintaining the genome integrity [18, 43, 44]. In addition to the unique function in MMR system, Exo 1 was also linked to carcinogenesis through its role in recombinational events, such as repairing of DNA double-strand breaks and maintaining of telomere stabilization [18, 19, 42]. Furthermore, Exo 1 has recently been shown to contribute to DNA damage-induced apoptosis [45, 46]. Recent findings indicated that mammalian Exo 1 is responsible for mutation prevention and the mice with Exo 1 inactivation have reduced survival time and increased risk for tumor development [19]. Moreover, Matakidou et al. [47] have showed that Lys/Lys genotype of Exo 1 E589K was associated with a significantly poorer prognosis and less favourable overall survival in lung cancer patients. The Exo 1 K589E polymorphism is non-synonymous SNP that result in replacement of amino acids, in turn, possibly affecting the protein functions. The amino acid change at codon 589 might influence the products of Exo 1 mRNA, because Jin et al. [20] reported that K589E polymorphism located at an exonic splicing enhancer region. Exo 1 deficiency would reduce the MMR capacity. Thus cells with Lys/Lys genotype may not be able to undergo DNA repair or apoptosis in response to DNA damage. Having these findings in mind, it is reasonable to suggest that individuals carrying the Lys/Lys genotype of Exo 1 E589K polymorphism may be susceptible to HCC.

The limitations of our study are as follows. First limitation of the present study is that it was hospital-based case–control study, and patients were selected at a single institution (Çukurova University, Balcalı Hospital) and thus may have been unrepresentative of HCC patients in the general population. In addition, it should be noted that the control subjects were recruited at the same hospital. However, in the control group, the agreement between the observed distributions of Exo 1 K589E genotype frequencies with the expected according to the Hardy–Weinberg equilibrium model suggested no selection bias. Second, our gene association analysis also carried limitations in statistical power. The numbers of patients were small in the some subgroup analyses stratified by sex and HCC etiology. Therefore, some subgroup analyses may not have sufficient statistical power to identify the association between these polymorphisms and HCC risk (especially statistical power <80% for female and non-viral-related HCC subgroup). For this reason, larger case–control studies stratified for sex and HCC etiology should be performed to clarify the association between the Exo 1 K589E polymorphism and HCC risk in the future. Third, we also limited our study to Turkish population due to variation in allele frequency between different ethnic groups has been observed for Exo 1 K589E polymorphism. Fourth, this study only focused on single locus on single gene without taking into consideration gene–environment, gene–gene interactions and interactions between different locuses on the same gene, which may affect individual susceptibility to HCC. Because of advances in high-throughput genotyping techniques, it is likely that future association studies on HCC will need to investigate multiple polymorphisms within Exo 1 gene and will need to use recently developed haplotype-based methods to evaluate the haplotypic effects. Fifth, due to the lack of data on Exo 1 expression according to K589E genotypes in our HCC group, future work need to be done in order to explore the correlation between levels of Exo 1 in normal liver and HCC tissues in the context of different genotypes of Exo 1 K589E polymorphism.

In conclusion, the present investigation indicated, for the first time, that the Exo 1 K589E polymorphism might confer genetic susceptibility that influences hepatocellular

carcinogenesis especially in men and non-viral-related HCC patients within the Turkish population, but we conclude that it cannot be attributed (solely) to the Exo 1 K589E polymorphism. Further independent studies are required to validate our findings in a larger series, as well as in patients of different ethnic origins and to better understand the Exo 1 K589E polymorphism and susceptibility to HCC. If our findings are confirmed in both larger series and other ethnic origins, genetic testing of Exo 1 K589E polymorphism may be useful in detecting high-risk individuals and the results may encourage the higher-risk population to have frequent medical examinations to detect early stage HCC. Furthermore, the knowledge of the mechanisms involved in HCC carcinogenesis may help to identify targets for the development of chemoprevention or therapeutic strategies.

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Conflict of interest All of the authors declare that there are no conflicts of interest.

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