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Original Article

Associations of LIM kinase1 (LIMK1) gene single nucleotide polymorphisms with prostate cancer susceptibility in Chinese population

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Abstract: Background: Prostate cancer is one of the most common cancers in men. LIM kinase1 (LIMK1) is a mediator in the process of cytoskeleton reorganization and cell motility. LIMK1 is related to progression, invasiveness and metastases of prostate cancer. However, the relationship between LIMK1 single nucleotide polymorphism (SNP) and the risk of prostate cancer has not been studied. Aim: The aim of our study is to determine the association between LIMK1 polymorphisms and the risk of prostate in a Chinese population. Methods: This case-control study consisted of 162 prostate cancer patients and 187 healthy control subjects. Five SNPs of LIMK1 including rs2269082, rs2269081, rs178409, rs6460071 and rs710968 were genotyped using iPLEX genotyping assays on a MassARRAY® platform. Results: No significant relationships were found between polymorphisms genotypes and the risk of prostate cancer. Also, no significant associations were found between genotypes and the individual factors such as Gleason Score, alcohol and cigarette consuming statuses. Conclusion: These polymorphisms of LIMK1 were not significantly associated with prostate cancer susceptibility in Chinese men.

Keywords: Prostate cancer, cancer risk, LIM kinase1 (LIMK1), single nucleotide polymorphism, gene variant

Introduction

Prostate cancer is the most common cancer in elderly males in Europe [1] and the second leading cause of cancer deaths in the USA [2]. A recent autopsy study suggestedthat more than 35% of prostate cancerwere observed in patients died from causes other than prostate cancer, in men aged above 60 years and 80 years, the positive findings reached nearly 40% and 60%, respectively [3]. Prostate cancer has been causing heavy social burden and becoming a big challenge as the aging population increased, especially in western countries [2]. Although Asia has the lowest incidence in the world, due to the large population in our continent, the quantity of prostate cancer patients appears fairly large [4].

Etiologies of prostate cancer are complex, however, it has been becoming clear that prostate cancer couldbe caused by multi-risk factors which mainly include family history, age, race, ethnicity, diet, environment agents, life style and hormones [5, 6]. Genetic background might also be risk factor for cancers, as to prostate cancer, some biomarkers such as miR-129, CCR7 and CXCR3 have been proposed to play crucial roles in the progression and metastasis of prostate cancer [7-9].

Single nucleotide polymorphism (SNP) denotes a single nucleotide variation which occurs at a certain position in the genome and consequently changes amino acid sequence in protein or influences gene splicing, transcription or RNA degradation so that affects the expression level of certain gene [10]. Studies have been carrying to explore the association between genetic variant and prostate cancer, actually, some promising SNPs have been reported to affect the onset risk and progression of pros-

Table 1. Primers for the investigated SNPs of LIMK1

SNP ID	Primer
rs2269082	5'-CCACCACACCCAGCTAATTTGGGTTACAC-3'[a]
	5'-TTACAGGTGTGAGCCACTGCCCCGCCTTAG-3'[b]
	5'-CTCACACCTGTAATCCCAACAGTTGGGGA-3'[c]
rs2269081	5'-CCCGCAGATCCACTCTGTGGGTAATTACC-3'[a]
	5'-TCTCACACCTGTGGCAGACATCTTAGCGC-3'[b]
	5'-CATTCGTGTGGGGTGAGCAGGTGTACACT-3'[c]
rs178409	5'-CCCGCAGATCCACTCTGTGGGTAATTACC-3'[a]
	5'-TACAAGTCCCCTTTATAACCAGGGATCAGG-3'[b]
	5'-TGAGGTGCTGCCTGCCCATGGCTGGGGTTG-3'[c]
rs6460071	5'-GGATCACCTGAGGCCCGTCTTCCAGAAG-3'[a]
	5'-CGATCTCTAGCCACAGAGCCAACTGGG-3'[b]
	5'-CTGGCTGCTCCCACCTTCCCGTGAGGCCT-3'[c]
rs710968	5'-CGCCCTTCATTCATTCGGCCCCTTCTAG-3'[a]
	5'-GCTGGTTTGGGTCTGCGCCACCTGG-3'[b]
	5'-GCCGCGAGCTCGGCGCGCCAGCCC-3'[c]

[a]Forward of amplicon primer. [b]Reserve of amplicon primer. [c]Extension primer.

Table 2. Clinical parameters of the prostate cancer patients and controls

	Controls, n (%) n=187	Patients, n (%) n=162	*P*-value
Age	65.67±5.63	65.03±5.03	0.31[a]
PSA	2.45±0.96	43.14±26.82	<0.001[a]
Alcohol			
NO	142 (75.94%)	112 (69.14%)	
YES	45 (24.06%)	50 (30.86%)	0.15[b]
Smoking			
NO	115 (61.50%)	88 (54.32%)	
YES	72 (38.50%)	74 (45.68%)	0.18[b]
Gleason			
<7		50 (31.00%)	
>7		112 (69.00%)	

PSA: prostate specific antigen. [a]Based on Student's t test. [b]Based on χ^2 test.

tate cancer which could explain the possible reason why not all the high-risk individuals exposed to risk factors develop to prostate cancer [11, 12].

Invasiveness of prostate canceris one of the most significant causes which leads to metastases, associated symptoms and even death, and an increased number of genes such as ERG and mTOR have been proposed to be associated with this process [13, 14]. The reorganization of actin cytoskeleton is a key contributor for prostate cancer cell to gain the morphology change and invasive ability [15]. LIM kinase1 (LIMK1) gene is dominantly expressed in central nervous system, it is a serine/threonine kinase which is a regulator of actin polymerization by means of phosphorylation and inactivation of the actin binding factor cofilin [16]. LIMK1 is demonstrated to be the contributor to form the key actin structures, and the regulator to dynamic assembly and disassembly of actin in the structures of membrane [17, 18]. A recent study also indicated that LIMK1 was overexpressed in prostate cancer and was crucial for the invasive property and growth of prostate cancer cells, and this effect might be not just mediated via the inactivation of phosphorylation of cofilin [19].

Some SPNs of LIMK1 have been reported to be related to cerebrovascular disorders [20-22], however, to the best of our knowledge, the association between SNPs of LIMK1 and prostate cancer has not yet been explored. In this study, we aimed to evaluate the relation of LIMK1 SNPs to the risk of prostate cancer.

Materials and methods

Subjects

Patients with histologically diagnosed prostate cancer between March 2014 and April 2016 at West China Hospital of Sichuan University were enrolled as patients group. Controls were recruited from non-prostate cancer and age-match healthy male without complaint of significant voiding symptoms (American Urological Association symptom score <8) [23] or prostate specific antigen (PSA) levels more than 4 ng/ml. Those control individuals who had the history of prostate surgery or other known cancer, or a family history of prostate cancer in first relatives were excluded. Subjects who smoked more than 10 cigarettes every week for more than six months were defined as smokers, and those who regularly consumed wine or beer more than 50 ml per week at least for six months were defined as alcoholic drinkers. This study was approved by the medical ethics review boards and written consent was obtained from each subject.

Genotyping

Three tag SNPs, rs2269082, rs2269081 and rs178409 were selected using SNP Tagger per-

Table 3. Genotypes frequency of SNPs and their risks for prostate cancer in patient and control groups

	Controls n=187	Patients n=162	*P*-value[a]	OR[b]	95% CI[b]	*P*-value[b]
rs2269082						
CC	11 (5.88%)	13 (8.02%)	0.43	1	(Reference)	
CT	55 (29.41%)	54 (33.33%)	0.43	1.20	(0.5-2.92)	0.68
TT	121 (64.71%)	95 (58.64%)	0.24	1.51	(0.65-3.5)	0.34
rs2269081						
CC	4 (2.14%)	6 (3.7%)	0.38	1	(Reference)	0.00
CA	47 (25.13%)	44 (27.16%)	0.67	1.60	(0.43-6.01)	0.48
AA	136 (72.73%)	112 (69.14%)	0.40	1.83	(0.51-6.55)	0.35
rs178409						
AA	155 (82.89%)	139 (85.8%)	-	1	(Reference)	
AG	32 (17.11%)	23 (14.2%)	0.46	1.25	(0.7-2.23)	0.21
rs6460071						
AA	6 (3.21%)	4 (2.47%)	0.68	1	(Reference)	
AG	43 (22.99%)	42 (25.93%)	0.52	0.68	(0.18-2.58)	0.57
GG	138 (73.8%)	116 (71.6%)	0.65	0.79	(0.22-2.87)	0.72
rs710968						
TT	9 (4.81%)	13 (8.02%)	0.22	1	(Reference)	
TC	26 (13.9%)	19 (11.73%)	0.55	1.98	(0.71-5.53)	0.19
CC	152 (81.29%)	130 (80.25%)	0.81	1.68	(0.71-4.05)	0.24

OR, odd ratio; CI, confident interval. [a]Differences of genotypes frequencies between patients and controls were calculated based on χ^2 test. [b]OR and 95% CI were calculated based on unconditional logistic regression analysis.

formed with HapMap Genome Browser by setting the minor allele frequency in the CHB population to be at least 0.2. Another two SNPs, rs6460071 and rs710968 were also included in this study. Genomic DNA was isolated from peripheral blood with QIAGEN Blood DNA kit (QIAGEN, Germany) according to the manufacturers' recommended protocols. The purity and concentration of isolated genomic DNA was evaluated with a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). We used an Assay Design 4.0 software (Sequenom, San Diego, CA, USA) to design the primers which were listed in **Table 1**. SNPs genotyping were performed using iPLEX genotyping assays on a MassARRAY® platform (Sequenom, San Diego, CA, USA). The threshold of DNA sample quality control was set at the level of 90%.

Statistical analysis

Categorical variables are expressed as percentage of studied subjects and continuous variables such as age and PSA are expressed as mean ± standard deviation (SD). Chis-squared (χ^2) test was used to compare the proportions of categorical variables andstudent t-test was used to compare values of mean age and PSA level between patients and control groups. Hardy-Weinberg equilibrium in the control subjects was assessed using the χ^2 test. Pearson's χ^2 test or Fisher's exact test were conducted to compare the frequencies of genotypes for SNPs between patients and control groups. Unconditional logistic regression was performed to assess the effect of polymorphisms for SNPs on the risk of prostate cancer. All the statistical analyses were performed using SPSS statistical package 20.0 (SPSS Inc., Chicago, USA) and $P<0.05$ was considered to be statistically significant.

Results

A total number of 162 patients and 187 controls were included in our study. As showed in **Table 2**, the patients group consisted of 50 individuals with Gleason Score <7 (31%) and 112 individuals with Gleason Score ≥7 (69%).

Table 4. Genotypes frequency of SNPs and their risks for prostate cancer in patients and controls with or without alcohol consumptions

	Controls n=187		Patients n=162									
	Alcohol (+) n=45	Alcohol (-) n=142	Alcohol (+) n=50	Alcohol (-) n=112	P^{a+}	OR^{b+}	(95% CI^{b+})	P^{b+}	$P1^{a-}$	OR^{b-}	(95% CI^{b-})	P^{b-}
rs2269082												
CC	2 (4.44%)	9 (6.34%)	3 (6.00%)	10 (8.93%)	0.73	1.00	(Refenence)		0.44	1.00	(Refenence)	
CT	13 (28.89%)	42 (29.58%)	19 (38.00%)	35 (31.25%)	0.35	1.03	(0.15-7.02)	0.98	0.94	1.33	(0.49-3.64)	0.57
TT	30 (66.67%)	91 (64.08%)	28 (56.00%)	67 (59.82%)	0.29	1.61	(0.15-10.2)	0.61	0.73	1.51	(0.58-3.9)	0.40
rs2269081												
CC	1 (2.22%)	3 (2.11%)	3 (6.00%)	3 (2.68%)	0.36	1.00	(Refenence)		0.77	1.00	(Refenence)	
CA	26 (57.78%)	21 (14.79%)	24 (48.00%)	20 (17.86%)	0.34	3.25	(0.35-30.01)	0.30	0.51	1.05	(0.19-5.83)	0.96
AA	18 (40.00%)	118 (83.10%)	23 (46.00%)	89 (79.46%)	0.37	2.35	(0.24-23.18)	0.47	0.37	1.33	(0.26-6.69)	0.73
rs178409												
AA	24 (53.33%)	131 (92.25%)	33 (66.00%)	106 (94.64%)		1.00	(Refenence)			1.00	(Refenence)	
AG	21 (46.67%)	11 (7.75%)	17 (34.00%)	6 (5.36%)	0.21	1.70	(0.74-3.88)	0.33	0.45	1.48	(0.53-4.12)	0.26
rs6460071												
AA	3 (6.67%)	3 (2.11%)	2 (4.00%)	2 (1.79%)	0.56	1.00	(Refenence)		0.85	1.00	(Refenence)	
AG	18 (40.00%)	25 (17.61%)	22 (44.00%)	20 (17.86%)	0.69	0.55	(0.08-3.55)	0.53	0.96	0.83	(0.13-5.47)	0.85
GG	24 (53.33%)	114 (80.28%)	26 (52.00%)	90 (80.35%)	0.90	0.62	(0.1-3.95)	0.61	0.99	0.84	(0.14-5.15)	0.85
rs710968												
TT	4 (8.89%)	5 (3.52%)	7 (14.00%)	6 (5.36%)	0.44	1.00	(Refenence)		0.48	1.00	(Refenence)	
TC	8 (17.78%)	18 (12.68%)	9 (18.00%)	10 (8.93%)	0.98	1.56	(0.33-7.33)	0.58	0.34	2.16	(0.53-8.79)	0.28
CC	33 (73.33%)	119 (83.80%)	34 (68.00%)	96 (85.71%)	0.57	1.70	(0.46-6.29)	0.43	0.67	1.49	(0.44-4.99)	0.52

OR, odd ratio; CI, confident interval. $^{a+}$Differences of genotypes frequencies between patients and controls with alcohol consumption were calculated based on χ^2 test. $^{b+}$OR and 95% CI were calculated between patients and controls with alcohol consumption based on unconditional logistic regression analysis. $^{a-}$Differences of genotypes frequencies between patients and controls without alcohol consumption were calculated based on χ^2 test. $^{b-}$OR and 95% CI were calculated between patients and controls without alcohol consumption based on unconditional logistic regression analysis.

The patients group revealed a significantly higher PSA level compared with controls (P< 0.001), no significant differences were found in terms of age, percentages of consuming alcohol or smoke (P>0.05).

The distributions of genotypes of rs2269082, rs2269081, rs178409, rs6460071 and rs710968 were consistent with Hardy-Weinberg equilibrium (P>0.05). Genotypes frequencies of each SNP were compared between patients and control groups, however, as showed in **Table 3**, no significant differences were found. Both patients and controls groups were then divided into subgroups according to the status of alcohol or smoke consumption. Genotypes frequencies of alcohol consumers, non-alcohol consumers, smoke consumers and non-smoke consumers were compared between patient and control groups, respectively. However, no significant differences were found in genotypes frequencies between these four subgroups, and genotypes of CT and TT in rs2269082, CA and AA in rs2269081, AG in rs178409, AG and GG in rs6460071, TC and CC in rs710968 were non-significantly associated with decreased or increased risk of prostate cancer.

We further divided the patients into two groups according to the patients' Gleason score, the majority of patients were with Gleason score ≥7 (n=112, 69%). Genotypes frequencies of each SNP in these two groups were compared with those in controls, respectively, whereas no significant differences were found, and the genotypes of CT and TT in rs2269082, CA and AA in rs2269081, AG in rs178409, AG and GG in rs6460071, TC and CC in rs710968 were not significantly decreased or increased the risk of prostate cancer both in patient group with Gleason Score <7 or Gleason score ≥7 (**Table 4**).

Discussion

LIMK1 is a serine/threonine kinase that contains two amino-terminal LIM domains in tandem as well as a PDZ domain [24]. LIMK1 belongs to Rho signaling pathway, phosphorylation of LIMK1 mediated by Rho-kinase (ROCK), p21 activated kinase1 (PAK1), PAK4 and myotonic dystrophy kinase-related Cdc42-binding kinaseα (MRCKα) is responsible for Rac- and Cdc42-induced actin cytoskeleton reorganization and focal adhesion complexes [25, 26]. Expression level of LIMK1 has been observed to be correlated with the aggressiveness of prostate cancer cells, high levels of LIMK1 were found in highly invasive prostate cancer cell lines [19]. High concentration of LIMK1 significantly increased tumorigenic and aggressive properties in prostate cancer cells, while inhibition of LIMK1 expression was observed to cause retarded effects on cell proliferation and invasion by arresting cells at G2/M phase, and these effects were found to act as a concentration-dependent manner [27].

It is cleared that missense and nonsense SNPs in the coding region could change the amino acid sequence of protein and then possibly influence the function of protein. For SNPs that are in non-coding regions may affect gene expression and disease susceptibility when locate in upstream or downstream from the gene [28]. Few SNPs of LIMK1 have been reported that relate to susceptibility of cerebrovascular diseases. Akagawa et al. found that rs6460071 and rs710968 increased the risk of intracranial aneurysms [22], and this conclusion was confirmed by Low et al. [20]. Another study carried by Yamada et al. also reported that rs710968 was significantly associated with intracerebral hemorrhage [21]. Although the expression level of LIMK1 has been identified to significantly proportionately affect the proliferated and invasive properties of prostate cancer cell, the associations between SNPs of LIMKs and prostate cancer are still lacking.

This is the first reported study of SNPs of LIMK1 in a Chinese population. In this study, we evaluated the associations of rs2269081, rs2269082, rs178409, rs6460071 and rs710968 with risks of prostate cancer. Among these SNPs, rs2269081, rs2269082 and rs178409 were considered as tag SNPs which locate in intron area. Both rs6460071 and rs710968 are upstream variants and as mentioned above, they have been reported that related to the risk of intracranial aneurysms via the possible mechanism of changing the expression of LIMK1. Our results showed that these candidate polymorphisms were not associated with a decreased risk of prostate cancer.

In this study, genotypic distribution of patients and controls revealed no significant differences, as shown in **Table 3**, all the *p*-value of

Table 5. Genotypes frequency of SNPs and their risks for prostate cancer in patients and controls of smoking or non-smoking

	Controls n=187		Patients n=162									
	Smoking (+) n=72	Smoking (-) n=115	Smoking (+) n=74	Smoking (-) n=88	P^{a+}	OR^{b+}	95% CI^{b+}	$P3^{b+}$	P^{a-}	OR^{b-}	95% CI^{b-}	P^{b-}
rs2269082												
CC	3 (4.17%)	8 (6.96%)	5 (6.76%)	8 (9.09%)	0.49	1	(Refenence)		0.58	1	(Refenence)	
CT	13 (18.06%)	42 (36.52%)	20 (27.03%)	34 (38.64%)	0.20	1.08	(0.22-5.33)	0.92	0.76	1.24	(0.42-3.63)	0.70
TT	56 (77.77%)	65 (56.52%)	49 (66.21%)	46 (52.27%)	0.12	1.90	(0.44-8.21)	0.39	0.61	1.41	(0.5-4.02)	0.52
rs2269081												
CC	1 (1.39%)	3 (2.61%)	4 (5.41%)	2 (2.27%)	0.18	1	(Refenence)		0.88	1.00	(Refenence)	
CA	23 (31.94%)	24 (20.87%)	25 (33.78%)	19 (21.59%)	0.81	3.68	(0.43-31.27)	0.23	0.90	0.84	(0.13-5.55)	0.86
AA	48 (66.67%)	88 (76.52%)	45 (60.81%)	67 (76.14%)	0.46	4.27	(0.54-33.64)	0.17	0.95	0.88	(0.14-5.38)	0.89
rs178409												
AA	61 (84.72%)	94 (81.74%)	65 (87.84%)	74 (84.09%)		1	(Refenence)			1.00	(Refenence)	
AG	11 (15.28%)	21 (18.26%)	9 (12.16%)	14 (15.91%)	0.58	1.30	(0.51-3.35)	0.14	0.66	1.18	(0.56-2.48)	0.27
rs6460071												
AA	2 (2.78%)	4 (3.48%)	2 (2.70%)	2 (2.27%)	0.98	1	(Refenence)		0.62	1.00	(Refenence)	
AG	8 (11.11%)	35 (30.43%)	9 (12.16%)	33 (37.50%)	0.84	0.89	(0.1-7.85)	0.92	0.29	0.53	(0.09-3.02)	0.47
GG	62 (86.11%)	76 (66.09%)	63 (85.14%)	53 (60.23%)	0.87	0.98	(0.13-7.21)	0.99	0.39	0.72	(0.13-4.03)	0.71
rs710968												
TT	2 (2.78%)	7 (6.09%)	3 (4.05%)	10 (11.36%)	0.67	1	(Refenence)		0.18	1.00	(Refenence)	
TC	10 (13.89%)	16 (13.91%)	8 (10.81%)	11 (12.50%)	0.57	1.88	(0.25-13.86)	0.54	0.77	2.08	(0.61-7.08)	0.24
CC	60 (83.33%)	92 (80.00%)	63 (85.14%)	67 (76.14%)	0.77	1.43	(0.23-8.78)	0.70	0.51	1.96	(0.72-5.34)	0.19

OR, odd ratio; CI, confident interval. $^{a+}$Differences of genotypes frequencies between smoking patients and controls were calculated based on χ^2 test. $^{b+}$OR and 95% CI were calculated between smoking patients and controls based on unconditional logistic regression analysis. $^{a-}$Differences of genotypes frequencies between non-smoking patients and controls were calculated based on χ^2 test. $^{b-}$OR and 95% CI were calculated between non-smoking patients and controls based on unconditional logistic regression analysis.

Table 6. Genotypes frequency of SNPs and their risks for prostate cancer in controls and patients with Gleason Scores <7 or ≥7

	Controls n=187	Patients									
		Gleason <7 n=50	Gleason ≥7 n=112	P[a]	OR[b]	95% CI[b]	P[b]	P[c]	OR[d]	95% CI[d]	P[d]
rs2269082											
CC	11 (5.88%)	5 (10.00%)	8 (7.14%)	0.30	1	(Reference)		0.67	1.00	(Reference)	
CT	55 (29.41%)	15 (30.00%)	39 (34.82%)	0.94	1.64	(0.5-5.4)	0.42	0.33	1.03	(0.38-2.78)	0.96
TT	121 (64.71%)	30 (60.00%)	65 (58.04%)	0.54	1.83	(0.6-5.6)	0.29	0.25	1.35	(0.52-3.52)	0.53
rs2269081											
CC	4 (2.14%)	2 (4.00%)	4 (3.57%)	0.46	1	(Reference)		0.46	1.00	(Reference)	
CA	47 (25.13%)	12 (24.00%)	32 (28.57%)	0.87	1.83	(0.31-11.01)	0.51	0.51	1.47	(0.34-6.26)	0.60
AA	136 (72.73%)	36 (72.00%)	76 (67.86%)	0.86	1.90	(0.34-10.52)	0.46	0.32	1.80	(0.45-7.28)	0.41
rs178409											
AA	155 (82.89%)	42 (84.00%)	97 (86.61%)		1	(Reference)			1.00	(Reference)	
AG	32 (17.11%)	8 (16.00%)	15 (13.39%)	0.85	1.08	(0.46-2.53)	0.17	0.39	1.34	(0.69-2.59)	0.24
rs6460071											
AA	6 (3.21%)	1 (2.00%)	3 (2.68%)	0.65	1	(Reference)		0.80	1.00	(Reference)	
AG	43 (22.99%)	9 (18.00%)	33 (29.46%)	0.45	0.78	(0.08-7.27)	0.83	0.21	0.65	(0.15-2.78)	0.56
GG	138 (73.80%)	40 (80.00%)	76 (67.86%)	0.37	0.58	(0.07-4.79)	0.61	0.27	0.91	(0.22-3.73)	0.89
rs710968											
TT	9 (4.81%)	3 (6.00%)	10 (8.93%)	0.73	1	(Reference)		0.16	1.00	(Reference)	
TC	26 (13.90%)	4 (8.00%)	15 (13.39%)	0.26	1.58	(0.29-8.55)	0.59	0.90	1.93	(0.64-5.76)	0.24
CC	151 (81.28%)	43 (86.00%)	87 (77.68%)	0.39	1.17	(0.3-4.51)	0.82	0.52	1.93	(0.76-4.86)	0.16

OR, odd ratio; CI, confident interval. [a]Differences of genotypes frequencies between controls and patients with Gleason Score <7 were calculated based on χ^2 test. [b]OR and 95% CI were calculated between controls and patients with Gleason Score <7 based on unconditional logistic regression analysis. [c]Differences of genotypes frequencies between controls and patients with Gleason Score ≥7 were calculated based on χ^2 test. [d]OR and 95% CI were calculated between controls and patients with Gleason Score ≥7 based on unconditional logistic regression analysis.

adjusted ORs for prostate cancer associated with heterozygous and mutant genotypes failed to reach a significant threshold (P>0.05). The results showed that these candidate polymorphisms were not associated with a decreased risk of prostate cancer.

Alcohol consumption has been considered as an increased risk factor for prostate cancer [29-31], however, conflicting conclusions such as decreased risk [32] and no relationship [33, 34] have also been reported by other observational studies. Previous studies have been found that some polymorphisms could cause astronger increased risk of prostate cancer in alcohol consumers [35, 36]. In order to better understand whether LIMK1 polymorphisms would be one of the risk factors for certain alcohol and non-alcohol consumersof prostate cancer susceptibility, we compared the difference of each genotype frequency in these two subgroups, respectively (**Table 4**). However, no significant differences of genotypes were found in both groups of different alcohol consumption status, respectively. Smoking is a common risk factor for cancers, an increased risk of prostate cancer has been identified among heavy smokers [37], and increased prostate cancer mortality was found among current smokers [37, 38]. Associations between polymorphisms and smoke status with regard to the risk of prostate cancer have also been reported [39, 40]. In our study, we compared the genotypes frequencies between control and patients groups based on their smoke status (**Table 5**). However, we failed to find any significant differences. We speculated that these candidate SNPs of LIMK1 might not cause a stronger increased or weaker decreased risk of prostate cancer in both alcohol and smoke consumers.

Prostate cancer with a high Gleason score is considered to be more aggressive, while high concentration of LIMK1 was also found to cause increased aggression of prostate cancer. Accordingly, we divided the patient group into two subgroups according to the Gleason score (**Table 6**). However, genotypes frequen-

cies showed no significant differences for each subgroup when compared with controls. The results indicated that these candidate polymorphisms might not be risk factor for prostate cancer either with low or high Gleason score.

To the best of our knowledge, this was the first study describing the association between LIMK1 polymorphisms and prostate cancer risk. However, there are some limitations in this article. Firstly, the studied sample was small and only the ethnic Chinese was included, and the number of sample became smaller after dividing into subgroups. Secondly, although age factor was adjusted when calculating OR value, the diet habits were not considered in this study. A larger population and genomic environmental as well as life habits combination studies are warranted to further confirm our finding.

Conclusion

The present study was the first to report that LIMK1 polymorphisms rs2269081, rs2269082, rs178409, rs6460071 and rs710968 may not be risk factors for a Chinese population. As the crucial role of LIMK1 in prostate cancer, further studies focus on more candidate SNPs in larger populations and more ethnic groups are needed.

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Disclosure of conflict of interest

None.

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