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Original paper

Study on the relationship between TagSNPs and haplotype of hCHK2 and esophageal cancer in Kazakh and Han in Xinjiang

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Aim of the study: To determine the association of hCHK2 rs2278022, rs2602431, and rs2970077 polymorphisms and haplotypes with susceptibility to esophageal cancer in Kazakh and Han in Xinjiang Uygur Autonomous Region.

Material and methods: Molecular epidemiology was carried out on 239 cases of esophageal cancer (132 Kazakh, 107 Han) and 513 controls (309 Kazakh, 204 Han) of Xinjiang. Polymorphisms of *hCHK2* at rs2278022, rs2602431 and rs2970077 were analyzed by polymerase chain reaction-ligase detection reaction (PCR-LDR). Haplotypes were estimated by the SHEsis software. Statistical differences in genotype/haplotype frequencies, and frequencies between the case group and the control group were estimated.

Results: 1) No significant difference was observed in the frequency of *hCHK2* at rs2278022, rs2602431 and rs2970077 between the cases and controls in Kazakh and Han ($P > 0.05$); 2) In Kazakh and Han, the distribution of haplotypes was not significantly different between esophageal cancer cases and controls ($P > 0.05$).

Conclusions: Polymorphisms of *hCHK2* at rs2278022, rs2602431 and rs2970077 and haplotypes are unlikely to be associated with the susceptibility to esophageal cancer in Kazakh and Han.

Key words: *hCHK2* gene, single nucleotide polymorphism, haplotype, esophageal cancer, Kazakh nationality.

Introduction

Esophageal cancer is one of the top ten most common malignant tumors in the world and the most common form of gastrointestinal cancer in developing countries [1], including Xinjiang, a province of China that has a high incidence of esophageal cancer [2]. Multiple genes are involved in the process of carcinogenesis, so a single gene polymorphism is insufficient to cause cancer. The accumulation of multiple altered gene functions can lead to changes in regulation of cell growth and proliferation, which could ultimately differentially affect the susceptibility of one individual to develop esophageal cancer from another. Therefore, based on previous studies, polymorphisms of *hCHK2* at rs2278022, rs2602431 and rs2970077 were analyzed by polymerase chain reaction-ligase detection reaction (PCR-LDR) of the Xinjiang Uygur Autonomous Region to compare differences in the distribution frequency of various single nucleotide polymorphism (SNP) genotypes and haplotypes and to determine the molecular mechanism behind the pathogenesis of esophageal cancer. Our work thus furthers our understanding of the disease by determining potential markers that could predict patient susceptibility to the development of esophageal cancer and facilitate the improvement of therapeutic and preventive measures.

Material and methods

Patients and controls

Samples of patients were collected from six hospitals (Xinjiang Autonomous Region People's Hospital, the First, Second, and Third Affiliated Hospital of Xinjiang Medical University, the Xinhua, and the Friendship Hospital of Ili State) during the period of 2005–2007. There were 239 (132 Kazakh, 107 Han) patients with primary esophageal cancer diagnosed by endoscopy, X-ray and pathology. A total of 513 healthy controls (309 Kazakh, 204 Han) were selected based on non-blood relationship, similar nationality and gender, age difference of ±5 years old, and over the same period of hospitalization due to non-tumor, non-autoimmune diseases. Informed consent was obtained from all participants prior to blood drawing (5 ml preserved in EDTA at –20°C).

Genotyping assays

DNA extraction Genomic DNA was extracted from 1 ml EDTA anti-coagulated peripheral blood samples by the improved Miller salting-out procedure [3].

Primer design

Primers were designed by the Primer 3.0 software (Table 1).

Multiplex PCR reaction system

A reaction volume of 20 μl contained template DNA 1 μl, 1 × buffer 2 μl, 3 mM Mg2 0.6 μl, 2 mM dNTP 2 μl, Taq DNA polymerase 0.3 μl, 1 × Q-Solution 4 μl, 0.2 pM forward and reverse primers 0.4 μl. The thermocycling conditions were as follows: 35 cycles of 95°C for 15 min, 94°C for 30 s, 56°C for 1 min, 72°C for 7 min.

Probe design

Upstream and downstream probes were designed based on the principles of the LDR probe design [4] (Table 2). The gene loci of the probes were as follows

Multi-LDR reaction

A reaction volume of 10 μl contained 1 × buffer 1 μl, 12.5 pmol/μl probe mixture 1 μl, 2 U ligase 0.05 μl, and 100 ng/μl PCR products 1 μl. The thermocycling conditions were as follows: 35 cycles of 95°C for 2 min, 94°C for 30 s, and 60°C for 2 min.

LDR genotyping

PCR products were sequenced by 377 DNA Sequencer (ABI, USA) to detect DNA fragments of various lengths as determined by the size of the product fragments. ROX was the internal reference marker.

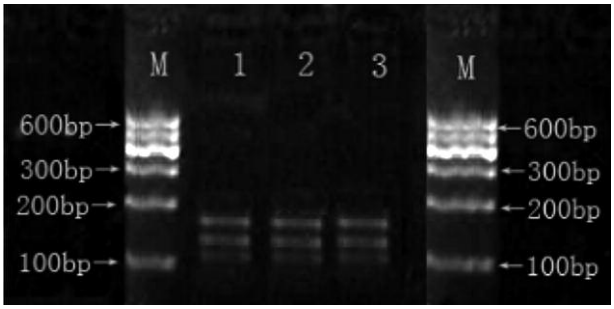
Statistical analysis

The goodness of fit between observed and estimated *hCHK2* rs2278022, rs2602431 and rs2970077 genotype frequencies according to the Hardy-Weinberg equilibrium was determined by the χ^2 test. Allele and genotype frequencies of the three sites in both the case and the control groups were tested by χ^2 test. The haplotype of *hCHK2* tagSNPs was determined and analyzed by the SHEsis software. Statistical analysis was conducted using the SPSS 15.0 software. *P*-values < 0.05 were considered significant.

Results

PCR amplification of *hCHK2* rs2278022, rs2602431 and rs2970077

PCR-amplified products of *hCHK2* were separated by agarose gel electrophoresis (Fig. 1).



M: Marker, 100bp DNA ladder; lane 1-3: PCR products of samples, hCHK2 rs2602431 (125 bp), rs2278022 (157 bp), and rs2970077 (184 bp)

Fig. 1. PCR products of *hCHK2*

Table 1. Forward and reverse primer sequence of *hCHK2* at the designated SNP sites

tagSNPs	Primer sequence		PCR length
	forward primer (5'-3')	reverse primer (5'-3')	
rs2278022	TGTCATGGTCGAAGAAAGTTG	AGGGATTGGTTGGTTGTTCA	157 bp
rs2602431	GGAAGGAAACTTGCCATTGT	CAGGGCAGTCATTTTCAACC	125 bp
rs2970077	AAACAACACAACAGGCTGGA	AATGGTCAAATGAATGCAGAA	184 bp

Table 2. LDR probe sequences of *hCHK2* at various gene loci

Probe name	Probe sequence (5'-3')	LDR length
rs2278022_modify	P-TAACTTCTGGTTTTGTGCACAGTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs2278022_C	TTTTTTTTTTTTTTTTTTTTCAATGATTCAATGGTCTTGTTGGG	90
rs2278022_T	TTTTTTTTTTTTTTTTTTTTTTCAATGATTCAATGGTCTTGTTGGA	92
rs2602431_modify	P-AGCTTATTAAAAATCCAGATGTCCATTTTTTTTTTTTTTTTT-FAM	
rs2602431_C	TTTTTTTTTTTTTTTTTTCAACCTTGGGACACATCTGTGACG	86
rs2602431_T	TTTTTTTTTTTTTTTTTTTTCAACCTTGGGACACATCTGTGACA	88
rs2970077R_modify	P-TAAACGAAATGTGGTGAGAATACTATTTTTTTTTTTTTTTTTTTT-FAM	
rs2970077R_A	TTTTTTTTTTTTTTTTTTTTTTAAGAGAAAAAGAGTATGAGTAGAT	94
rs2970077R_G	TTTTTTTTTTTTTTTTTTTTTTTTAAGAGAAAAAGAGTATGAGTAGAC	96

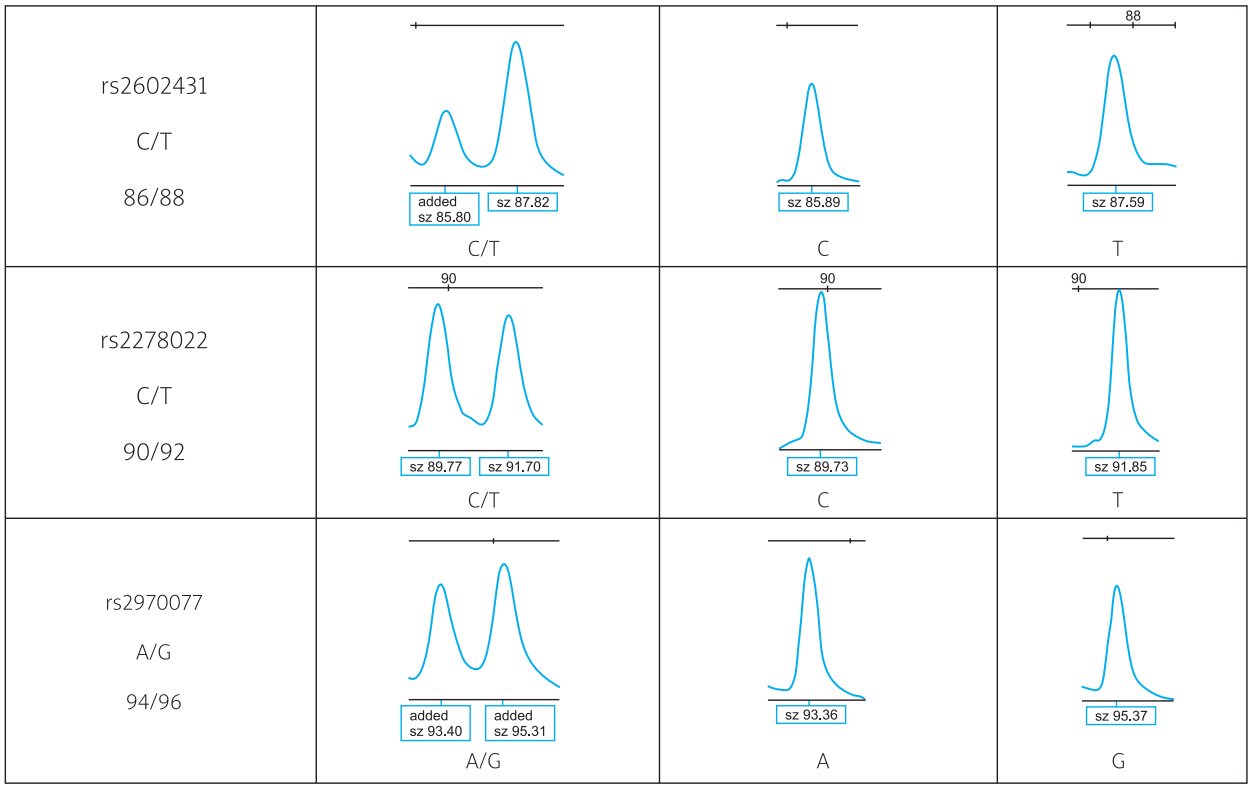


Fig. 2. The genotype of *hCHK2* rs2602431, rs2278022 and rs2970077 gene polymorphisms

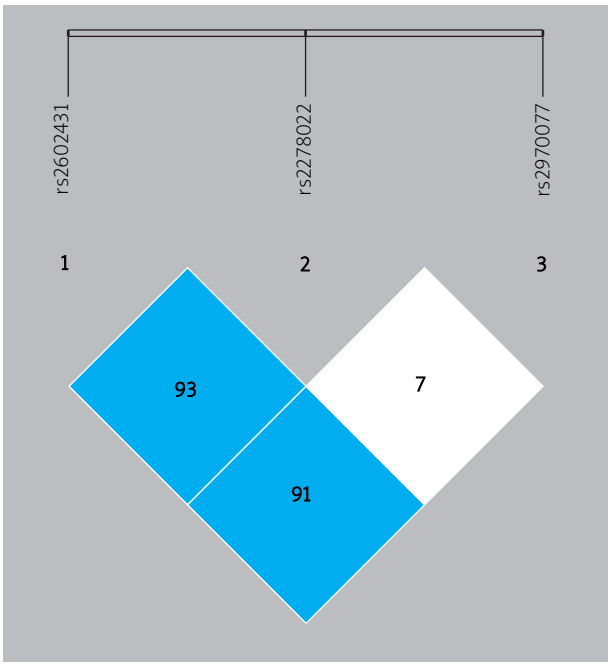


Fig. 3. Schematic diagram of linkage disequilibrium for *hCHK2* found in Kazakh population

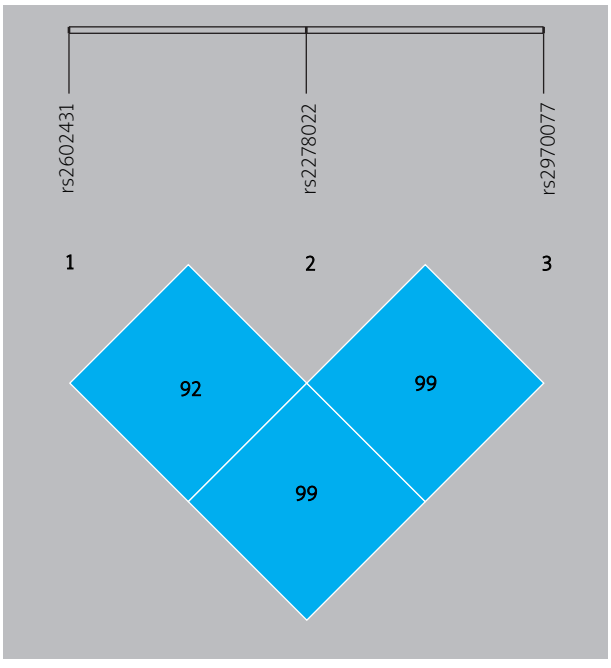


Fig. 4. Schematic diagram of linkage disequilibrium for *hCHK2* found in Han population

Sequencing results

The sequences of *hCHK2* rs2278022, rs2602431 and rs2970077 were analyzed by an automated sequencer to determine their genotype (Fig. 2).

Correlation between hCHK2 tagSNPs and esophageal cancer in Kazakh and Han

The effect of *hCHK2* rs2278022, rs2602431 and rs2970077 on the susceptibility of the case and control groups to esophageal cancer is summarized in Table 3. Genotype fre-

Table 3. Correlation of *hCHK2* SNPs with susceptibility to esophageal cancer in Kazakh and Han

tagSNP position	Genotype	Kazakh nationality		χ^2	*P*	Han nationality		χ^2	*P*
		cases (%)	controls (%)			cases (%)	controls (%)		
rs2278022	genotypes TT	93 (78.15)	188 (74.60)	0.75	0.69	57 (58.16)	110 (60.77)	1.20	0.55
	TC	25 (21.01)	60 (23.81)			35 (35.71)	65 (35.91)		
	CC	1 (0.84)	4 (1.59)			6 (6.12)	6 (3.31)		
	TT	93 (78.15)	188 (74.60)	0.55	0.46	57 (58.16)	110 (60.77)	0.18	0.67
	TC +CC	26 (21.85)	64 (25.40)			41 (41.84)	71 (39.23)		
	Total	119	252			98	181		
	allele genes C	27 (11.34)	68 (13.49)	0.67	0.41	47 (23.99)	77 (21.27)	0.54	0.46
	T	211 (88.66)	436 (86.51)			149 (76.01)	285 (78.73)		
rs2602431	genotypes CC	70 (58.33)	155 (62.75)	0.68	0.71	43 (43.43)	75 (42.86)	2.17	0.34
	CT	42 (35.00)	78 (31.58)			45 (45.45)	89 (50.86)		
	TT	8 (6.67)	14 (5.67)			11 (11.11)	11 (6.29)		
	CC	70 (58.33)	155 (62.75)	0.67	0.42				
	CT +TT	50 (41.67)	92 (37.25)						
	Total	120	247			99	175		
	allele genes T	58 (24.16)	106 (21.46)	0.68	0.41	67 (33.84)	111 (31.71)	0.26	0.61
	C	182 (75.84)	388 (78.54)			131 (66.16)	239 (68.29)		
rs2970077	genotypes CC					83 (86.46)	151 (82.51)	0.44	0.80
	CT					13 (13.54)	30 (16.39)		
	TT					0 (0.00)	2 (1.09)		
	CC	98 (82.35)	217 (85.43)	0.59	0.44	83 (86.46)	151 (82.51)	0.72	0.40
	CT+TT	21 (17.65)	37 (14.57)			13 (13.54)	32 (17.49)		
	Total	119	254			96	183		
	allele genes T	21 (8.82)	38 (7.48)	0.40	0.53	13 (6.77)	34 (9.29)	1.71	0.19
	C	217 (91.18)	470 (92.52)			179 (93.23)	332 (90.71)		

Table 4. Distribution of haplotypes in *hCHK2* SNPs between case and control groups of the Kazakh population

Haplotype	Cases (%)	Controls (%)	χ^2	*P*	OR	OR 95%CI
C-C-C	1.16 (0.50)	2.23 (0.50)	–	–	–	–
C-C-T	1.07 (0.50)	0.00 (0.00)	–	–	–	–
C-T-C	178.78 (75.80)	383.34 (77.60)	0.19	0.66	0.92	0.63–1.34
C-T-T	0.00 (0.00)	2.43 (0.50)	–	–	–	–
T-C-C	22.71 (9.60)	59.22 (12.00)	0.85	0.36	0.79	0.47–1.31
T-C-T	2.07 (0.90)	1.55 (0.30)	–	–	–	–
T-T-C	12.35 (5.20)	15.22 (3.10)	2.09	0.15	1.75	0.81–3.77
T-T-T	17.87 (7.60)	30.01 (6.10)	0.61	0.43	1.27	0.69–2.34

Note: Listed in the order of rs2602431, rs2278022 and rs2970077

quencies of these genetic polymorphisms calculated from the control group were in Hardy-Weinberg equilibrium (χ^2-test, $P > 0.05$) [5]. No significant difference was observed in the frequency of *hCHK2* at rs2278022, rs2602431 and rs2970077 between the case and control groups of Kazakh and Han.

Linkage disequilibrium test on hCHK2 tagSNPs

The outcomes of the linkage disequilibrium test on *hCHK2* tagSNPs are presented in Figures 3 and 4.

Table 5. Distribution of haplotypes in *hCHK2* SNPs between case and control groups in the Han population

Haplotype	Cases (%)	Controls (%)	χ^2	*P*	OR	OR 95%CI
C-C-C	3.05 (2.00)	2.05 (1.00)	–	–	–	–
C-T-C	126.95 (66.80)	236.95 (67.70)	0.03	0.87	0.97	0.66–1.43
T-C-C	43.95 (23.10)	69.95 (20.00)	0.77	0.38	1.21	0.79–1.86
T-T-C	3.05 (1.60)	8.05 (2.30)	–	–	–	–
T-T-T	13.00 (6.80)	33.00 (9.40)	1.04	0.31	0.71	0.36–1.38

Note: Listed in the order of rs2602431, rs2278022 and rs2970077

Correlation between haplotype of *hCHK2* tagSNPs and esophageal cancer in Kazakh and Han

hCHK2 rs2602431, rs2278022 and rs2970077 can form eight haplotypes. In the Kazakh groups, only four haplotypes were defined by the SHEsis software. The frequency distributions of these four haplotypes (CTC, TCC, TTC, and TTT) in the case and control groups were 75.80% and 77.60%, 9.60% and 12.00%, 5.20% and 3.10%, and 7.60% and 6.10%, respectively. The frequency distribution between the two groups was not significant ($P > 0.05$). In the Han groups, five haplotypes were identified, though only three were defined by the SHEsis software. The frequency distributions of these three haplotypes (CTC, TCC, and TTT) in the case and control groups were 67.70% and 66.80%, 23.10% and 20.00%, and 6.80% and 9.40%, respectively. The frequency distribution between the two groups was also not statistically significant ($P > 0.05$). The results are summarized in Tables 4 and 5.

Discussion

The cell cycle checkpoint kinase 2 (*CHK2*) gene is located on chromosome 22q12.1 and contains 14 exons. Its full-length cDNA is 1731 bp. In the event of DNA damage or replication block, CHK2 is activated and acts on various downstream target proteins. Ultimately, CHK2 activates cell cycle checkpoints at G1/S and/or G2/M to block cell cycle progression and to initiate the transcription of repair genes to promote the repair of cell damage. Many scholars believe that CHK2 plays a role in tumor suppression. Dysregulation of CHK2 would result in a defective DNA damage response, leading to the development of cancer.

Previous studies have focused on whether *hCHK2* polymorphism at the 84th codon A252G increases patient susceptibility to cancer. These studies show that the *hCHK2* A252G polymorphism leads to higher risk of head and neck squamous cell carcinoma and familial multiple tumor syndromes [6]. Studies by Jonine and others have shown that *CHK2*1100delC* increases susceptibility to breast cancer [7]. Given these findings, we thus chose to analyze three *CHK2* tagSNPs by Hapmap. We have shown here that in either the population of Kazakh or Han, there was no statistical difference between the case and control groups in terms of *hCHK2* rs2278022, rs2602431 and rs2970077 genotypes or their allele frequency distribution. Our findings suggest that these polymorphisms do not affect the occurrence of esophageal cancer. It is possible that polymorphism of a single gene may weakly affect tumor development. To address this possibility, we therefore analyzed *CHK2* haplotypes. Data from our analysis of single loci revealed that there is no significant difference between polymorphisms of *hCHK2* rs2602431, rs2278022 and rs2970077 and esophageal cancer. It is important to note that this finding does not rule out the possibility that the presence of two or more of these polymorphisms in an individual would render the patient more susceptible to esophageal cancer.

Our current study also shows that the Kazakh population has at least eight haplotypes formed by three SNPs sites at *hCHK2* rs2602431, rs2278022 and rs2970077. However, the haplotypes of which the frequency in both the case and control groups were greater than 0.03 were only CTC, TCC, TTC, or TTT. The frequency distributions for the case and control group were 75.80% and 77.60%, 9.60% and 12.00%, 5.20% and 3.10%, and 7.60% and 6.10%, respectively. The distribution of haplotypes was not significantly different between the case and control groups ($P > 0.05$). In the Han population, *hCHK2* rs2602431, rs2278022 and rs2970077 SNPs with haplotype frequencies greater than 0.03 are CTC, TCC, and TTT types. The frequency distributions for the case and control groups were 67.70% and 66.80%, 23.10% and 20.00%, and 68.00% and 9.40%, respectively. The distribution of haplotypes was not significantly different between the case and control groups ($P > 0.05$). Our findings show that haplotypes of *hCHK2* rs2602431, rs2278022 and rs2970077 do not affect the incidence of esophageal cancer in either Kazakh or Han populations. Our study did not address how multiple haplotypes may affect susceptibility to cancer. Since multiple genetic alterations can result in complex changes in biology, further studies are needed to determine how multiple haplotypes within one individual can drive cancer susceptibility.

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The authors declare no conflict of interest.

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