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Review Article

The Association between Four Common Polymorphisms in microRNA and Risk of Hepatocellular Carcinoma: An Updated Meta-Analysis

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Abstract

Background: Many epidemiological studies have explored the relationship between single-nucleotide polymorphism and hepatocellular carcinoma (HCC). However, the results remain controversial. We performed a large-scale meta-analysis to draw a more precise estimation of the aforementioned association.

Methods: Studies on the association between microRNA (MIR) polymorphisms and HCC risk that had been published up to Sep 30, 2021 were identified by searching the PubMed, Cochrane Library, Google Scholar, Web of Science, and Chinese Biomedical Literature electronic databases and the Excerpta Medical Database. The association between MIR polymorphisms and HCC risk was assessed using odds ratios (ORs) and their 95% confidence intervals (CIs).

Results: Overall, 29 studies, with a total of 9,263 cases and 10,875 controls, were included in our meta-analysis. MicroRNA149 (MIR149) significantly decreased the risk of developing HCC on the overall population (homozygous model CC vs. TT: OR = 0.703, 95% CI = 0.549-0.899, *P* = 0.005), and microRNA 196 (MIR196) significantly decreased the risk of developing HCC on the overall population (recessive model TT vs. CT+CC: OR = 0.864, 95% CI = 0.751-0.993, *P* = 0.04) and on Caucasians (OR = 0.613, 95% CI = 0.414-0.907, *P* = 0.014).

Conclusion: The MIR149 and MIR196 polymorphisms are the protect factors of developing HCC. The conduct of multi-center and multi-region studies with gene-gene, gene-environment should be considered.

Keywords: Hepatocellular carcinoma; MicroRNA; Polymorphism; Susceptibility; Meta-analysis

Introduction

Liver cancer ranked in the sixth and the fourth in all cancers in new cancer and cancer death around the world in 2018. Approximately 841,000 was newly diagnosed with liver cancer, and 782,000 have died from the disease (1). It was also estimated to become the third leading cause of cancer-related death among the Chinese in 2015 (2). Hepatocellular carcinoma (HCC) is the major type of primary liver cancer, accounting for approximately 75%~85%, which the main

pathogenic factors including hepatitis B virus (HBV) and hepatitis C virus (HCV) (3), aflatoxin-contaminated foodstuffs, excessive drinking, smoking (4), obesity, and type 2 diabetes (5, 6). These known risk factors, however, cannot fully result in the overall occurrence of HCC.

Recently, epigenetics may change the carcinogenesis of gene transcription, chromosomal stability, and cell differentiation (7-9). The key factors regulating multiple development, differentiation, and cell proliferation. MicroRNAs are small non-coding RNAs that regulate the genetic translation by controlling the expression of pivotal proteins involved in cancer-associated pathways in several ways (10).

Of all the miRNAs, miRNA146a (MIR146a), miRNA149 (MIR149), miRNA196a2 (MIR196a2) and miRNA499a (MIR499a), which lie in chromosomes 5q33.3, 2q37.3, 12q13.13, and 20q11.22 (https://www.ncbi.nlm.nih.gov/gene), respectively, are the most commonly studied in relation to HCC. These miRNAs influence tumor and immune cell proliferation, apoptosis, migration (11) and metastasis (12) by regulating multiple signaling, such as Signal Transducers and Activators of Transcription (STAT) signaling (13) and nuclear factor kB (NF-κB) signaling (14). Single-nucleotide polymorphism (SNP) mainly refers to the DNA sequence polymorphism caused by single-nucleotide variation. Several studies have reported the relationship between SNP in miRNAs and HCC risk, but their results remain controversial in MIR146 (15-19), MIR149 (15, 16), MIR196 (20-22) and MIR499 (15-17). Therefore, to acquire a more precise evaluation, we analyzed all relevant studies to evaluate the possible affiliation of four common polymorphisms with HCC risk.

Materials and Methods

Search strategy

A comprehensive literature search of studies published in the PubMed, Excerpta Medical Database (Embase), Cochrane Library, Google Scholar, Web of Science, and Chinese Biomedical Literature (CBM) electronic databases up to Sep 30, 2021 was conducted. The following MeSH terms were used for the search to be able to obtain more comprehensive and relevant articles: "Carcinoma, Hepatocellular", "Polymorphism, Single Nucleotide", "MIRN146 microRNA", "MIRN149 microRNA", "MIRN196 microRNA" and "MIRN499 microRNA" and its relative Entry Terms. There was no restraint in the time period, sample size, population, or language. Furthermore, all eligible studies were retrieved, and their references were checked for other additional eligible studies. All steps were performed according to Preferred Reporting Items for Meta-Analyses (PRISMA) guidelines (23).

Eligibility criteria

To be included in the meta-analysis, all the studies had to satisfy the following inclusion criteria: 1) the association between one or more SNPs in MIR146/149/196/499 and HCC; 2) case-control studies; 3) sufficient data to access the odds ratio (OR) with their 95% confidence intervals (95% CIs), and 4) control population without malignant tumor.

Exclusion criteria

Studies were excluded from the meta-analysis if any one of the following existed: 1) no control population; 2) unrelated our theme; and 3) insufficient information.

Data extraction

The two authors of the present study independently extracted data from the eligible studies to guarantee the accuracy and precision of the extracted messages. The extracted information included the first author name, publication year, country, ethnicity, genotyping methods, source of control, the HCC ascertainment, the diverse genotype frequencies of the cases and controls, and the *P* value for the control population in Hardy Weinberg equilibrium (HWE).

Quality assessment

We evaluated the quality of all the papers on the basis of the Newcastle−Ottawa Quality Assessment Scale (NOS) (24). The NOS scores, range from 0 to 9. The studies were considered "high-quality" studies if the scores more than 7 (Table 1).

Statistical analysis

Heterogeneity was applied through the Q test and I^2 statistics for each combined analysis, where $P<0.1$ or $I^2>50\%$ were considered significantly heterogeneous. A random-effects model was then used to evaluate the different ORs. Otherwise, a fixed-effect model was applied if there was only marginal heterogeneity or if there was no heterogeneity at all. Then logistic meta-regression to explore the sources of heterogeneity if heterogeneity was detected. The following characteristics were included as covariates in the analysis: ethnicity, genotyping method, quality score, source of controls, and HWE in the control. Subgroup analysis was performed if the logistic meta-regression analysis showed one or more of the aforementioned conditions. Simultaneously, to identify the potentially influential studies, sensitivity analysis was also conducted to determine if the effect estimate was robust. HWE in the control population was tested with a goodness-of-fit chi-squared test. Given the existence of denotation bias, the publication bias was assessed using a Begg's funnel plot and the Egger's regression asymmetry test. All the statistics were analyzed using Stata version 16.0 (Stata Corp., College Station, Texas, USA), and all the *P* values in the two sides that were less than 0.05 were considered to show statistical significance.

Results

Characteristics of study

Based on the search strategy, 229 studies were identified from all the electronic databases. As shown in Fig. 1, after precluding 193 irrelevant studies as judged from their titles and/or abstracts, the remaining 36 articles were reviewed for a more detailed evaluation. Based on the exclusion criteria, 7 studies were excluded: 1 about a review (25), 3 for insufficient data to evaluate OR and 95%CI (26-28), 2 for control group with HCC (29, 30), and 1 for case group uncontained HCC(31). Consequently, 29 studies met the inclusion criteria in this meta-analysis. Most study come from Asian, including 28 English articles (15-22, 32-51) and 1 Chinese study (52). The number of articles of MIR146, MIR149, MIR196 and MIR499 is 18, 6, 19 and 19, respectively Thus, 29 articles containing 62 studies were included in this meta-analysis (Table 1).

Table 1: Characteristics of all the eligible studies

Reference	*Ethnicity*	*Method-Genotyping*	*Control Source*	*sample size (case/control)*	*Genotype Distribution (case/control)*			*Quality Score*	*HWE*
MIR146 rs2910164					CC	CG	GG		
(19)	Asian	PCR-RFLP	PB	479/504	158/197	241/249	80/58	6	0.297
(34)	Caucasian	PCR-RFLP	PB	222/222	10/11	75/67	137/144	9	0.684
(52)	Asian	PIRA-PCR	PB	963/852	319/303	450/386	156/151	8	0.352
(16)	Asian	PCR-RFLP	HB	159/201	57/74	88/103	14/24	8	0.423
(18)	Asian	PCR-RFLP	HB	100/100	28/33	45/46	27/21	5	0.801
(50)	Asian	PCR-RFLP	PB	186/483	67/158	86/254	33/71	6	0.16
(17)	Asian	PCR-RFLP	PB	172/185	82/78	62/71	28/36	8	0.0345
(46)	Asian	MassARRAY	HB	997/998	331/367	503/475	163/156	7	0.994
(42)	Asian	RT-PCR	HB	314/407	149/159	165/244	0/3	7	0.001
(49)	Asian	PCR-RFLP	PB	266/281	73/97	153/154	40/30	8	0.025
(35)	Asian	PCR-RFLP	HB	188/337	84/141	82/146	22/50	8	0.487

(37)	Asian	PCR-RFLP	PB	184/184	58/47	83/85	43/52	8	0.593
(22)	Asian	PCR-RFLP	HB	274/328	94/123	145/169	35/36	9	0.145
(39)	Asian	PCR-RFLP	PB	266/266	29/19	86/81	151/166	8	0.133
(43)	Asian	MassARRAY	HB	103/423	40/167	46/190	17/66	6	0.622
(47)	Asian	PCR-RFLP	HB	175/302	52/137	86/135	37/30	7	0.927
(15)	Caucasian	PCR-RFLP	PB	100/120	14/13	22/33	64/74	7	0.018
(48)	Asian	TaqMan	HB	584/923	194/359	297/432	84/130	6	1
MIR149					TT	TC	CC		
rs2292832									
(16)	Asian	PCR-RFLP	HB	159/201	81/83	64/97	14/21	8	0.64
(35)	Asian	RT-PCR	HB	188/337	139/246	36/64	13/27	8	0.001
(45)	Asian	PCR-RFLP	PB	152/304	67/113	72/148	13/43	8	0.886
(22)	Asian	PCR-RFLP	HB	274/328	66/72	133/156	75/100	9	0.751
(39)	Asian	PCR-RFLP	PB	266/266	45/34	130/124	91/108	8	0.985
(15)	Caucasian	PCR-RFLP	PB	100/120	42/35	58/41	20/24	7	0.236
MIR196					CC	CT	TT		
rs11614913									
(40)	Asian	RT-PCR	PB	310/222	78/42	150/102	82/78	5	0.703
(21)	Asian	PCR–LDR	HB	361/391	82/92	179/197	100/102	6	0.987
(52)	Asian	PIRA-PCR	HB	963/852	156/151	450/386	319/303	8	0.352
(32)	Caucasian	PCR-RFLP	PB	185/185	77/58	86/87	22/40	9	0.79
(16)	Asian	PCR-RFLP	HB	159/201	34/45	84/107	41/49	8	0.653
(46)	Asian	MassARRAY	HB	996/995	208/181	449/417	277/239	7	1
(36)	Asian	RT-PCR	PB	1021/1012	207/220	505/485	309/307	7	0.55
(42)	Asian	RT-PCR	HB	314/407	45/71	209/214	60/121	7	0.365
(49)	Asian	PCR-RFLP	PB	266/281	93/66	139/160	34/55	8	0.06
(35)	Asian	PCR-RFLP	HB	188/337	41/70	81/167	66/100	8	1
(22)	Asian	PCR-RFLP	HB	274/328	46/27	147/165	81/136	9	0.06
(39)	Asian	PCR-RFLP	PB	266/266	84/113	131/123	51/30	8	0.99
(43)	Asian	MassARRAY	HB	103/423	27/103	48/203	28/117	6	0.723
(47)	Asian	PCR-RFLP	HB	175/302	25/42	85/138	65/122	7	0.957
(38)	Asian	RT-PCR	HB	109/105	25/18	64/52	20/35	5	0.985
(44)	Caucasian	TaqMan	PB	60/150	25/80	32/53	3/17	7	0.221
(20)	Caucasian	RT-PCR	HB	75/75	37/30	32/35	6/10	8	1
(15)	Caucasian	PCR-RFLP	PB	100/120	26/41	57/59	17/20	7	0.988
(48)	Asian	TaqMan	HB	584/923	113/158	281/474	181/289	6	0.308
MIR499					AA	AG	GG		
rs3746444									
(33)	Caucasian	PCR-RFLP	PB	222/222	45/47	87/93	90/82	7	0.111
(16)	Asian	PCR-RFLP	HB	159/201	109/120	47/74	3/7	8	0.555
(18)	Asian	PCR-RFLP	HB	100/100	36/54	40/36	24/10	7	0.563
(50)	Asian	PCR-RFLP	PB	186/483	141/371	41/100	4/12	5	0.26
(17)	Asian	PCR-RFLP	PB	172/185	128/123	37/48	7/14	8	0.02
(51)	Asian	PCR-RFLP	PB	185/203	136/139	44/52	5/13	5	0.043
(42)	Asian	RT-PCR	HB	314/407	195/301	117/101	2/4	8	0.367
(49)	Asian	PCR-RFLP	PB	266/281	184/204	59/61	23/16	9	0.002
(35)	Asian	PCR-RFLP	HB	188/337	119/281	60/55	9/1	8	0.661
(41)	Asian	MassARRAY	HB	984/969	724/765	241/179	19/25	9	0.002
(45)	Asian	PCR-RFLP	PB	152/304	98/218	32/62	22/24	8	3.80E-07
(37)	Asian	PCR-RFLP	PB	184/184	128/117	39/43	17/24	7	2.90E-06

(22)	Asian	PCR-RFLP	HB	274/328	147/188	98/112	29/28	6	0.171
(39)	Asian	PCR-RFLP	PB	266/266	150/166	92/83	24/17	7	0.336
(47)	Asian	PCR-RFLP	HB	175/302	115/197	49/87	11/18	8	0.151
(44)	Caucasian	TaqMan	PB	60/150	28/57	23/66	9/27	6	0.594
(20)	Caucasian	RT-PCR	HB	75/75	41/31	32/30	2/14	6	0.398
(15)	Caucasian	PCR-RFLP	PB	100/120	21/37	40/32	39/51	7	3.18E-06
(48)	Asian	TaqMan	HB	584/923	409/669	154/230	12/22	8	0.915

PB, Population–based; HB, Hospital–based; HWE, Hardy–Weinberg equilibrium in control population; PCR–RFLP, Polymerase chain reaction-restriction fragment length polymorphism; RT-PCR, reverse transcription-polymerase chain reaction; DM, diabetes mellitus; HBP: high blood pressure

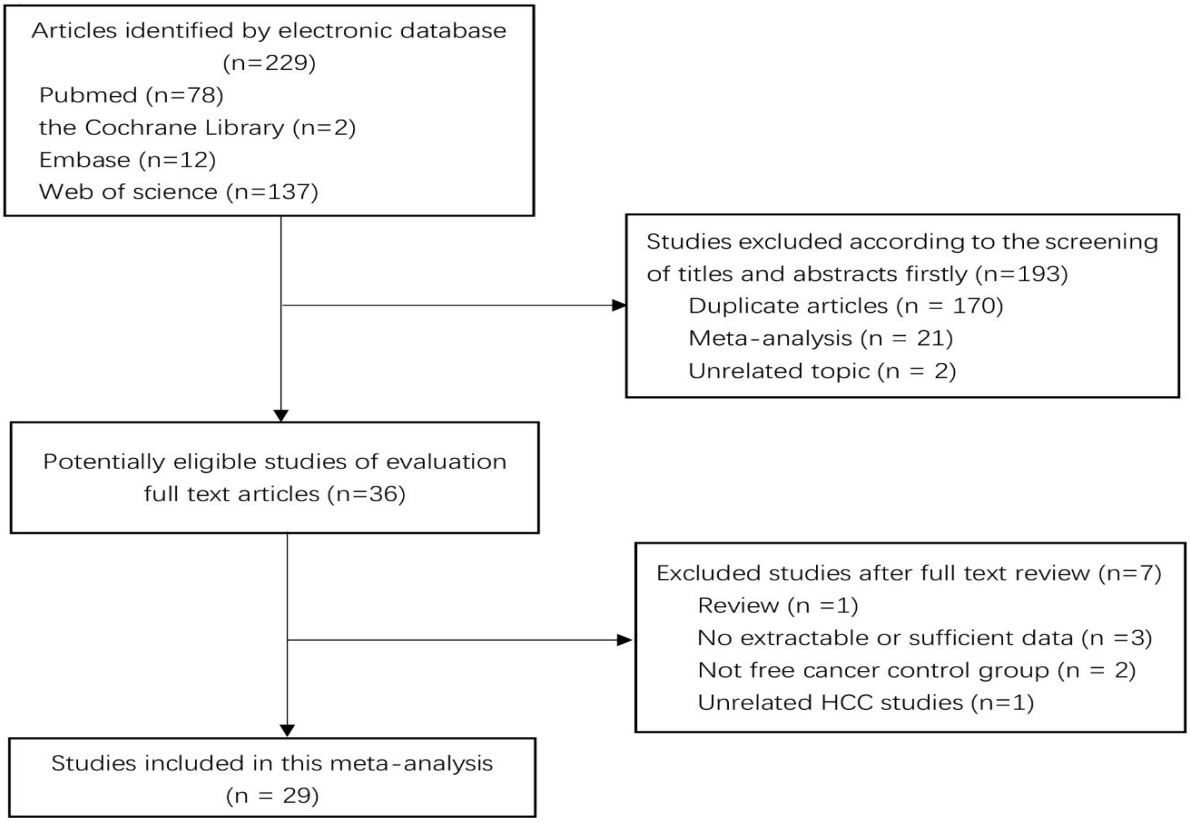

Fig. 1: Flow graph of included essay in this meta-analysis

Results of meta-analysis

For MIR146 polymorphism, it was not associated with overall HCC: dominant model CG+GG vs CC (OR=1.057, 95%CI= 0.939-1.190, *P*=0.36) (Fig. 2a), recessive model GG vs CG+CC (OR=1.043, 95%CI=0.947-1.148, *P*=0.397), homozygous model GG vs CC (OR=1.08, 95%CI= 0.997-1.171, *P*=0.61), and heterozygous model CG vs CC (OR=1.104, 95%CI=0.919-1.326, *P*=0.29), respectively (Table 2). Similar results were also found in different subgroups, including ethnicity, genotyping methods, quality score, source of controls and HWE in control (data not shown). Due to the apparent heterogeneity in the dominant and homozygous models, the random-effects model was used for the comparison models while the fix-effects model was used for the remaining models.

For MIR149 polymorphism, our results showed a significant decreased HCC risk in dominant model TC+CC vs TT (OR=0.817, 95%CI=0.686-0.973, *P*=0.024), recessive model CC vs TC+TT (OR=0.767, 95%CI=0.628-0.938, *P*<0.001) and homozygous model CC vs TT (OR=0.703, 95%CI=0.549-0.899, *P*=0.005) (Fig. 2b), respectively. As no obvious heterogeneity was observed, the fix-effects model was used to analyze all the comparison data in this polymorphism (Table 2).

Table 2: Results of the association between MIR polymorphism and HCC risk in the meta-analysis

Comparison	*Population*	***No. of study***	***Test of association***				***Heteorogencity***		
			OR	95%CI	*P*-value	Model	P_Q value	I^2(%)	P_{bias}
rs2910164									
CG+GG vs CC	Overall	18	1.057	(0.939-1.190)	0.36	R	0.008	50.3	0.24
	Caucasian	2	0.893	(0.494-1.618)	0.71	R	0.518	0	0.317
	Asian	16	1.062	(0.939-1.202)	0.338	R	0.004	55	0.293
GG vs CG+CC	Overall	18	1.043	(0.947-1.148)	0.397	F	0.04	40.1	0.974
	Caucasian	2	0.944	(0.688-1.294)	0.72	F	0.492	0	0.317
	Asian	16	1.053	(0.952-1.166)	0.315	F	0.025	45.4	0.55
GG vs CC	Overall	18	1.08	(0.997-1.171)	0.61	R	0.097	31.7	0.448
	Caucasian	2	0.877	(0.459-1.678)	0.692	R	0.302	6.3	0.583
	Asian	16	1.084	(0.999-1.175)	0.061	R	0.076	35.9	0.139
CG vs CC	Overall	18	1.104	(0.919-1.326)	0.29	F	0.004	53.7	0.166
	Caucasian	2	0.908	(0.496-1.662)	0.754	F	0.669	0	0.317
	Asian	16	1.118	(0.919-1.360)	0.264	F	0.002	58.4	0.205
rs2292832 T/C									
TC+CC vs TT	Overall	6	0.817	(0.686-0.973)	0.024	F	0.784	0	0.795
	Caucasian	1	1	(0.573-1.744)	1	F	-	-	-
	Asian	5	0.799	(0.664-0.961)	0.017	F	0.757	0	-
CC vs TC+TT	Overall	6	0.767	(0.628-0.938)	0	F	0.893	0	0.38
	Caucasian	1	0.633	(0.326-1.230)	0.178	F	-	-	-
	Asian	5	0.782	(0.634-0.965)	0.022	F	0.861	0	-
CC vs TT	Overall	6	0.703	(0.549-0.899)	0.005	F	0.888	0	0.473
	Caucasian	1	0.694	(0.330-1.461)	0.337	F	-	-	-
	Asian	5	0.704	(0.542-0.914)	0.008	F	0.789	0	-
TC vs TT	Overall	6	0.866	(0.718-1.045)	0.133	F	0.778	0	0.375
	Caucasian	1	1.179	(0.646-2.150)	0.591	F	-	-	-
	Asian	5	0.838	(0.688-1.021)	0.079	F	0	80.3	-
rs11614913 C/T									
CT+TT vs CC	Overall	19	0.937	(0.817-1.074)	0.348	R	0.001	57.3	0.411
	Caucasian	4	0.992	(0.607-1.621)	0.973	R	0.026	67.7	0.409
	Asian	15	0.933	(0.808-1.076)	0.338	R	0.003	57.4	0.224

TT vs CT+CC	Overall	19	0.864	(0.751-0.993)	0.04	R	0	62.5	0.122
	Caucasian	4	0.613	(0.414-0.907)	0.014	R	0.388	0.9	0.881
	Asian	15	0.895	(0.775-1.032)	0.128	R	0	65.7	0.393
TT vs CC	Overall	19	0.835	(0.693-1.006)	0.058	R	0	67.4	0.103
	Caucasian	4	0.627	(0.343-1.148)	0.13	R	0.153	43	0.828
	Asian	15	0.869	(0.717-1.055)	0.156	R	0	65.2	0.178
CT vs CC	Overall	19	0.986	(0.903-1.077)	0.755	F	0.015	45.9	0.766
	Caucasian	4	1.061	(0.800-1.407)	0.638	F	0.041	63.7	0.485
	Asian	15	0.978	(0.892-1.073)	0.644	F	0.037	43.4	0.386
rs3746444 A/G									
AG+GG vs AA	Overall	19	1.132	(0.961-1.334)	0.137	R	0	76.1	0.329
	Caucasian	4	0.934	(0.609-1.433)	0.755	R	0.086	54.6	0.724
	Asian	15	1.175	(0.983-1.404)	0.076	R	0	72.7	0.614
GG vs AG+AA	Overall	19	1.008	(0.777-1.308)	0.95	R	0.002	55.1	0.38
	Caucasian	4	0.765	(0.430-1.361)	0.362	R	0.035	65.2	0.059
	Asian	15	1.09	(0.802-1.481)	0.681	R	0.006	54.5	0.822
GG vs AA	Overall	19	1.042	(0.776-1.397)	0.786	R	0.001	59.1	0.391
	Caucasian	4	0.759	(0.368-1.568)	0.457	R	0.021	69.3	0.092
	Asian	15	1.122	(0.803-1.568)	0.502	R	0.002	58.5	0.946
AG vs AA	Overall	19	1.143	(0.982-1.329)	0.084	R	0	59.6	0.263
	Caucasian	4	1.036	(0.659-1.630)	0.877	R	0.103	51.5	0.738
	Asian	15	1.163	(0.988-1.368)	0.069	R	0.001	62.5	0.307

OR, odds ratio; CI, confidence intervals; NPR: Not PCR-RFLP, R, random effects model; F, fixed effects model, P_{bias}, P values of publication bias

With regard to MIR196 polymorphism, there was a negative association with HCC risk in recessive model TT vs CT+CC on overall population (OR= 0.864, 95%CI= 0.751-0.993, *P*=0.04) and Caucasian (OR= 0.613, 95%CI=0.414-0.907, *P*=0.014) (Fig. 2c), while the rest models were not associated with HCC risk. Random-effects model was used to compare dominant, recessive and homozygous models due to the obvious heterogeneity.

For MIR499 polymorphism, there were any significant association with HCC risk in overall population and all genetic model, such as dominant model AG+GG vs AA (OR=1.129, 95%CI=0.965-1.321, *P*=0.154) (Fig. 2d), recessive model GG vs AG+AA, homozygous model GG vs AA and heterozygous model AG vs AA (Table 2), respectively. The random-effects model was thus used for all the comparison data owing to its visible heterogeneity.

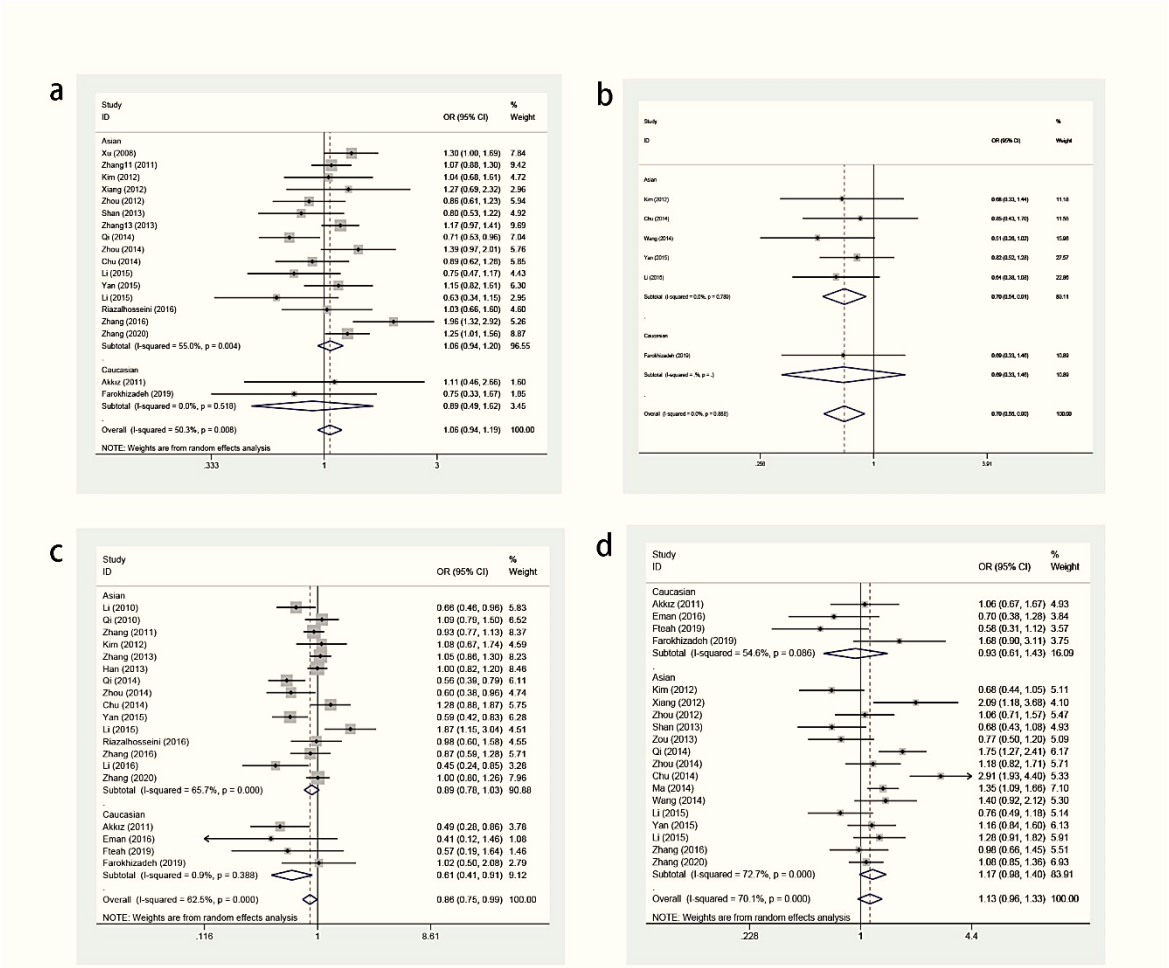

Fig. 2: Forest plot of the association between MIR Polymorphism and HCC Risk (a. dominant model in MIR146: CG+GG vs CC; b. homozygous model in MIR149: CC vs TT; C. recessive model in MIR196: TT vs CT+CC; d: dominant model in MIR499: AG+GG vs AA)

Heterogeneity analysis

Due to the manifest heterogeneity in MIR146/196/499 polymorphism, we conducted meta-regression and subgroup analyses to find the factors of heterogeneity. For the MIR146 polymorphism, the meta-regression analysis showed that the ethnicity, genotyping methods, quality score, source of controls, and HWE in the control were not influenced by the heterogeneity. To deduce further the heterogeneity, we performed Galbraith plot analysis to search the outliers. The studies by Qi et al. (42) and Zhang et al. (47) were outliers in CG+GG vs CC while the studies by Qi et al. (42) and Xu et al. (19) were in GG vs CC. When extracting the two studies respectively, all I^2 values were apparently reduced and P_Q values were more than 0.10 in overall populations. The significance of the summary ORs for the MIR146 polymorphism in all models of overall population was not influenced by omitting the two studies.

For the MIR196 polymorphism, the meta-regression analysis showed that heterogeneity has nothing to do with the ethnicity, genotyping methods, quality score, source of controls, and HWE of the control in the dominant, recessive, and homozygous models. But the Galbraith plot analysis showed that studies by three studies (22, 37, 49) were outliers in different models. After extracting the three studies respectively, all I^2 val-

ues greatly reduced and P_Q values were greater than 0.10 in overall population. The significance of the summary ORs for the MIR196 polymorphism in different models of the overall and subgroup analyses was not influenced by the omission of these studies.

For MIR499 polymorphism, the factors, such as ethnicity, genotyping methods, quality score, source of controls and HWE of control, were also caused the heterogeneity. Therefore, Galbraith plots showed that the seven studies (16-18, 20, 35, 45, 51) were outliers. When extracting the six studies respectively, all I^2 values were apparently reduced and P_Q values were more than 0.10 in recessive model, homozygous model and heterozygous model.

Sensitivity analysis

There were some studies that were inconsistent with HWE in the MIR146/196/499 polymorphism, sensitivity analysis was performed to determine if there was any study that might affect the final results of the susceptible risks. Each of the studies was deleted each time in the pooled OR, which was not materially altered with or without these studies (Fig. 3), revealing that our results were statistically robust.

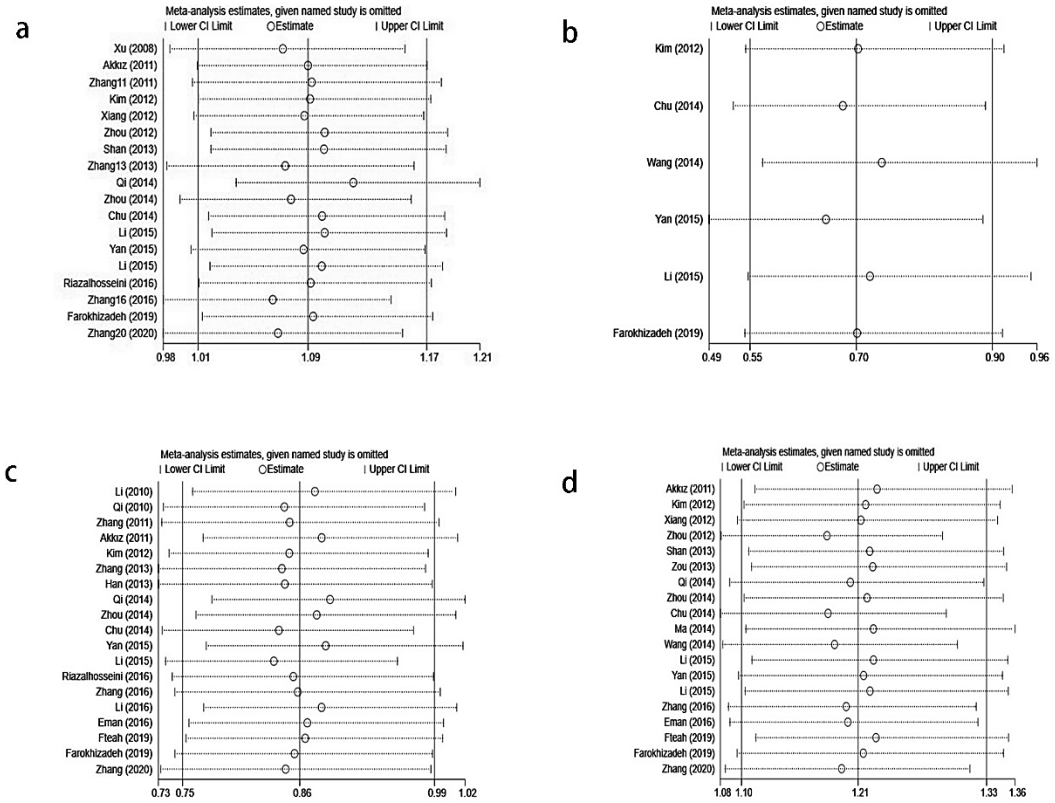

Fig. 3: Influence analysis of different MIR Polymorphism in the overall meta-analysis (a. dominant model in MIR146: CG+GG vs CC; b. homozygous model in MIR149: CC vs TT; C. recessive model in MIR196: TT vs CT+CC; d: dominant model in MIR499: AG+GG vs AA)

Publication bias

Begg's funnel plot and Egger's test were used to assess the publication bias in all comparison models. The shape of the Begg's funnel plots (Fig. 4) were symmetrical and Egger's test indicated that there was no publication bias in all polymorphism. Therefore, the results suggested no evidence of publication bias in our meta-analysis.

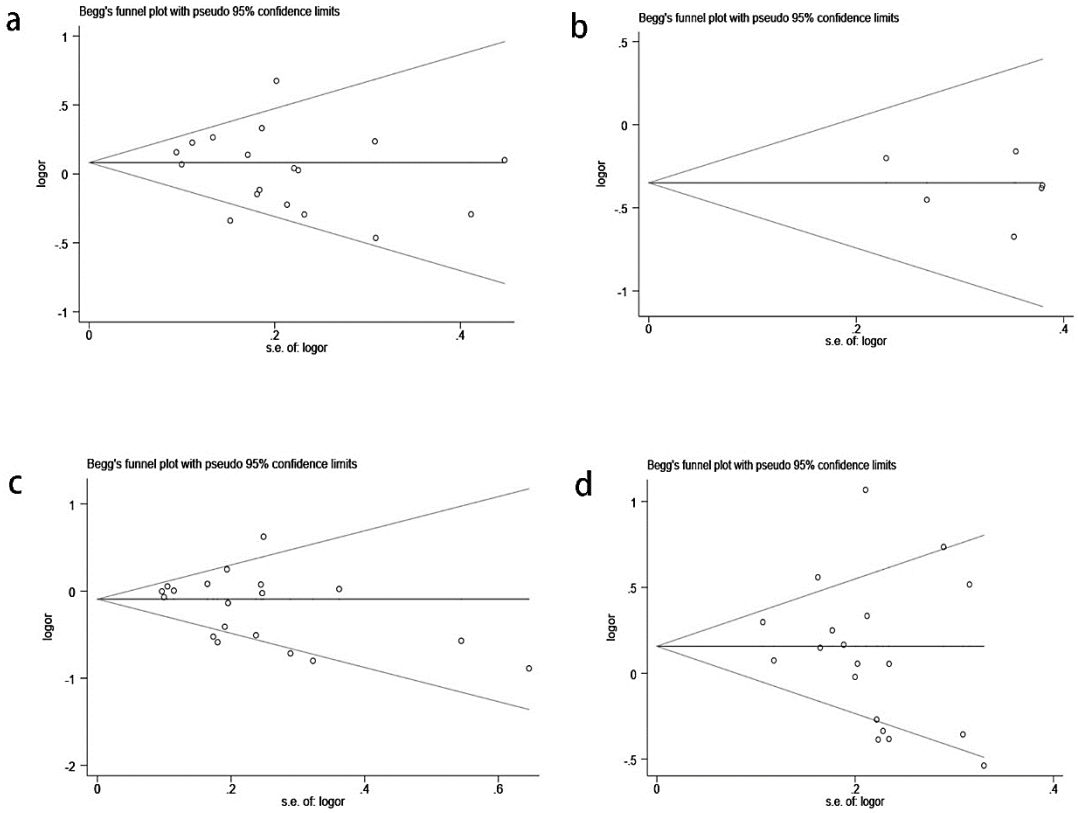

Fig. 4: Funnel plot to assess the publication bias of the meta-analysis in different MIR (a. dominant model in MIR146: CG+GG vs CC; b. homozygous model in MIR149: CC vs TT; C. recessive model in MIR196: TT vs CT+CC; d: dominant model in MIR499: AG+GG vs AA)

Discussion

Recent studies have focused on the SNP in miRNA and conducted their feasible function in the risk of HCC. Most of such studies have shown that SNP is the critical reason for the development, differentiation, and cell proliferation (25) of HCC. Tumor and immune cells influence the proliferation, apoptosis, migration, and metastasis of HCC by regulating STATA and NF-κB signaling (13) 14). The function of SNP is so different that each HCC patients have a different prognosis, progress and treatment effects. A deeper survey of the relationship between SNP and HCC would help clinicians evaluate the risk of overall survival rate and tumor development in HCC. Among these SNPs, MIR146/149/196/499 are the most widely discussed and investigated. Previous case-control studies proposed that these SNP was the cause of the progress and development of HCC (18, 35, 37, 47), some studies recommended that they are the protective factor of HCC (20, 22, 32), while some investigations could not find any relationship between them (17, 35, 40, 43, 44). The contradictory results may be due to several factors, including ethnicity, genotyping methods, paper quality, source of controls and HWE in control. Several meta-analyses have also explored the association between these SNP and HCC risk, but their results are controversial as the results of single-center studies. The power of single study was too low to make an accurate conclusion. Thus, it is extremely necessary to probe the association between HCC and SNP. Hence, we performed an updated and comprehensive meta-analysis to further understand the relationship between SNP and HCC risk, hoping to provide

guidance for evaluating the susceptibility of HCC. From the results of the 29 initial studies, we demonstrated that the mutation of MIR149 and MIR196 polymorphism was likely to the protected factor of HCC. In MIR149 polymorphism, the shift from TT to CC might decrease the HCC risk by about 10.1~45.1%. While in MIR196 polymorphism, recessive model TT vs CT+CC on overall population and Caucasian may decreased the risk of HCC by about 0.007%~24.9% and 0.093%~58.6%. Although the new studies in our meta-analysis increased by six studies (15, 20, 43, 44, 47, 48), no risk association was found form four SNPs, which was consistent with previous studies on MIR499 (53) but inconsistent with the results of the previous studies on MIR146 (54, 55) and MIR196 (55). However, we found that MIR149 decreases the risk of HCC, which is inconsistent with the results of all original investigation owing to including more studies.

Studies deviated the rule of HWE may be due to genetic or methodological reasons. In our study, the number of studies were inconsistent with HWE in MIR146 (15, 17, 42, 49) and MIR499 (15, 17, 38, 41, 45, 51) were four and seven. Therefore, we performed subgroup analysis by HWE in controls, the results still persistent after removing the studies deviated HWE (Supplementary Table 2), indicating that this element makes no difference in the overall estimates in our studies.

Finally, no publication bias was found in all trials after the analysis of Begg's funnel plot and Egger's test, which means that our study was robust and reliable, as mentioned earlier. Significant heterogeneity was observed in many of the genetic model in MIR146, MIR196 and MIR499, which might have been due to the sample size, ethnicity, genotyping methods, quality score, source of controls, and HWE in control. However, there was any significant when analysis in subgroup and meta-regression. Other reasons, such as the cancer diagnostic criteria or the hepatitis virus infection status, may have caused the significance. Regrettably, due to insufficient original data we could not further investigate the main reasons for the heterogeneity. Further clinical trials should analyze the risk of HCC with different hepatitis virus.

Our study had some limitations that should be taken into consideration. Firstly, heterogeneity widely existed in most of the genetic models because some of the eligible studies lacked information to establish a powerful correlation. Secondly, the number of studies for the subgroup of Caucasian populations was so small that we make a real conclusion. Besides, only seven studies in MIR149 were eligible for inclusion in our meta-analysis, and the small samples might not be able to establish a real connection. It is necessary to expand investigation by using a bigger sample in different regions and country. Thirdly, we mainly concentrated on SNP and the risk of HCC rather than considering the possible interactions between gene and environment.

Conclusion

The risk of HCC is associated with MIR149 in overall population and MIR196 in overall population and Caucasian. Besides, no significant connection was found between MIR146 and MIR499 and risk in HCC. Larger studies, further clinical trials and the relation between gene and environment are needed to support our study's findings.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare no conflicts of interest in this work.

Data Availability Statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

References

1. Bray F, Ferlay J, Soerjomataram I, et al (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin,* 68:394-424.
2. Chen W, Zheng R, Baade PD, et al (2016). Cancer statistics in China, 2015. *CA Cancer J Clin,* 66:115-32.
3. Singal AG, El-Serag HB (2015). Hepatocellular Carcinoma From Epidemiology to Prevention: Translating Knowledge into Practice. *Clin Gastroenterol Hepatol,* 13:2140-2151.
4. El-Serag HB (2011). CURRENT CONCEPTS Hepatocellular Carcinoma. *N Engl J Med,* 365:1118-1127.
5. Dyson J, Jaques B, Chattopadyhay D, et al (2014). Hepatocellular cancer: The impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol,* 60:110-117.
6. Asrani SK, Devarbhavi H, Eaton J, Kamath PS (2019). Burden of liver diseases in the world. *J Hepatol,* 70:151-171.
7. Tam WL, Weinberg RA (2013). The epigenetics of epithelial-mesenchymal plasticity in cancer. *Nat Med,* 19:1438-49.
8. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH (2019). An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. *J Cell Physiol,* 234:5451-5465.
9. Iorio MV, Croce CM (2017). MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol Med,* 9:852.
10. Dhanasekaran R, Bandoh S, Roberts LR (2016). Molecular pathogenesis of hepatocellular carcinoma and impact of therapeutic advances. *F1000Res,* 5:F1000 Faculty Rev-879.
11. Hao Y, Wang J, Zhao L (2015). The effect and mechanism of miR196a in HepG2 cell. *Biomed Pharmacother,* 72:1-5.
12. Dong J, Teng F, Guo W, et al (2018). lncRNA SNHG8 Promotes the Tumorigenesis and Metastasis by Sponging miR-149-5p and Predicts Tumor Recurrence in Hepatocellular Carcinoma. *Cell Physiol Biochem,* 51:2262-2274.
13. Liu Z, Qin Q, Wu C, et al (2018). Downregulated NDR1 protein kinase inhibits innate immune response by initiating an miR146a-STAT1 feedback loop. *Nat Commun,* 9:2789.
14. Lu Y, Hippen KL, Lemire AL, et al (2016). miR-146b antagomir-treated human Tregs acquire increased GVHD inhibitory potency. *Blood,* 128:1424-35.
15. Farokhizadeh Z, Dehbidi S, Geramizadeh B, et al (2019). Association of MicroRNA Polymorphisms With Hepatocellular Carcinoma in an Iranian Population. *Ann Lab Med,* 39:58-66.
16. Kim WH, Min KT, Jeon YJ, et al (2012). Association study of microRNA polymorphisms with hepatocellular carcinoma in Korean population. *Gene,* 504:92-7.
17. Shan YF, Huang YH, Chen ZK, et al (2013). miR-499A>G rs3746444 and miR-146aG>C expression and hepatocellular carcinoma risk in the Chinese population. *Genet Mol Res,* 12:5365-71.
18. Xiang Y, Fan S, Cao J, et al (2012). Association of the microRNA-499 variants with susceptibility to hepatocellular carcinoma in a Chinese population. *Mol Biol Rep,* 39:7019-23.
19. Xu T, Zhu Y, Wei QK, et al (2008). A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. *Carcinogenesis,* 29:2126-31.
20. Fteah AM, Ahmed AI, Mosaad NA, et al (2019). Association of MicroRNA 196a and 499 Polymorphisms with Development of Cirrhosis and Hepatocellular Carcinoma Post-HCV Infection in Egyptian Patients. *Asian Pac J Cancer Prev,* 20:3479-3485.

21. Qi P, Dou TH, Geng L, et al (2010). Association of a variant in MIR 196A2 with susceptibility to hepatocellular carcinoma in male Chinese patients with chronic hepatitis B virus infection. *Hum Immunol,* 71:621-6.
22. Yan P, Xia M, Gao F, et al (2015). Predictive role of miR-146a rs2910164 (C>G), miR-149 rs2292832 (T>C), miR-196a2 rs11614913 (T>C) and miR-499 rs3746444 (T>C) in the development of hepatocellular carcinoma. *Int J Clin Exp Pathol,* 8:15177-83.
23. Page MJ, McKenzie JE, Bossuyt PM, et al (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ,* 372:n71.
24. Wells G, Shea B, O'Connell J (2014). The Newcastle-Ottawa Scale (NOS) for Assessing The Quality of Nonrandomised Studies in Meta-analyses. *Ottawa Health Research Institute Web site,* 7.
25. Chen ZY, Chen X, Wang ZX (2016). The role of microRNA-196a in tumorigenesis, tumor progression, and prognosis. *Tumour Biol*, doi: 10.1007/s13277-016-5430-2.
26. Kim HY, Yoon JH, Lee HS, et al (2014). MicroRNA-196A-2 polymorphisms and hepatocellular carcinoma in patients with chronic hepatitis B. *J Med Virol,* 86:446-53.
27. Parlayan C, Ikeda S, Sato N, et al (2014). Association analysis of single nucleotide polymorphisms in miR-146a and miR-196a2 on the prevalence of cancer in elderly Japanese: a case-control study. *Asian Pac J Cancer Prev,* 15:2101-7.
28. Al-Qahtani AA, Al-Anazi MR, Nazir N,et al (2017). Association of single nucleotide polymorphisms in microRNAs with susceptibility to hepatitis B virus infection and HBV-related liver complications: A study in a Saudi Arabian population. *J Viral Hepat,* 24:1132-1142.
29. Xu X, Ling Q, Wang J, et al (2016). Donor miR-196a-2 polymorphism is associated with hepatocellular carcinoma recurrence after liver transplantation in a Han Chinese population. *Int J Cancer,* 138:620-9.
30. Zhu X, Hou R, Ma A, Yang S, Pan X (2018). Associations of miR-146a, miR-149, miR-196a2, and miR-499 Polymorphisms with Ischemic Stroke in the Northern Chinese Han Population. *Med Sci Monit,* 24:7366-7374.
31. Khanizadeh S, Hasanvand B, Nikoo HR, et al (2019). Association between miRNA-146a rs2910164 (G/C) polymorphism with the susceptibility to chronic HBV infection and spontaneous viral clearance in an Iranian population. *J Med Virol,* 91:1063-1068.
32. Akkiz H, Bayram S, Bekar A, et al (2011). A functional polymorphism in pre-microRNA-196a-2 contributes to the susceptibility of hepatocellular carcinoma in a Turkish population: a case-control study. *J Viral Hepat,* 18:e399-407.
33. Akkiz H, Bayram S, Bekar A, et al (2011). Genetic variation in the microRNA-499 gene and hepatocellular carcinoma risk in a Turkish population: lack of any association in a case-control study. *Asian Pac J Cancer Prev,* 12:3107-12.
34. Akkiz H, Bayram S, Bekar A, et al (2011). No association of pre-microRNA-146a rs2910164 polymorphism and risk of hepatocellular carcinoma development in Turkish population: a case-control study. *Gene,* 486:104-9.
35. Chu YH, Hsieh MJ, Chiou HL, et al (2014). MicroRNA gene polymorphisms and environmental factors increase patient susceptibility to hepatocellular carcinoma. *PLoS One,* 9:e89930.
36. Han Y, Pu R, Han X, et al (2013). Associations of pri-miR-34b/c and pre-miR-196a2 polymorphisms and their multiplicative interactions with hepatitis B virus mutations with hepatocellular carcinoma risk. *PLoS One,* 8:e58564.
37. Li D, Peng JJ, Tan Y, et al (2015). Genetic variations in microRNA genes and susceptibility to hepatocellular carcinoma. *Genet Mol Res,* 14:1926-31.
38. Li J, Cheng G, Wang S (2016). A Single-Nucleotide Polymorphism of miR-196a2T>C rs11614913 Is Associated with Hepatocellular Carcinoma in the Chinese Population. *Genet Test Mol Biomarkers,* 20:213-5.
39. Li X, Li K, Wu Z (2015). Association of four common SNPs in microRNA polymorphisms with the risk of hepatocellular carcinoma. *Int J Clin Exp Pathol,* 8:9560-6.
40. Li XD, Li ZG, Song XX, Liu CF (2010). A variant in microRNA-196a2 is associated with susceptibility to hepatocellular carcinoma in

Chinese patients with cirrhosis. *Pathology,* 42:669-73.
41. Ma Y, Wang R, Zhang J, et al (2014). Identification of miR-423 and miR-499 polymorphisms on affecting the risk of hepatocellular carcinoma in a large-scale population. *Genet Test Mol Biomarkers,* 18:516-24.
42. Qi JH, Wang J, Chen J, et al (2014). High-resolution melting analysis reveals genetic polymorphisms in microRNAs confer hepatocellular carcinoma risk in Chinese patients. *BMC Cancer,* 14:643.
43. Riazalhosseini B, Mohamed Z, Apalasamy YD, et al (2016). Association between microRNA-196A2 and microRNA-146A polymorphisms and progression to cirrhosis and hepatocellular carcinoma in patients with viral hepatitis B. *Pharmacogenet Genomics,* 26:74-9.
44. Toraih EA, Fawzy MS, Elgazzaz MG, et al (2016). Combined Genotype Analyses of Precursor miRNA196a2 and 499a Variants With Hepatic and Renal Cancer Susceptibility a Preliminary Study. *Asian Pac J Cancer Prev,* 17:3369-3375.
45. Wang XH, Wang FR, Tang YF, et al (2014). Association of miR-149C>T and miR-499A>G polymorphisms with the risk of hepatocellular carcinoma in the Chinese population. *Genet Mol Res,* 13:5048-54.
46. Zhang J, Wang R, Ma YY, et al (2013). Association between single nucleotide polymorphisms in miRNA196a-2 and miRNA146a and susceptibility to hepatocellular carcinoma in a Chinese population. *Asian Pac J Cancer Prev,* 14:6427-31.
47. Zhang LH, Hao BB, Zhang CY, et al (2016). Contributions of polymorphisms in miR146a, miR196a, and miR499 to the development of hepatocellular carcinoma. *Genet Mol Res,* 15: 10.4238/gmr.15038582.
48. Zhang S, Chen L, Wang Y, et al (2020). Investigation of the Association of miRNA-499, miRNA-146a, miRNA-196a2 Loci with Hepatocellular Carcinoma Risk: A Case-Control Study Involving 1507 Subjects. *DNA Cell Biol,* 39:379-388.
49. Zhou B, Dong LP, Jing XY, et al (2014). Association between miR-146aG>C and miR-196a2C>T polymorphisms and the risk of hepatocellular carcinoma in a Chinese population. *Tumour Biol,* 35:7775-80.
50. Zhou J, Lv R, Song X, et al (2012). Association between two genetic variants in miRNA and primary liver cancer risk in the Chinese population. *DNA Cell Biol,* 31:524-30.
51. Zou HZ, Zhao YQ (2013). Positive association between miR-499A>G and hepatocellular carcinoma risk in a Chinese population. *Asian Pac J Cancer Prev,* 14:1769-72.
52. Zhang XW, Pan SD, Feng YL, et al (2011). [Relationship between genetic polymorphism in microRNAs precursor and genetic predisposition of hepatocellular carcinoma]. *Zhonghua Yu Fang Yi Xue Za Zhi,* 45:239-43.
53. Zheng L, Zhuang C, Zhao J, Ming L (2017). Functional miR-146a, miR-149, miR-196a2 and miR-499 polymorphisms and the susceptibility to hepatocellular carcinoma: An updated meta-analysis. *Clin Res Hepatol Gastroenterol,* 41:664-676.
54. Dong S, Miao AY, Lei W, et al (2017). miR-146a rs2910164 and hepatocellular carcinoma: a meta-analysis. *Minerva Med,* 108:287-292.
55. Tian T, Wang M, Zhu W, et al (2017). MiR-146a and miR-196a-2 polymorphisms are associated with hepatitis virus-related hepatocellular cancer risk: a meta-analysis. *Aging (Albany NY),* 9:381-392.