Cellular Physiology and Biochemistry

Cell Physiol Biochem 2018;48:149-157
DOI: 10.1159/000491671
Published online: July 12, 2018
Accepted: May 20, 2018

Original Paper

The Association Between Three Genetic Variants in MicroRNAs (Rs11614913, Rs2910164, Rs3746444) and Prostate Cancer Risk

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Key Words
Microrna • Polymorphism • Prostate cancer • Meta-analysis

Abstract
Background/Aims: MicroRNAs (miRNAs) are a class of small non-coding RNA molecules which play a significant role in transcriptional and translational regulation. Published data on the association between the miRNA SNPs and prostate cancer (PCa) risk are somewhat inconclusive. ***Methods:*** We performed a meta-analysis of all available studies including 2,227 patients and 2,331 control subjects to evaluate the impact of three common genetic variants of microRNAs in prostate cancer risk. Odds ratios (ORs) with 95% confidence intervals (CIs) were utilized to investigate the strength of the association. ***Results:*** For miR-499 polymorphism, a significant association was observed between the rs3746444 A>G polymorphism and PCa risk in heterozygote comparison and dominant genetic model, in particular in Asian population subgroup. For miR-146a polymorphism, the rs2910164 CC genotype was associated with decreased PCa risk in Asian population in homozygote comparison. In addition, rs2910164 CC genotype had a weekly higher percentage value in subgroup of Gleason score < 7. Similar results were also indicated in localized prostate cancer in subgroup analysis by tumor stage. For miR-196a2 polymorphism, no association was observed between this variant and PCa risk in the overall group. However, in stratified analysis by ethnicity, we found that rs11614913 T allele was a risk factor for Asian PCa patients. ***Conclusions:*** Polymorphisms of miR-196a2 rs11614913, miR-146a rs2910164, and miR-499 rs3746444 may contribute to the risk for developing prostate cancer in Asian descendants. Moreover, miR-146a rs2910164 polymorphism was related to PCa prognosis.

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Introduction

Prostate cancer (PCa) is one of the most common malignant tumor among men in the United States [1] However, the exact etiology underlying the development and progression of PCa is still poorly understood. Published researches indicated that both environmental and genetic factors may contribute to the development and progression of PCa [2, 3]. Single nucleotide polymorphism (SNP) is thought to be the most common type of genetic variation and has been indicated to be associated with the PCa risk [4, 5]. Genome wide association studies (GWAS) have indicated that more than 100 SNPs associated with predisposition to PCa. However, the molecular mechanisms of these SNPs remain poorly defined [6].

MicroRNAs (MiRNAs) are a family of small single-stranded short (17-25 nucleotides) RNAs, which are evolutionarily well conserved but are non-proteincoding [7-9]. These RNAs are predicted to regulate gene expression at the posttranscriptional level through binding to 3'-untranslated region (3'-UTR) of the target mRNAs and consequently lead to mRNA cleavage or translational repression [10, 11]. Published studies have indicated that miRNAs regulate the expression of roughly 10–30% of the all human genes [12], contributing to excessive physiologic and pathologic process, including apoptosis, proliferation, and immune response, which are known to play critical roles in carcinogenesis [13-15].

To date, many epidemiological studies have demonstrated the relationship between SNPs in miRNAs and cancer susceptibility, including gastric cancer [16, 17], breast cancer [18, 19], bladder cancer [20, 21] and other cancers [22-24]. Present studies indicated that miR-146a rs2910164 polymorphism marginally decreased the gastric cancer risk [25] and the miR-499 A>G (rs3746444) polymorphism might be related to susceptibility to cancer [26]. However, there is inconsistent and little data regarding the impact of miRNA gene polymorphisms on prostate cancer susceptibility. Some researchers found that rs2910164 minor allele C confers reduced risk of PCa in Chinese Han population [27], whereas another researches revealed no evidence of association between this variant and PCa risk, nor with the relevant parameters of cancer prognosis in North Indian population [28]. Therefore, a meta-analysis of all eligible published case-control studies [27-32] was conducted to evaluate the effect of three MiRNA SNPs (miR-146a rs2910164, miR-196a2 rs11614913, miR-499 rs3746444) on PCa risk. Additionally, interaction between miR-146a rs2910164 polymorphism and several PCa prognostic parameters were also evaluated.

Materials and Methods

Search strategy and identification of eligible studies

PubMed database searches were carried out utilizing the following keywords: 'microRNA 146a/196a2/499' or 'mir-146a/196a2/499', 'prostate cancer' and 'polymorphism' (last search updated on May 01, 2017). Review articles and bibliographies of other relevant studies were also screened by a hand search. All the studies would be included if they met the following criteria: (a) full-text study; (b) utilizing an unrelated case–control design; (c) study published in English; (d) sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI); (e) availability of genotypes or allele frequencies.

Data extraction and quality assessment

Data were collected on the genotype of mir-146a/196a2/499 according to prostate cancer. For each publication, all data complying with the selection criteria were reviewed and extracted independently by two of the investigators. In the case of a conflict, an agreement should be reached by the discussion between two reviewers. If they could not reach a consensus, discrepancies would be adjudicated by a third reviewer until the consensus was achieved on every item. For each study, the following characteristics were extracted: first author's last name, year of publications, country of origin, ethnicity, study-design (sources of controls), sample size in cases and controls, the number of cases and controls with variant allele and wild type, and genotyping methods respectively.

Statistical analysis

The strength of association between the three microRNA SNPs and prostate cancer risk was measured by odds ratios (ORs) with 95% confidence intervals (CIs). To evaluate the wild-type homozygote (WW), the risk of the rare allele homozygote (RR) and heterozygous (WR) genotypes on prostate cancers were assessed. Moreover, we tested the prostate cancer susceptibility under a dominant model (RR+WR vs. WW) and recessive model (RR vs. WR+WW). Furthermore, subgroup analysis was stratified by ethnicity (Asian and Caucasian), source of control (population-based and hospital-based), Gleason score and tumor stage. Heterogeneity across the studies was assessed by utilizing the Chi-square-based Q-statistic test and it was considered significant when *P* value of heterogeneity less than 0.05. The data were combined utilizing both random effects (the DerSimonian and Laird method) models and fixed-effects (the Mantel-Haenszel method). When the heterogeneity existed, a random-effect model was conducted [33, 34]; otherwise, the fixed-effect model was performed to pool the results [35]. We used *Z*-test to calculate the statistical significance of the pooled OR and a *P* value of < 0.05 was considered significant. Moreover, a sensitivity analysis was applied to evaluate the stability of the results. Funnel plots and Egger's linear regression tests were performed to determine whether the publication bias was exist [36]. All the statistical tests for this meta-analysis were conducted with STATA version 11.0 (Stata Corporation College Station, TX, USA).

Results

Study characteristics

A total of six eligible papers according to the inclusion criteria were enrolled in this study (Fig. 1). Study characteristics of the eligible studies are pooled in Table 1. We checked the three microRNAs' Minor Allele Frequency (MAF) reported for the main worldwide populations. For rs11614913: American (AMR), 0.389; African (AFR), 0.140; East Asian (EAS), 0.458; South Asian (SAS), 0.258; and European (EUR), 0.410. For rs3746444: AMR, 0.134; AFR, 0.169; EAS, 0.145; SAS, 0.267; and EUR, 0.194. For rs2910164: EUR, 0.239; AFR, 0.391; and China (CHN), 0.354 (Fig. 2 a-c). For rs2910164 polymorphism, five studies with available data were enrolled in the pooled analysis. In the subgroup of ethnicity, four were performed in Asian descendants and one was in European descendants. Hospital based controls were carried out in four of the studies. Furthermore, four publications containing available genotype frequency information investigated PCa prognostic parameters. The association between rs2910164 polymorphism and PCa risk stratified by disease stage (localized: T1-2N0M0; advanced: T3-4NxMx or TxN1Mx or TxNxM1) and pathologic grade (Gleason score <7 and ⩾7) is shown in Table 3-4. For rs11614913 polymorphism, three studies with available data were enrolled. There were two studies of Asian descendent and one of Caucasian ethnicity. Hospital based controls were carried out in two of the studies. For rs3746444 polymorphism, three studies covered Serbia, India and Iran related to prostate

Table 1. Basic information for included studies of the association between three miRs' polymorphisms and PCA susceptibility. HB: hospital-based; PB: population-based; PCR-RFLP: polymerase chain reaction and restrictive fragment length polymorphism; HRMA: high resolution melting analysis; T-ARMS-PCR: tetra amplification refractory mutation system-polymerase chain reaction; qRT-PCR: real-time quantitative reverse transcription-PCR; MAF: Minor Allele Frequency

Author	Year	Origin	Ethnicity	Design	Case	Control	Case				Control				Genotype methods
miR-196a2 rs11614913							TT	TC	CC	MAF	TT	TC	CC	MAF	
Nikolic [31]	2015	Serbia	Caucasian	PB	351	309	40	161	150	0.343	41	147	121	0.371	HRMA
George [28]	2011	India	Asian	HB	159	230	3	101	55	0.336	10	114	106	0.291	PCR-RFLP
Hashemi [30]	2016	Iran	Asian	HB	169	182	17	88	64	0.361	12	93	77	0.321	T-ARMS-PCR
miR-499 rs3746444							GG	GA	AA		GG	GA	AA		
Nikolic [31]	2015	Serbia	Caucasian	PB	355	307	18	147	190	0.258	17	110	180	0.235	PCR-RFLP
George [28]	2011	India	Asian	HB	159	230	13	98	48	0.390	34	92	104	0.348	PCR-RFLP
Hashemi [30]	2016	Iran	Asian	HB	169	182	25	82	62	0.391	33	64	85	0.357	PCR-RFLP
miR-146a rs2910164							CC	CG	GG		CC	CG	GG		
Nikolic [32]	2014	Serbia	Caucasian	PB	286	199	12	90	184	0.199	7	63	129	0.193	Taqman
George [28]	2011	India	Asian	HB	159	230	76	79	4	0.726	116	107	7	0.737	PCR-RFLP
Hashemi [30]	2016	Iran	Asian	HB	169	182	13	131	25	0.464	11	147	24	0.464	T-ARMS-PCR
Xu [27]	2010	China	Asian	HB	251	280	48	135	68	0.460	76	150	54	0.539	qRT-PCR
Chen [29]	2014	China	Asian	HB			18	54(CG+GG)							PCR-RFLP

cancer were included. To analyze polymorphisms, genotyping by the classical genotyping method (polymerase chain reaction-restriction fragment length polymorphism, PCR-RFLP) were carried out by all of the studies, in which two were hospital-based and one was population-based.

Quantitative synthesis

For miR-146a polymorphism, no significant risk association was observed when all the eligible studies were pooled into the analysis: heterozygote comparison (fixed-effects OR = 0.87, 95% CI = 0.68 – 1.12, $P_{heterogeneity}$ = 0.639, P = 0.292), homozygote comparison (fixed-effects OR = 0.71, 95% CI = 0.49 – 1.05, $P_{heterogeneity}$ = 0.222, P = 0.084) and the dominant genetic model (fixed-effects OR = 0.85, 95%CI = 0.66–1.08, $P_{heterogeneity}$ = 0.386 P = 0.180) (Table 2). However, in the subgroup analysis by ethnicity, obvious associations between rs2910164 G>C polymorphism and prostate cancer risk were observed in Asian descendants for homozygote comparison (fixed-effects OR = 0.64, 95%CI = 0.42 – 0.98, $P_{heterogeneity}$ = 0.221, P = 0.040), but not in European descendants. Furthermore, the rs2910164 CC genotype had a weekly higher percentage value in subgroup of Gleason score < 7 (OR = 1.62, 95% CI = 1.07–2.47, $P_{heterogeneity}$ = 0.220, P = 0.023 Table 5). Similar results were also identified in localized prostate cancer in subgroup analysis by tumor stage (OR = 1.64, 95% CI = 1.00–2.68, $P_{heterogeneity}$ = 0.609, P = 0.048 Table 6). For miR-499 polymorphism, a significant association was observed between the rs3746444 A>G polymorphism and PCa risk in heterozygote comparison (fixed-effects OR = 1.60, 95%CI = 1.28–2.01, $P_{heterogeneity}$ = 0.090, P < 0.001) and dominant genetic model (fixed-effects OR = 1.45, 95%CI = 1.17–1.80, $P_{heterogeneity}$ = 0.257, P = 0.001) (Table 2), in particular in Asian population subgroup (heterozygote comparison OR = 2.03, 95%CI = 1.47 – 2.79, $P_{heterogeneity}$ = 0.404, P < 0.001; dominant genetic model OR = 1.70, 95%CI = 1.26 - 2.30, $P_{heterogeneity}$ = 0.450, P = 0.001). For miR-196a2 polymorphism, no association was observed between rs11614913 C>T variant and PCa risk in the overall group: heterozygote comparison (fixed-effects OR = 1.14, 95% CI = 0.91 – 1.42, $P_{heterogeneity}$ = 0.054, P = 0.262), homozygote comparison (fixed-effects OR = 0.93, 95% CI = 0.62 – 1.38, $P_{heterogeneity}$ = 0.215, P = 0.704) and the dominant genetic model (fixed-effects OR = 1.11, 95%CI = 0.90–1.38, $P_{heterogeneity}$ = 0.056 P = 0.335) (Table 2). However, in stratified analysis by ethnicity, we found that rs11614913 T allele was a risk factor for Asian PCa patients (heterozygote comparison OR = 1.41, 95%CI = 1.04 – 1.91, $P_{heterogeneity}$ = 0.193, P = 0.027; dominant genetic model OR = 1.40, 95%CI = 1.04 – 1.89, $P_{heterogeneity}$ = 0.333, P = 0.027), but not in European descendants (heterozygote comparison OR = 0.88, 95%CI = 0.64 – 1.23, P = 0.459; dominant genetic model OR = 0.86, 95%CI = 0.63 – 1.18, P = 0.351).

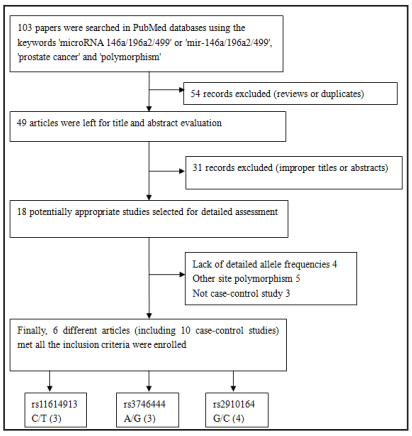

Fig. 1. Flow diagram of the strategy of literature search among the associated studies.

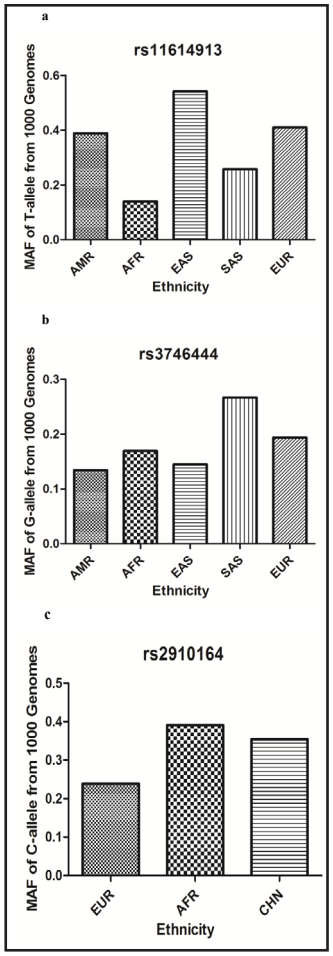

Fig. 2. Minor allele frequencies for three miRs' polymorphisms in the controls stratified by ethnicity. Vertical line, minor allele frequency; Horizontal line, ethnicity type.

Table 2. Total and stratified subgroup analysis for three miRs' polymorphisms and PCA susceptibility

Variables	N	Case/ Control	OR(95%CI) P_h P	OR(95%CI) P_h P	OR(95%CI) P_h P
miR-196a2 rs11614913			TC vs. CC	TT vs. CC	TT+TC vs. CC
Total	3	679/721	1.14(0.91-1.42) 0.054 0.262	0.93(0.62-1.38) 0.215 0.704	1.11(0.90-1.38) 0.056 0.335
Ethnicity					
Caucasian	1	351/309	0.88(0.64-1.23) - 0.459	0.79(0.48-1.29) - 0.345	0.86(0.63-1.18) - 0.351
Asian	2	328/412	1.41(1.04-1.91) 0.193 0.027	1.24(0.64-2.42) 0.173 0.526	1.40(1.04-1.89) 0.333 0.027
miR-499 rs3746444			GA vs. AA	GG vs. AA	GG+GA vs. AA
Total	3	683/719	1.60(1.28-2.01) 0.090 <0.001	0.96(0.65-1.42) 0.888 0.844	1.45(1.17-1.80) 0.257 0.001
Ethnicity					
Caucasian	1	355/307	1.27(0.92-1.74) - 0.149	1.00(0.50-2.01) - 0.993	1.23(0.90-1.68) - 0.187
Asian	2	328/412	2.03(1.47-2.79) 0.404 <0.001	0.94(0.59-1.51) 0.641 0.808	1.70(1.26-2.30) 0.450 0.001
miR-146a rs2910164			CG vs. GG	CC vs. GG	CC+CG vs. GG
Total	4	865/891	0.87(0.68-1.12) 0.639 0.292	0.71(0.49-1.05) 0.222 0.084	0.85(0.66-1.08) 0.386 0.180
Ethnicity					
Caucasian	1	286/199	1.00(0.68-1.48) - 0.994	1.20(0.46-3.14) - 0.707	1.02(0.70-1.49) - 0.912
Asian	3	579/692	0.79(0.56-1.10) 0.650 0.164	0.64(0.42-0.98) 0.221 0.040	0.74(0.53-1.02) 0.506 0.063

Table 3. Basic information for the association between miR-146a rs2910164 polymorphism in PCA by Gleason score

Author	Origin	Ethnicity	Gleason score<7		Gleason score>7	
			CC	CG+GG	CC	CG+GG
Nikolic [32]	2015	Serbia	11	149	1	112
George [28]	2011	India	24	25	52	58
Hashemi [30]	2016	Iran	7	50	6	106
Xu [27]	2010	China	16	50	32	153

Table 4. Basic information for the association between miR-146a rs2910164 polymorphism in PCA by tumor stage

Author	Origin	Ethnicity	Localized		Advanced	
			CC	CG+GG	CC	CG+GG
Nikolic [32]	2015	Serbia	7	96	4	156
Xu [27]	2010	China	31	105	17	98
Hashemi [30]	2016	Iran	9	113	4	43
Chen [29]	2014	China	15	41	3	13

Table 5. Relationship between miR-146a rs2910164 polymorphism and PCA prognosis by Gleason score subgroup

miR-146a	Genotype	Gleason <7	Gleason ⩾7	OR(95%CI)	P_h	P	Egger's test	Begg's test
rs2910164	CC	58	91					
	CG+GG	274	429	1.62(1.07-2.47)	0.220	0.023	t=3.86,p=0.061	z=1.70,p=0.089

Table 6. Relationship between miR-146a rs2910164 polymorphism and PCA prognosis by tumor stage subgroup

miR-146a	Genotype	Localized	Advanced	OR(95%CI)	P_h	P	Egger's test	Begg's test
rs2910164	CC	62	28					
	CG+GG	355	310	1.64(1.00-2.68)	0.609	0.048	t=1.43,p=0.290	z=0.34,p=0.734

Publication bias

The Egger's test and Begg's funnel plot were conducted to assess the publication bias of literatures. No obvious evidence of publication bias was found in miR-196a2 rs11614913 (TC vs. CC, t = 1.17, *P* = 0.450; TT vs. CC, t = 1.04, *P* = 0.296 ; TT+TC vs. CC, t = 1.36, *P* = 0.404), miR-499 rs3746444 (GA vs. AA, t = 1.32, *P* = 0.412; GG vs. AA, t = 1.56, *P* = 0.363; GG+GA vs. AA, t = 2.30, *P* = 0.055), and miR-146a rs2910164 (CG vs. GG, t = 4.55, *P* = 0.138; CC vs. GG, t = 2.44, *P* = 0.248; CC+CG vs. GG, t = 5.12, *P* = 0.123). The shape of the funnel plots seemed asymmetrical in heterozygote comparison for three miRs' polymorphisms, suggesting no publication bias (Fig. 3a-c).

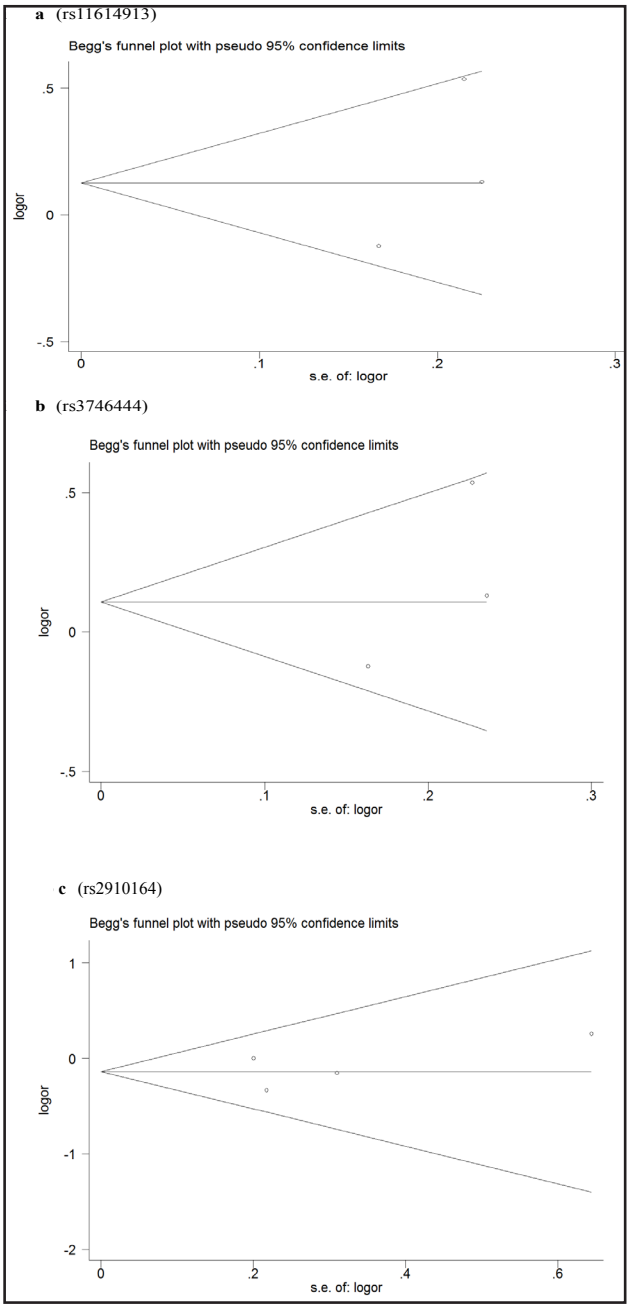

Fig. 3. Begg's funnel plot of standard error for assessing publication bias.

Discussion

Genetic susceptibility to malignant tumors has led to accumulating attention to the studies of polymorphism genes involved in process of carcinogenesis. Previous researches have shown evidence that miRNAs are involved in various crucial biological processes through imperfect pairing with target mRNAs of protein-coding genes [37, 38]. Meta-analysis is used to combine the results based on individual research to yield summary conclusions, especially when results from single case-control studies were incomprehensive and conflicting. Several meta-analyses have been performed on the miRNA SNPs associated with the risk of overall cancer [39-41]. However, none of the meta-analyses has focused on prostate cancer independently for short of eligible data. In the present study, novel case-control studies from the last years were enrolled and some new findings have been indicated.

Our results demonstrated that polymorphisms of miR-196a2 rs11614913, miR-146a rs2910164, and miR-499 rs3746444 may contribute to the risk for developing prostate cancer in Asian descendants. Tumor stage and Gleason score could be considered as prognostic factors for prostate cancer, if the Tumor stage is higher than T2c or Gleason score is more than 7, individuals must have a worse prognosis and this cancer will show more aggressive. The present study indicated that individuals who carried rs2910164 CC genotype had a high percentage in Gleason score less than 7 and localized PCa group, manifesting that

rs2910164 G>C polymorphism was partly related to PCa prognosis. The rs11614913 C>T polymorphism present in the miR-196a2 has been indicated to be associated with various carcinogenesis [42-44]. Previous meta-analysis support that the rs11614913 TT genotype was associated with a decreased risk for breast cancer and lung cancer, although this variant was not associated with gastric cancer and hepatocellular carcinoma [42]. In the present study, we demonstrated that the rs11614913 TT genotype were associated with increased risk of prostate cancer in Asian descendants but not in Europeans. Published studies reported that rs3746444 G allele was an increased cancer risk factor in Chinese population, especially for breast cancer [45], our results indicated a significant association between the rs3746444 A>G polymorphism and PCa risk, in particular in Asian population subgroup. Nevertheless, this meta-analysis still has some limitations. Firstly, although all eligible studies were enrolled, the relatively small sample size of studies could lead to reduced statistical power. For only one study was based on Caucasian descendants. Secondly, the detailed information such as age, environmental factors and life-style was not considered. Furthermore, the available data containing PCa prognostic parameters in miR-499 rs3746444 and miR-196a2 rs11614913 polymorphisms was not compatible. In addition, the present analysis did not assess any potential gene-environment interaction or gene-gene interaction due to lack of relevant published data.

In conclusion, this meta-analysis evaluated the association of three miRNA polymorphisms and prostate cancer risk. Our results showed evidence that miR-146a rs2910164 CC genotype was associated with decreased prostate cancer risk in Asian population. Moreover, this variant was probably related to PCa outcome. The miR-196a2 rs11614913 T allele was a risk factor for Asian PCa patients. Furthermore, the miR-499 polymorphism rs3746444 A>G polymorphism was a risk factor for prostate cancer, especially in the Asian population. Further well-designed large studies are warranted to clarify the possible roles of these polymorphisms in more details.

Acknowledgements

This work was supported by grants from Changzhou Science and Technology International Cooperation Grant (No. : CZ20160017), Jiangsu '333 Project' Scientific Research Grant (No. : BRA 2016118), Changzhou High-Level Medical Talents Training Project (Number: 2016CZBJ035), Jiangsu Post-Doc Scientific Research Grant (No. :1701184C), Youth talent project of Wuxi Commission of Health and Family Planning (No. : QNRC043), Wuxi Commission of Health and Family Planning (No.: Q201746, Z201712, jzyx03), Science and Technology Bureau of Wuxi City (No. : CSE31N1605).

Disclosure Statement

The authors declare that they have no competing interests.

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