REVIEW

Association of Endothelial Nitric Oxide Synthase Gene Polymorphisms with Susceptibility to Prostate Cancer: a Comprehensive Systematic Review and Meta-Analysis

Mehdi Abedinzadeh[1], Seyed Alireza Dastgheib[2]*, Hadi Maleki[1], Naeimeh Heiranizadeh[3], Mohammad Zare[3], Jamal Jafari-Nedooshan[3], Saeed Kargar[3], Hossein Neamatzadeh[4,5]

Purpose: A variety of studies have evaluated the association of polymorphisms at endothelial nitric oxide synthase (eNOS) gene with risk of prostate cancer. However, the results remain inconclusive. This meta-analysis was performed to derive a more precise estimation between eNOS polymorphisms and prostate cancer risk.

Materials and Methods: A comprehensive literature search was conducted using PubMed, EMBASE, Wed of Science, Elsevier, Cochrane Library, SciELO, SID, WanFang, VIP, CBD and CNKI database up to March 20, 2020. Odds ratios with 95% confidence intervals were used to assess the strength of the associations.

Results: A total of 22 case-control studies including 12 studies with 4,464 cases and 4,347 controls on +894G>T, five studies with 589 cases and 789 controls on VNTR 4a/b, and five studies with 588 cases and 692 controls on -786T > C were selected. Overall, pooled data showed a significant association between eNOS 894G>T, VNTR 4a/b, and -786T > C polymorphisms and an increased risk of prostate cancer in the global population. When stratified by ethnicity, a significant association was found between eNOS +894G>T and -786T>C polymorphisms and risk of prostate cancer in Caucasians.

Conclusion: Our results indicated that eNOS 894G>T, VNTR 4a/b, and -786T>C polymorphisms were associated with risk of prostate cancer in the global population as well as Caucasian population.

Keywords: prostate Cancer; nitric oxide synthase; polymorphism; meta-analysis

INTRODUCTION

Prostate cancer is the second most common cancer and the third leading cause of cancer death in men in United States[(1)]. It is suggested that approximately 161,360 men will have been diagnosed with prostate cancer and 26,000 men will have died of the disease in 2017 in the United States[(2)]. Although, African-American males have the highest mortality and morbidity rates of prostate cancer in the world, the global burden of this disease is raising globally[(3,4)]. Although the occurrence rate of prostate cancer is rare in men younger than 40 years, but its morbidity increases with age more rapidly than any other malignancies in men[(5,6)].

The exact etiology of prostate cancer is poorly understood[(4)]. However, with the remarkable advances in high-throughput technologies of molecular biology of cancer, genetic risk factors of prostate cancer have been intensively investigated[(7,8)], and polymorphisms of endothelial nitric oxide synthase (eNOS) gene were on focus[(9,10)]. Nitric oxide (NO) is mainly produced by the catalyzing action of the 3 nitric oxide synthase (NOS3) family enzymes via the conversion of L-arginine[(11,12)]. NO is an intracellular messenger that plays a vital role in vascular system, homoeostasis, and bone turnover [(13)]. Low NO release can cause several cardiovascular diseases, such as atherosclerosis, hypertension and thrombosis, while high circulating NO concentration is generally toxic[(14,15)]. Moreover, NO has been suggested plays an effective role in different cancer related processes including angiogenesis, apoptosis, invasion, and metastasis[(10,11,16)].

The human eNOS gene is located on chromosome 7q35-36, comprises 26 exons and spanning 21 kb of genomic DNA[(10)]. The 894G>T (rs1799983, Glu298Asp), intron VNTR 4a/b (a-deletion allele with 27 bp VNTR in intron 4), and -786T>C (rs2070744) are the most clinically relevant polymorphisms in the eNOS gene so far described[(9)]. Several studies have evaluated the association of the eNOS 894G>T, VNTR 4a/b, and -786T>C polymorphisms with risk of prostate cancer in different populations[(17–29)]. However, those studies results are inconsistent and inconclusive, might be due to small sample size, different characteristics of populations,

[1]Department of Urology, Shahid Rahnamoun Hospital, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[2]Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Department of General Surgery, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4]Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[5]Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
*Correspondence: Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences , Shiraz, Iran
Tel: +989177148573, Fax: +983518248382, E-mail: dastgheibsa@gmail.com.
Received July 2019 2019 & Accepted April 2020

low statistical power, different genotyping methods and clinical heterogeneity of the patients. Therefore, we have performed this systematic review and meta-analysis to clarify the association of 894G>T, VNTR 4a/b, and -786T>C polymorphisms at eNOS gene with susceptibility to prostate cancer.

MATERIALS AND METHODS

Literature Search

The ethical approval was not required for this study, as it is a systematic review and meta-analysis. This work was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. We conducted a comprehensive literature search on electronic databases including PubMed, EMBASE, Wed of Science, Elsevier, Google Scholar, Cochrane Library, SciELO, SID, Wan-Fang, VIP, Chinese Biomedical Database (CBD) and Chinese National Knowledge Infrastructure (CNKI) databases to identifying all relevant studies on association of eNOS 894G>T, VNTR 4a/b, and -786T>C polymorphisms with prostate cancer risk up to March 20, 2020. Terms used for the research were ("Prostate Cancer" OR "Prostate Carcinoma") AND ("Endothelial Nitric Oxide Synthase" OR ''eNOS'' OR ''Nitric Oxide Synthase 3'' OR ''NOS3'' OR ''Constitutive NOS'' OR ''Endothelial NOS'') AND (''894G>T'' OR ''rs1799983'' OR ''Glu298Asp'') AND (''27-bp repeat insertion (b)/deletion (a) in intron 4'' OR ''Intron 4 b/a VNTR'' '' intron 4a/4b'' OR ''rs61722009'') AND (''-786T>C'' OR ''rs2070744'') AND (''Gene'' OR ''Allele'' OR ''Genotype'' OR ''Polymorphism'' OR ''Mutation'' OR ''Variation'' OR ''Variant''). We also identified additional studies with the "Related Articles" option and list of references. In the current meta-analysis, publications written in English, Farsi, Portuguese and Chinese were eligible. The search was limited to human studies.

Inclusion and Exclusion Criteria

Studies included in this meta-analysis had to meet the following criteria: 1) studies with case-control design; 2) studies evaluating the association between 894G>T, VNTR 4a/b, and -786T>C polymorphisms of eNOS gene and prostate cancer risk; 3) having detailed data to calculate the odds ratio (OR) and 95% confidence interval (CI). Accordingly, the following exclusion criteria were also used: 1) studies did not provide adequate data to estimate the association between eNOS polymorphisms and prostate cancer risk; 2) case only studies or studies without controls; 3) in vitro and animal studies; 4) linkage studies and family based studies such as twins and sibling studies; 5) case reports, abstracts, reviews, posters, commentaries, editorials, conference articles, proceedings and previous meta-analyses; and 6) repeating or overlapping studies. We defined overlapping data to studies that used the same published case-control studies to generate the same results with the exact same population sample size as well. Thus, if more than one study was published by the same author(s) using repeated or overlapped data, the most complete one or more recently published study was selected.

Data Extraction

All the data was collected independently by two authors according to the inclusion criteria. Then, in order to guarantee the veracity of collected data, two authors checked the collected data achieved an agreement. If there was a dispute regarding inclusion data, a third author was invited to resolve the issue. The following data were collected from each study: first author, year of publication, country of origin, ethnicity, source of healthy controls (hospital based or population based), genotyping methods, sample size, genotype and allele frequencies of cases and controls, genotype distribution in cases and controls, minor allele frequencies (MAFs) and p value for Hardy-Weinberg equilibrium (HWE) in healthy controls. The patient ethnicities were categorized as Caucasian, Asian, African, and mixed. The ''mixed'' group means mixed or unknown populations. Disagreements about eligibility were resolved through a discussion between the two investigators.

Quality Assessment

The quality of the case-control studies included in the current meta-analysis was evaluated by two authors using Newcastle-Ottawa Scale (NOS). Primary contents to be assessed include selection of study subjects (4 scores in total); inter-group comparability (2 scores in total); exposure factors or outcomes (3 scores in total). Low-quality studies: 0 to 4 points; high-quality studies: 5 to 9 points.

Statistical Analysis

The strength of association between the eNOS 894G>T, VNTR 4a/b, and -786T>C polymorphisms and risk of prostate cancer was measured by odds ratios (ORs) with 95% confidence intervals (CIs). The Z-test was used to assess the pooled OR, in which p-value less than 0.05 was considered as statistically significant. The association was estimated under all five genetic models, i.e., allele (B vs. A), homozygous model (BB vs. AA), heterozygous model (BA vs. AA), dominant model (BB+BA vs. AA), and recessive model (BB vs. BA+AA), which ''A'' represent the ''wild allele'' and ''B'' represent ''mutant allele'', respectively. In this meta-analysis the Cochran's χ^2 based Q-statistic test was used to appraise the between-studies heterogeneity, where test result was $P < 0.1$ indicated the presence of heterogeneity. Moreover, the I2 value was used to quantify the effect of heterogeneity, with the range of 0 to 100% (0%-40% meant no risk of heterogeneity, 30%-50% meant a low risk of heterogeneity, 60%-90% meant substantial heterogeneity, and 75%-100% meant considerable heterogeneity). If obvious heterogeneity was observed among the studies, the random-effects model (the DerSimonian and Laird method) was used to calculate the pooled OR and 95% CI. Otherwise, the fixed-effects model (the Mantel-Haenszel method) was adopted for the meta-analysis. Hardy-Weinberg equilibrium (HWE) in the healthy subjects was assessed using Fisher's exact test, which a p-value < 0.05 was considered significant. Subgroup analyses according to the ethnicity were also performed to evaluate the association and heterogeneity. To check the stability of the results, a sensitivity analysis was performed by omitting each individual study in turn from the all selected studies and reanalyzing the pooled OR for the remainder. Moreover, the sensitivity analysis was performed by excluding HWE-violating studies. Publication bias was assessed by the funnel plots and the Egger's linear regression test. Additionally, if publication bias was seen, the "trim and fill" method which conservatively imputes hypothetical negative unpublished studies to

Table 1. Characteristics of the studies included in the meta-analysis.

First Author/Year	Country (Ethnicity)	SOC	Genotyping Methods	Case/Control	Cases					Controls					NOS	MAFs	HWE
					Genotype			Allele		Genotype							
894G>T					GG	TT	TT	G	T	GG	GT	TT	G	T			
Medeiros 2002	Portugal (Caucasian)	HB	PCR-RFLP	125/153	49	61	15	159	91	70	65	18	205	101	7	0.330	0.623
Marangoni 2006	Brazil(Mixed)	HB	PCR-RFLP	84/76	30	50	4	110	58	35	34	7	104	48	6	0.315	0.751
Jacobs 2008	USA(Caucasian)	PB	TaqMan	1420/1446	659	632	129	1950	890	682	600	164	1964	928	9	0.320	0.065
Lee 2009a	USA(Caucasian)	PB	TaqMan	1088/1293	517	468	103	502	674	607	557	129	1771	815	9	0.315	0.947
Lee 2009b	USA(Caucasian)	PB	TaqMan	97/373	77	20	0	174	20	280	88	5	648	98	6	0.131	0.510
Chen 2011	China(Asian)	NS	PCR-RFLP	78/88	64	12	2	140	16	66	21	1	153	23	6	0.130	0.633
Ziaei 2012	Iran(Caucasian)	Mixed	Sequencing	78/87	44	23	11	111	45	48	33	6	129	45	6	0.258	0.912
Safarinejad 2013	Iran(Caucasian)	HB	PCR-RFLP	170/340	120	48	2	288	52	248	89	3	585	95	7	0.139	0.101
Brankovic 2013	Serbia(Caucasian)	HB	PCR-RFLP	150/250	76	65	9	217	83	132	99	19	363	137	7	0.274	0.945
Polat 2016	Turkey(Caucasian)	HB	PCR-RFLP	50/50	1	22	27	24	76	29	17	4	75	25	6	0.250	0.502
Ceylan 2016	Turkey(Caucasian)	HB	PCR-RFLP	40/75	20	17	3	57	23	47	23	5	117	33	6	0.220	0.358
Diler 2016	Turkey(Caucasian)	HB	PCR-RFLP	84/116	6	55	23	67	101	65	41	10	171	61	7	0.262	0.342
VNTR 4a/b					**bb**	**ab**	**aa**	**b**	**a**	**bb**	**ab**	**aa**	**b**	**a**			
Medeiros 2002	Portugal(Caucasian)	HB	PCR-RFLP	125/153	87	32	6	206	44	121	29	3	271	35	7	0.114	0.434
Safarinejad 2013	Iran(Caucasian)	HB	PCR-RFLP	170/340	101	54	15	256	84	249	88	3	586	94	7	0.138	0.112
Sanli 2011	Turkey(Caucasian)	PB	PCR-RFLP	137/158	92	40	5	114	50	104	48	6	256	60	7	0.189	0.885
Polat 2016	Turkey(Caucasian)	HB	PCR-RFLP	50/50	41	7	2	89	11	36	12	2	84	16	6	0.160	0.442
Diler 2016	Turkey(Caucasian)	HB	PCR-RFLP	84/116	65	16	3	146	22	83	31	2	197	35	6	0.150	0.646
-786T>C					**TT**	**TC**	**CC**	**T**	**C**	**TT**	**TC**	**CC**	**T**	**C**			
Safarinejad 2013	Iran(Caucasian)	HB	PCR-RFLP	170/340	52	93	25	197	143	150	159	31	459	221	7	0.325	0.223
Brankovic 2013	Serbia(Caucasian)	HB	PCR-RFLP	150/100	54	68	28	176	124	34	51	15	119	81	7	0.405	0.562
Polat 2016	Turkey(Caucasian)	HB	PCR-RFLP	50/50	32	11	7	75	25	21	24	5	66	34	6	0.340	0.623
Diler 2016	Turkey(Caucasian)	HB	PCR-RFLP	84/116	30	30	24	90	78	47	56	13	150	82	6	0.353	0.542
Sugie 2016	Japan(Asian)	NS	PCR-RFLP	134/86	65	48	21	178	90	54	27	5	135	37	7	0.215	0.514

Abbreviations: SOC: source of controls; HB: Hospital-Based; PB: Population-Based; NS: Not Stated; PCR: Polymerase Chain Reaction; RFLP: Restriction Fragment Length Polymorphism; NOS: Newcastle-Ottawa Scale; MAF: Minor Allele Frequency; HWE: Hardy-Weinberg Equilibrium.

mirror the positive studies that cause funnel plot asymmetry was used to further analyses the possible effect of publication bias. All statistical analyses were performed using Comprehensive Meta-Analysis (CMA) Software version 2.0 (Biostat, Englewood, USA). All tests were two-sided, and the $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of Included Studies

A flow diagram summarizing the process of study selection was shown in **Figure 1**. Searches of the electronic databases and manually searching references returned 186 studies. Among them, 78 studies were ex-

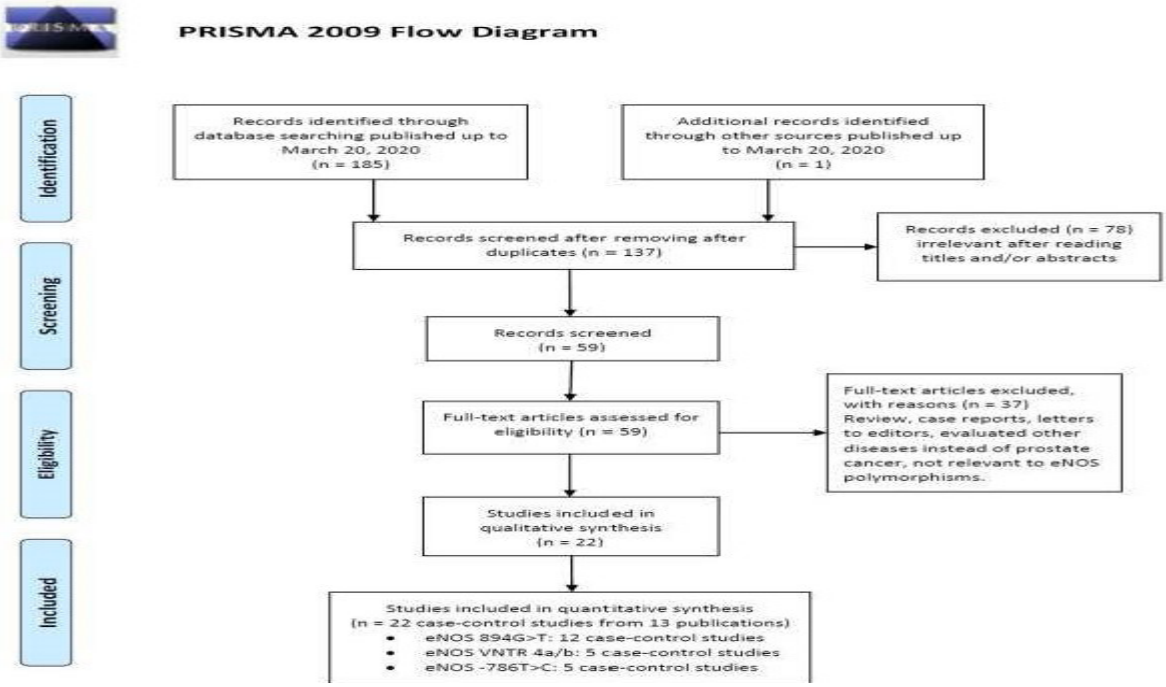

Figure 1. Flow diagram for inclusion of the studies in the meta-analysis.

Table 2. Summary risk estimates for association between eNOS polymorphisms and risk of prostate cancer.

Polymorphism	Genetic Model	Type of Model	Heterogeneity		Odds Ratio			Publication Bias		
			I^2 (%)	P_H	OR	95% CI	ZOR	POR	PBeggs	PEggers
+894G>T										
Overall	T vs. G	Random	88.25	≤0.001	1.340	1.039-1.727	2.256	0.024	0.086	0.102
	TT vs. GG	Random	81.37	≤0.001	1.679	0.966-2.918	1.836	0.066	0.450	0.106
	TG vs. GG	Random	78.17	≤0.001	1.299	0.991-1.702	1.894	0.058	0.114	0.106
	TT+TG vs. GG	Random	81.15	≤0.001	1.323	1.004-1.745	1.987	0.047	0.023	0.062
	TT vs. TG+GG	Random	72.17	≤0.001	1.357	0.886-2.077	1.405	0.160	0.537	0.154
Ethnicity										
Caucasian	T vs. G	Random	90.28	≤0.001	1.421	1.071-1.885	2.437	0.015	0.020	0.076
	TT vs. GG	Random	84.57	≤0.001	1.825	0.998-3.337	1.953	0.051	0.283	0.100
	TG vs. GG	Random	80.52	≤0.001	1.345	1.003-1.803	1.982	0.048	0.049	0.077
	TT+TG vs. GG	Random	83.78	≤0.001	1.387	1.023-1.880	2.105	0.035	0.020	0.047
	TT vs. TG+GG	Random	76.26	≤0.001	1.447	0.917-2.281	1.589	0.112	0.474	0.124
VNTR 4a/b										
Overall	a vs. b	Random	73.45	0.005	1.193	0.783-1.825	0.825	0.409	0.462	0.136
	aa vs. bb	Random	58.97	0.045	2.393	0.840-6.814	1.634	0.102	0.806	0.577
	ab vs. bb	Fixed	44.18	0.127	1.160	0.903-1.490	1.161	0.246	0.226	0.129
	aa+ab vs. bb	Random	65.67	0.020	1.130	0.735-1.738	0.558	0.577	0.220	0.117
	aa vs. ab+bb	Fixed	53.07	0.074	2.504	1.309-4.788	2.775	0.006	0.806	0.659
-786T>C										
Overall	C vs. T	Random	65.33	0.021	1.387	0.954-2.016	1,715	0.086	0.086	0.018
	CC vs. TT	Fixed	26.35	0.246	2.019	1.399-2.913	3.752	≤0.001	0.806	0.737
	CT vs. TT	Random	73.26	0.005	0.946	0.567-1.579	-0.212	0.832	0.220	0.038
	CC+CT vs. TT	Random	83.18	≤0.001	0.860	0.417-1.776	-0.407	0.684	0.086	0.045
	CC vs. CT+TT	Fixed	0.00	0.398	1.915	1.365-2.686	3.759	≤0.001	1.000	0.596
Ethnicity										
Caucasian	C vs. T	Random	76.70	0.005	1.265	0.845-1.895	1.141	0.254	0.734	0.481
	CC vs. TT	Fixed	33.26	0.213	1.843	1.222-2.779	2.916	0.004	1.000	0.748
	CT vs. TT	Random	78.21	0.003	0.970	0.519-1.814	-0.095	0.924	0.308	0.110
	CC+CT vs. TT	Random	77.81	0.004	1.118	0.626-1.996	0.376	0.707	0.308	0.164
	CC vs. CT+TT	Fixed	0.00	0.603	1.680	1.149-2.457	2.676	0.007	1.000	0.679

cluded because they were duplications, review articles, case reports, meta-analyses, irrelevant to eNOS polymorphisms and prostate cancer risk, and did not provide enough genotype information. Finally, a total of 22 case-control studies in 13 publications[17–29] with 4,618 cases with prostate cancer and 5,856 healthy subjects were included in this meta-analysis. Detailed characteristics and genotype distribution of eligible studies are listed in **Table 1**. The relevant research was published between August 2002 and April 2016. Prostate cancer cases in the selected studies ranged from 50 to 1420. Of those 22 case-control studies, 12 studies with 3,464 cases and 4,347 controls were on eNOS 894G>T, five studies with 566 cases and 817 controls were on eNOS VNTR 4a/b, and five studies with 588 cases and 692 controls were on eNOS -786T>C polymorphism. In terms of ethnicity, eleven were performed on a Caucasian population, one on a mixed and two on an Asian population. The studies were carried out in Portugal (n=2), Brazil (n=1), USA (n=3), China (n=1), Iran (n=4), Serbia (n=2), Turkey (n=8) and Japan (n=1). The control sources of the 15 studies were hospital-based (HB), four studies were population-based (PB), one study was mixed (HB and PB), and one study did not state. Three molecular techniques including RFLP-PCR, TaqMan and direct sequencing were used to genotype the eNOS polymorphisms. The genotypes and minor allele frequency (MAF) distributions for eNOS polymorphisms in cases and controls were presented in **Table 1**. The distribution of genotypes in the healthy controls was consistent with the Hardy-Weinberg equilibrium (**Table 1**).

Quantitative Data Synthesis

eNOS 894G>T

Table 2 listed the main results of the meta-analysis for association between eNOS 894G>T polymorphism and prostate cancer risk. When all the eligible studies were pooled into the meta-analysis of eNOS 894G>T polymorphism, significantly increased risk of prostate cancer was observed under two genetic models, i.e., allele (T vs. G: OR = 1.340, 95% C = 1.039-1.727, $p = 0.024$) and dominant (TT+GT vs. GG: OR = 1.323, 95% CI 1.004-1.745, p = 0.047). Moreover, we performed subgroup analysis based on ethnicity among Caucasians. Assessment of stratified analysis by ethnicity in other populations is not meaningful due to limited number of studies included in this study (**Table 1**). When stratified by ethnicity, there was a significant association between eNOS 894G>T polymorphism and an increased risk of prostate cancer in Caucasians under three genetic models, i.e., allele (T vs. G: OR = 1.421, 95% C = 1.071-1.885, $p = 0.015$, Figure 2A), heterozygote (GT vs. GG: OR = 1.345, 95% CI 1.003-1.803, $p = 0.048$) and dominant (TT+GT vs. GG: OR = 1.387, 95% CI 1.023-1.880, $p = 0.035$).

eNOS VNTR 4a/b

The summary results for the association between eNOS VNTR 4a/b polymorphism and prostate cancer risk are shown in Table 2. When all the eligible studies were pooled into the meta-analysis of eNOS VNTR 4a/b polymorphism, significantly an increased risk of prostate cancer was observed under the recessive genetic model (aa vs. ab+bb: OR = 2.504, 95% CI 1.309-4.788, $p = 0.006$, Fig 2B). Assessment of stratified analysis by ethnicity is not meaningful due to limited number of studies included in this study (**Table 1**).

eNOS -786T>C

Table 2 also listed the main results for the association between eNOS -786T>C polymorphism and prostate cancer risk. Overall, the pooled data indicated a significant association between the eNOS 894G>T polymor-

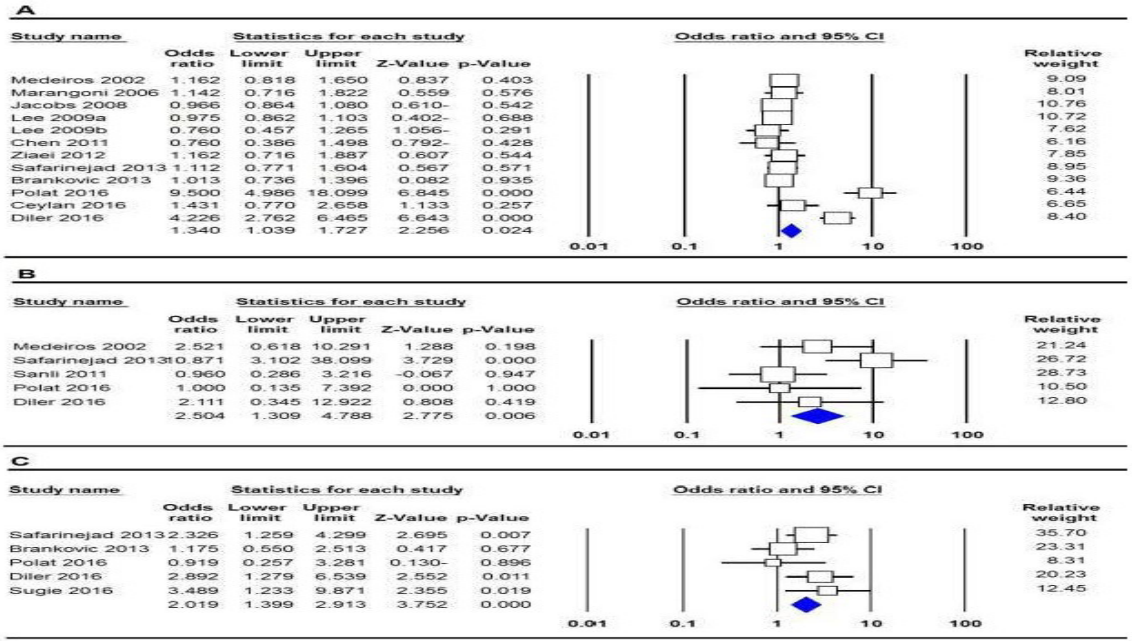

Figure 2. Forest plots for association of eNOS 894G>T, VNTR 4a/b, and -786T>C polymorphisms with prostate cancer risk. A: +894G>T (allele model: T vs. G); B: VNTR 4a/b (recessive model: aa vs. ab+bb); and C: -786T>C (recessive model: CC vs. CT+TT).risk. A: +894G>T (allele model: T vs. G); B: VNTR 4a/b (recessive model: aa vs. ab+bb); and C: -786T>C (recessive model: CC vs. CT+TT).

phism and an increased risk of prostate cancer under two genetic models, i.e., homozygote (CC vs. TT: OR = 2.019, 95% CI 1.399-2.913, $p \leq 0.001$) and recessive (CC vs. CT+TT: OR = 1.915, 95% CI 1.365-2.686, $p \leq 0.001$, **Figure 2C**). Moreover, we performed subgroup analysis based on ethnicity among Caucasians. Assessment of stratified analysis by ethnicity in other populations is not meaningful due to limited number of studies included in this study (**Table 1**). Stratified analysis showed an increased risk of prostate cancer in Caucasian population under two genetic models, i.e., homozygote (CC vs. TT; OR = 1.843, 95% CI 1.222-2.779, $p = 0.004$) and recessive (CC vs. CT+TT; OR = 1.680, 95% CI 1.149-2.457, $p = 0.007$).

Between-Study Heterogeneity

We found significant between-study heterogeneity for eNOS 894G>T, VNTR 4a/b, and -786T>C polymorphisms in overall population under almost genetic models and thus the random-effect model was applied to calculate their combined OR (**Table 2**). Therefore, a subgroup analysis by ethnicity was performed to explain the potential source of heterogeneity. As shown in Table 2, when subgroup analyses were performed, the between-study heterogeneity did not change considerably. The results revealed that ethnicity might not be the major source of heterogeneity in the current meta-analysis.

Sensitivity Analysis

Sensitivity analysis was performed to identify the influence of each study on the pooled OR by consecutively omitting one study each time in the overall population. The sensitivity analysis for eNOS 894G>T, VNTR 4a/b, and -786T>C polymorphisms revealed that no individual study did not significantly affect the pooled data. Hence, results of the sensitivity analysis indicated that our results are statistically stable and reliable.

Publication Bias

The Begg's and Egger's linear regression tests were used to investigate the potential publication bias for association between eNOS polymorphisms and prostate cancer risk in the overall population. **Table 2** lists the publication bias assessment method with its respective P-value for each test. The shapes of the funnel plots did not show any evidence of publication bias under all five genetic models in the overall population for eNOS 894G>T and VNTR 4a/b polymorphisms. For example, Figure 3 showed funnel plot of publication bias test for association of eNOS 894G>T (allele model: T vs. G), VNTR 4a/b (homozygote model: aa vs. bb) and -786T>C (recessive model: CC+CT vs. TT) polymorphisms with prostate cancer risk. However, the shapes of the funnel plots revealed obvious asymmetry for -786T>C polymorphism under the dominant model (TT+TG vs. GG: PBeggs = 0.023; PEggers = 0.062). Moreover, Egger's test found a publication bias under the genetic model, suggesting that there was an obvious publication bias for association between eNOS -786T>C polymorphism and prostate cancer. Thus, we used the Duval and Tweedie nonparametric ''trim and fill'' method to adjust the pooled risk for association between eNOS -786T>C polymorphism and prostate cancer under the dominant model (**Figure 4**). However, the "trim and fill" method did not significantly change conclusions, indicating that our results were statistically robust.

DISCUSSION

Although several case-control studies have been conducted to assess the roles of eNOS gene polymorphisms to the prostate cancer susceptibility in different populations, contradictory results were reported due to the relatively small sample size of individual studies and sampling effects. For example; Ziaei et al. did not observe an association between eNOS 894G>T poly-

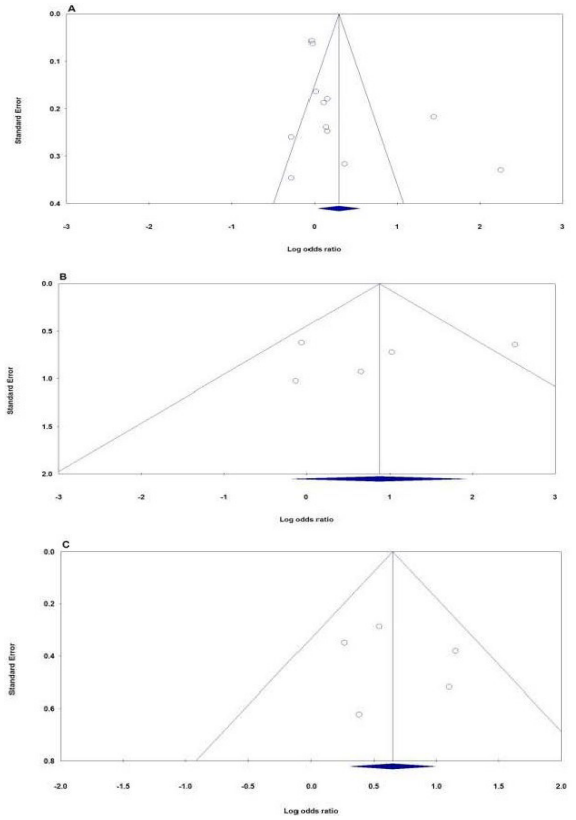

Figure 3. Begg's funnel plot of publication bias test for association of eNOS 894G>T, VNTR 4a/b, and -786T>C polymorphisms with prostate cancer risk. A: 894G>T (allele model: T vs. G); B: VNTR 4a/b (homozygote model: aa vs. bb); and C: 786T>C (recessive model: CC+CT vs. TT).

morphism and prostate cancer risk in 95 prostate cancer patients and 111 benign prostate hyperplasia in an Iranian population[25]. Similarly, two studies by Polat et al., and Ceylan et al., also found no association between eNOS 894G>T and the 4 VNTR polymorphism and prostate cancer risk, respectively[30,31]. However, in a case-control study with 125 prostate cancer patients and 153 controls, Medeiros et al., reported that the eNOS 894G>T polymorphism was associated with an increased risk of prostate cancer risk in a Caucasian population[17]. Safarinejad et al. also showed that two eNOS -786T>C and VNTR 4a/b polymorphisms might modify the individual susceptibility to prostate cancer in an Iranian population[26]. Therefore, the current meta-analysis based on 22 case-control studies was performed to provide a more precise estimation of the association between eNOS 894G>T, VNTR 4a/b, and -786T>C polymorphisms and prostate cancer risk. Our pooled results showed that eNOS −786T>C, VNTR 4a/b, and -786T>C polymorphisms were significantly associated with risk of prostate cancer.

The 894G>T polymorphism is one of the most important identified functional polymorphisms on the eNOS gene. As this polymorphism is located in a coding region, it might be in relation to altered eNOS protein and functional changes of the endothelium by an amino acidic substitution at position 298 (Glu298Asp)[30]. Our pooled results support the role of 894G>T polymorphism in pathogenesis of prostate cancer. In addition, epidemiological studies have showed that the -786T>C polymorphism, a 5' flanking region polymorphism of the eNOS gene, is associated with different disease. In the present meta-analysis, the overall analysis showed a significant association between the eNOS -786T>C polymorphism and prostate cancer risk in the homozygote and recessive models, identifying that the C allele of eNOS -786T>C polymorphism had a statistically significant increased prostate cancer risk. As this polymorphism located in promoter region of eNOS gene, it may affect eNOS expression and then lowers eNOS mRNA and serum NO levels. Our pooled results were inconsistent with two previous meta-analysis by Nikolić et al., and Gao et al. on 894G>T polymorphism[10,31]. Nikolić et al., included nine case-control studies and one case-only study on eNOS 894G>T and four studies on -786T>C. Their results suggested that -786T>C polymorphism were associated with increased prostate cancer risk, while the 894G>T polymorphism did not associated with risk and progression of prostate cancer. However, the previous meta-analyses results regarding the eNOS 894G>T polymorphism and prostate cancer risk essentially remains an open field, as the number of studies included was considerably smaller than that needed to achieve robust and conclusive results. Moreover, Nikolić et al., and Gao et al. did not perform subgroup analysis. In the present meta-analysis, by including only 12 case-control studies for quantitative synthesis, we found that both eNOS 894G>T and -786T>C polymorphisms were associated with susceptibility to prostate cancer. Moreover, stratified analysis indicated that the Caucasians carriers of the minor alleles of eNOS 894G>T and -786T>C polymorphisms might have high risk of prostate cancer.

The polymorphism of eNOS VNTR 4a/b (VNTR 4a/4b) gene consists of the two alleles of eNOS 4a with 4 tandem 27-repeats and eNOS 4b with 5 repeats in the intron 4. The polymorphism of eNOS VNTR 4a/b gene has been associated with many vascular diseases including hypertension, diabetic retinopathy, and diabetic nephropathy in various populations. In 2002, Medeiros et al., first reported that the eNOS VNTR 4a/b polymorphism is associated with threefold increase risk of prostate cancer risk in a Portuguese population[17]. In 2015, in a meta-analysis of three case-control studies an increased risk of prostate cancer was observed for eNOS VNTR 4a/b polymorphism[31]. The present meta-analysis based on five case-control studies found a significantly increased risk of prostate cancer for eNOS VNTR 4a/b polymorphism, which was partially consistent with the previous meta-analysis. However, the larger number of studies included leading to an increased statistical power.

Between-study heterogeneity is common in meta-analysis for genetic association studies[32–34]. Therefore, exploring the potential sources of between-study heterogeneity is an essential component of meta-analysis [35–37]. The between-study heterogeneity might arise from study quality, characteristics such as study design, sample size, inclusion criteria, ethnicity, clinical heterogeneity, and different genotyping methods and lifestyle factors[38–41]. In the case of prostate cancer, the screening policy also varies between countries. These different screening policies might also be responsible for the between study heterogeneity. In this study, there was a significant heterogeneity for eNOS gene poly-

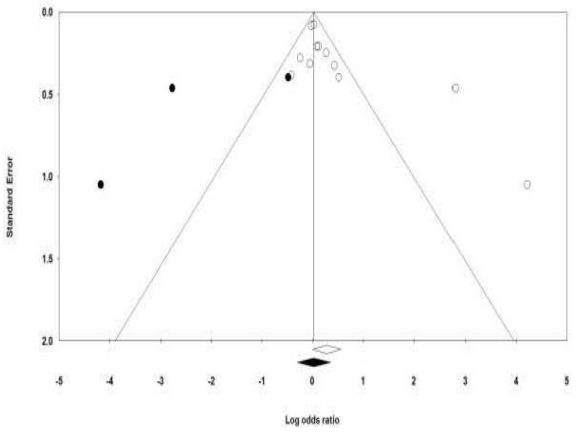

Figure 4. Begg's funnel plot of publication bias test before (hollow circles) and after (filled circles) trim-and-fill method for eNOS -786T>C polymorphisms and prostate cancer risk under heterozygote model (CT vs. TT)

morphisms. Therefore, meta-regression and subgroup analyses were performed to explore the sources of between-study heterogeneity. However, the results indicated that ethnicity was not the source of heterogeneity in the current meta-analysis

Some limitations of our meta-analysis should be considered when interpreting the results. First, although we collected all the eligible studies, sample size of the included studies was small, especially for stratified analyses by ethnicity, which may have limited the statistical power to find conclusions. Second, we included only published study in English in this meta-analysis, published studies in other languages, ongoing studies and unpublished data were not included, which may cause publication bias. Third, among those 22 studies included in this meta-analysis, most of studies were conducted in Caucasians, only two studies were in Asians and one study in mixed. Thus, the findings from this meta-analysis might be applicable to Caucasians. Future studies containing the full range of possible ethnic differences are required to avoid selection bias. Fourth, in this meta-analysis evidence of heterogeneity and publication bias was observed, which both might distort the conclusion of our results. Fifth, due to the unavailability of other detailed information our results were based on single-factor estimates without adjustments for other risk factors such as age, gender, life style, environmental factors and other variables. Finally, further evaluation of prostate cancer risk should pay more attention to the potential interactions among gene-gene, gene-environment, and even different polymorphisms of the eNOS gene.

CONCLUSIONS

The current meta-analysis indicates that eNOS 894G>T, VNTR 4a/b and -786T>C polymorphisms were significantly associated with an increased risk of prostate cancer in the overall population, especially in Caucasians.

CONFLICTING INTEREST

The authors declared no potential conflicts of interest with respect to the research or publication of this article.

REFERENCES

1. Abedinzadeh M, Zare-Shehneh M, Neamatzadeh H, Abedinzadeh M, Karami H. Association between MTHFR C677T polymorphism and risk of prostate cancer: Evidence from 22 studies with 10,832 cases and 11,993 controls. Asian Pac J Cancer Prev. 2015;16:4525-30.
2. Abedinzadeh M, Ghodsian M, Dastgheib SA, Jafari-Nedooshan J, Zare M, Heiranizadeh N, et al. Association of interlukine-18 polymorphisms with susceptibility to prostate cancer in Iranian population. Neoplasma. 2020;pii: 190616N513.
3. Liu S, Cai H, Cheng W, Zhang H, Pan Z, Wang D. Association of VDR polymorphisms (Taq I and Bsm I) with prostate cancer: a new meta-analysis. J Int Med Res. 2017;45:3-10.
4. Weng H, Li S, Huang J-Y, He Z-Q, Meng X-Y, Cao Y, et al. Androgen receptor gene polymorphisms and risk of prostate cancer: a meta-analysis. Sci Rep. 2017;7:40554;
5. Fang C, Guo Z-Q, Chen X-Y, Liu T-Z, Zeng X-T, Wang X-H. Relationship between SRD5A2 rs9282858 polymorphism and the susceptibility of prostate cancer. Medicine (Baltimore). 2017;96:e6791.
6. Taghavi A, Mohammadi-Torbati P, Kashi AH, Rezaee H, Vaezjalali M. Polyomavirus hominis 1(BK virus) infection in prostatic tissues: Cancer versus hyperplasia. Urol J 2015;12:2240–4.
7. Shen MM, Abate-Shen C. Molecular genetics of prostate cancer: new prospects for old challenges. Genes Dev. 2010;24:1967-2000.
8. Yang Z, Goldstein AS, Jiaoti H. The molecular basis for ethnic variation and histological subtype differences in prostate cancer. Sci China Life Sci. 2013;56:780–7.
9. Nikolić ZZ, Pavićević DLS, Romac SP, Brajušković GN. Genetic variants within endothelial nitric oxide synthase gene and prostate cancer: a meta-analysis. Clin Transl Sci. 2015;8:23–31.
10. Azarpira MR, Ghilian MM, Sobhan MR, Mahdinezhad-Yazdi M, Aghili K, Ahrar H, et al. Association of eNOS 27-bp VNTR, 894G>T and 786T>C polymorphisms with susceptibility to Legg-Calve-Perthes Disease in Iranian children. J Orthop. 2019;16:137–40.
11. Korde Choudhari S, Chaudhary M, Bagde S, Gadbail AR, Joshi V. Nitric oxide and cancer: a review. World J Surg Oncol. 2013;11:118.
12. Rath M, Muller I, Kropf P, Closs EI, Munder M. Metabolism via Arginase or Nitric Oxide Synthase: Two Competing Arginine Pathways in Macrophages. Front Immunol. 2014;5:532.
13. Zhou ZC, Gu SZ, Wu J, Liang QW. VEGF, eNOS, and ABCB1 genetic polymorphisms may increase the risk of osteonecrosis of the femoral head. Genet Mol Res. 2015;14:13688-

98.

14. Kaur R, Matharoo K, Raina P, Sikka R, Bhanwer AJS. Interactive role of endothelial nitric oxide synthase gene polymorphisms in T2D with CAD and CAD patients of Punjab (North-West India). Int J Diabetes Dev Ctries. 2017;37:286–97.
15. Abbasi H, Dastgheib SA, Hadadan A, Karimi-Zarchi M, Javaheri A, Meibodi B, et al. Association of Endothelial Nitric Oxide Synthase 894G > T Polymorphism with Preeclampsia Risk: A Systematic Review and Meta-Analysis based on 35 Studies. Fetal Pediatr Pathol. 2020:1-16. doi: 10.1080/15513815.2019.1710880. [Epub ahead of print]
16. Gohari M, Dastgheib SA, Noorishadkam M, Lookzadeh MH, Mirjalili SR, Akbarian-Bafghi MJ, et al. Association of eNOS and ACE Polymorphisms with Retinopathy of Prematurity: A Systematic Review and Meta-Analysis. Fetal Pediatr Pathol. 2019:1-12. doi: 10.1080/15513815.2019.1652378. [Epub ahead of print]
17. Medeiros R, Morais A, Vasconcelos A, Costa S, Pinto D, Oliveira J, et al. Endothelial nitric oxide synthase gene polymorphisms and genetic susceptibility to prostate cancer. Eur J Cancer Prev. 2002;11:343-50.
18. Marangoni K, Neves AF, Cardoso AM, Santos WK, Faria PC, Goulart LR. The endothelial nitric oxide synthase Glu-298-Asp polymorphism and its mRNA expression in the peripheral blood of patients with prostate cancer and benign prostatic hyperplasia. Cancer Detect Prev. 2006;30:7-13.
19. Diler SB, Öden A. The T -786C, G894T, and Intron 4 VNTR (4a/b) Polymorphisms of the Endothelial Nitric Oxide Synthase Gene in Prostate Cancer Cases. Genetika. 2016;52(2):249-54.
20. Sanli O, Kucukgergin C, Gokpinar M, Tefik T, Nane İ, Seckin S. Despite the lack of association between different genotypes and the presence of prostate cancer, endothelial nitric oxide Synthase a/b (eNOS4a/b) polymorphism may be associated with advanced clinical stage and bone metastasis. Urologic Oncology: Seminars and Original Investigations. 2011;29:183–8.
21. Sugie S. Effects of the Endothelial Nitric Oxide Synthase (Enos) T786c Genotype on the Risk of Prostate Cancer in Japanese Population. J Urol Nephrol Open Access. 2016;2:01–5.
22. Jacobs EJ, Hsing AW, Bain EB, Stevens VL, Wang Y, Chen J, et al. Polymorphisms in Angiogenesis-Related Genes and Prostate Cancer. Cancer Epidemiol Biomarkers Prev. 2008;17:972–7.
23. Lee K-M, Kang D, Park SK, Berndt SI, Reding D, Chatterjee N, et al. Nitric oxide synthase gene polymorphisms and prostate cancer risk. Carcinogenesis. 2009;30:621–5.
24. ZG Chen, W Zhou, ZH Tao WC. Association of endothelial nitric oxide synthase gene polymorphism with prostate cancer. Zhong Guo Nan Ke Xue Za Zhi. 2011;25:18–21.
25. Ziaei SAM, Samzadeh M, Jamaldini SH, Afshari M, Haghdoost AA, Hasanzad M. Endothelial Nitric Oxide Synthase Glu298Asp Polymorphism as a Risk Factor for Prostate Cancer. Int J Biol Markers. 2013;28:43-8.
26. Safarinejad MR, Shafiei N, Safarinejad S. The role of endothelial nitric oxide synthase (eNOS) T-786C, G894T, and 4a/b gene polymorphisms in the risk of idiopathic male infertility. Mol Reprod Dev. 2010;77:720–7.
27. Branković A, Brajušković G, Nikolić Z, Vukotić V, Cerović S, Savić-Pavićević D, et al. Endothelial nitric oxide synthase gene polymorphisms and prostate cancer risk in Serbian population. Int J Exp Pathol. 2013;94:355–61.
28. Polat F, Turaçlar N, Yilmaz M, Bingöl G, Cingilli Vural H. eNOS gene polymorphisms in paraffin-embedded tissues of prostate cancer patients. Turk J Med Sci. 2016;46:673-9.
29. Ceylan GG, Ceylan C, Gülmemmedov B, Tonyalı Ş, Odabaş O, Gözalan A, et al. Polymorphisms of eNOS, catalase, and myeloperoxidase genes in prostate cancer in Turkish men: preliminary results. Genet Mol Res. 2016;15.
30. Zhao C, Yan W, Zu X, Chen M, Liu L, Zhao S, et al. Association between endothelial nitric oxide synthase 894G>T polymorphism and prostate cancer risk: a meta-analysis of literature studies. Tumour Biol. 2014;35:11727-33.
31. Gao X, Wang J, Wang W, Wang M, Zhang J. eNOS Genetic Polymorphisms and Cancer Risk. Medicine (Baltimore). 2015;94:e972.
32. Soleimani-Jadidi S, Abbasi H, Javaheri A, Behforouz A, Zanbagh L, Meibodi B, et al. Cumulative Evidence for Association of IL-10 -1082G > A Polymorphism with Susceptibility to Recurrent Pregnancy Loss: A Systematic Review and Meta-Analysis. Fetal Pediatr Pathol. 2020:1-15. doi: 10.1080/15513815.2020.1716903. [Epub ahead of print]
33. Karimi-Zarchi M, Moghimi M, Abbasi H, Hadadan A, Salimi E, Morovati-Sharifabad M, et al. Association of MTHFR 677C > T polymorphism with susceptibility to ovarian and cervical cancers: A systematic review and meta-analysis. Asian Pac J Cancer Prev. 2019;20:2569-2577.
34. Jafari-Nedooshan J, Moghimi M, Zare M, Heiranizadeh N, Morovati-Sharifabad M, Akbarian-Bafghi MJ, et al. Association of Promoter Region Polymorphisms of IL-10 Gene with Susceptibility to Lung Cancer:

Systematic Review and Meta-Analysis. Asian Pac J Cancer Prev. 2019;20:1951–7.

35. Aflatoonian M, Sivandzadeh G, Morovati-Sharifabad M, Mirjalili SR, Akbarian-Bafghi MJ, Neamatzadeh H. Associations of IL-6 -174G>C and IL-10 -1082A>G polymorphisms with susceptibility to celiac disease: evidence from a meta-analysis and literature review. Arq Gastroentero. 2019;56:323–8.

36. Moghimi M, Sobhan MR, Jarahzadeh MH, Morovati-Sharifabad M, Aghili K, Ahrar H, et al. Association of GSTM1, GSTT1, GSTM3, and GSTP1 Genes Polymorphisms with Susceptibility to Osteosarcoma: a Case-Control Study and Meta-Analysis. Asian Pac J Cancer Prev. 2019;20:675–82.

37. Moghimi M, Kargar S, Jafari MA, Ahrar H, Jarahzadeh MH, Neamatzadeh H, et al. Angiotensin Converting Enzyme Insertion/ Deletion Polymorphism is Associated with Breast Cancer Risk: A Meta-Analysis. Asian Pac J Cancer Prev. 2018;19:3225–31.

38. Namazi A, Forat-Yazdi M, Jafari M, Farahnak S, Nasiri R, Foroughi E, et al. Association of interleukin-10 -1082 A/G (rs1800896) polymorphism with susceptibility to gastric cancer: meta-analysis of 6,101 cases and 8,557 controls. Arq Gastroenterol. 2018;55:33–40.

39. Sobhan MR, Mahdinezhad-Yazdi M, Aghili K, Zare-Shehneh M, Rastegar S, Sadeghizadeh-Yazdi J, et al. Association of TNF-α-308 G > A and -238G > A polymorphisms with knee osteoarthritis risk: A case-control study and meta-analysis. J Orthop. 2018;15:747–53.

40. Farbod M, Karimi-Zarchi M, Heiranizadeh N, Seifi-Shalamzari N, Akbarian-Bafghi MJ, Jarahzadeh MH, et al. Association of TNF-α -308G>A Polymorphism with Susceptibility to Cervical Cancer and Breast Cancer: a Systematic Review and Meta-analysis. Klin Onkol. 2019;32:170–80.

41. Niktabar SM, Latifi SM, Moghimi M, Jafari-Nedooshan J, Aghili K, Miresmaeili SM, et al. Association of Vitamin D receptor gene polymorphisms with risk of cutaneous melanoma. A meta-analysis based on 40 case-control studies. Dermatol Rev/Przegl Dermatol. 2019; 106:268–279.