REVIEW ARTICLE

Relevance of hMLH1 -93G>A, 655A>G and 1151T>A polymorphisms with colorectal cancer susceptibility: a meta-analysis based on 38 case-control studies

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http://dx.doi.org/10.1590/1806-9282.64.10.942

SUMMARY

OBJECTIVE: *There has been increasing interest in the study of the association between human mutL homolog 1 (hMLH1) gene polymorphisms and risk of colorectal cancer (CRC). However, results from previous studies are inconclusive. Thus, a meta-analysis was conducted to derive a more precise estimation of the effects of this gene.*

METHODS: *A comprehensive search was conducted in the PubMed, EMBASE, Chinese Biomedical Literature databases until January 1, 2018. Odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of the association.*

RESULTS: *Finally, 38 case-control studies in 32 publications were identified met our inclusion criteria. There were 14 studies with 20668 cases and 19533 controls on hMLH1 -93G>A, 11 studies with 5,786 cases and 8,867 controls on 655A>G and 5 studies with 1409 cases and 1637 controls on 1151T>A polymorphism. The combined results showed that 655A>G and 1151T>A polymorphisms were significantly associated with CRC risk, whereas -93G>A polymorphism was not significantly associated with CRC risk. As for ethnicity, -93G>A and 655A>G polymorphisms were associated with increased risk of CRC among Asians, but not among Caucasians. More interestingly, subgroup analysis indicated that 655A>G might raise CRC risk in PCR-RFLP and HB subgroups.*

CONCLUSION: *Inconsistent with previous meta-analyses, this meta-analysis shows that the hMLH1 655A>G and 1151T>A polymorphisms might be risk factors for CRC. Moreover, the -93G>A polymorphism is associated with the susceptibility of CRC in Asian population.*

KEYWORDS: *colorectal cancer, hMLH1, polymorphism; Meta-analysis.*

DATE OF SUBMISSION: 19-Jan-2018
DATE OF ACCEPTANCE: 24-Mar-2018
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INTRODUCTION

Colorectal cancer (CRC) is one of the most frequent malignant tumours of the digestive tract in human, especially in the Western world.[1,2] CRC ranks among the three most common cancers in terms of both cancer incidence and cancer-related deaths in most developed countries. More than one million cases of CRC are diagnosed in the worldwide every year.[1-3] The cross cultural and migrant studies suggest that the majority of CRC cases (≈85%) is related to environmental factors including smoking, drinking, meat consumption, less activity, exposure to aryl amines, and heterocyclic amines.[4-6]

The MMR genes encode a family of highly conserved proteins, including MLH1, MSH2, MSH6, and PMS2.[7,8] MMR systems promote genetic stability by repairing DNA replication errors, inhibiting recombination between non-identical DNA sequences, and participating in responses to DNA damage.[9,10] MLH1 protein physically interacts with other MMR components; although the exact role of MLH1 gene remains elusive, MLH1-deficiency is associated with cancer predisposition.[11] To date, most of the causative mutations have been identified in MLH1 gene. Mutations in the gene for MLH1 are estimated to account for nearly 40% of the more than 400 known MMR gene mutations, and prevalence of mutations in MLH1 in Western countries is between 1 of 1000.[12] Standardized incidence ratios (SIRs) for carriers of hMLH1 mutations, when compared with the general population is 68 and the relative risk for CRC for first-degree relatives of mutation carriers compared with first degree relatives of non-carriers is 8.1.[13,14]

Molecular epidemiological and pooling analyses studies have reported the association of hMLH1 -93G>A, 655A>G and 1151T>A with CRC risk,[15-46] but the results remain inconsistent and inclusive. Inconsistencies in results may be caused by differences in study design, population, or different statistical methods. Meta-analysis is a powerful tool for summarizing the different studies. It can not only overcome the problem of small size and inadequate statistical power of genetic studies of complex traits, but also provide more reliable results than a single case-control study. However, the previous meta-analysis on hMLH1 polymorphisms with CRC risk has shown conflicting conclusions. Because, several published studies were not included in the meta-analysis and additional original studies with larger sample sizes have been published since then. We therefore performed a meta-analysis to make a more precise assessment the association between hMLH1 polymorphisms with CRC risk, by adding more studies implemented in recent years.

METHODS

Search Strategy

A comprehensive literature search was performed using the MEDLINE (National Library of Medicine), EMBASE (Excerpta Medica), CANCERLIT (National Cancer Institute), Web of Science (Thomson-Reuters), Cochrane Library, Chinese Biomedical Literature Database and Google scholar for all relevant articles published up to January 1, 2018 that evaluated the association between the hMLH1 gene polymorphisms and risk of CRC. The following terms were included in the search: ''Colorectal cancer" or ''CRC", ''human mutL homolog 1" or ''hMLH1",''-93G>A" or ''rs1800734", ''655A>G" or ''p.Ile219Val" or ''rs1799977", ''1151T>A" or ''p.Val384Asp" or ''rs63750447", "polymorphism", "mutation", "variant", "gene", "genotype", "SNP", and "allele". The search was not restricted by the publication year or language. Furthermore, in order to identify potentially relevant studies, we manually searched reference lists of eligible studies, reviews and related meta-analyses. If there were multiple reports of the same sample or overlapping data only the study with the largest sample sizes or the most recent one was included following careful examination.

Inclusion and Exclusion Criteria

Studies were selected according to the following inclusion criteria: (1) full-text published studies; (2) epidemiological studies with case-control or cohort design; (3) investigating the association between hMLH1 polymorphisms and CRC risk; (4) providing sufficient genotype data or information that could help infer the results in the studies to calculate the odd ratios (ORs) with a 95% confidence interval (CI). The exclusion criteria were as follows: (1) studies with only case population (no control population); (2) studies without detail genotype frequencies, which were unable to calculate odds ratio; (3) duplicate of previous publication.

Data Extraction

Information was carefully extracted from all eligible studies independently by two investigators ac-

cording to the inclusion criteria. For each study the following information was extracted: name of first author, publication year, country of origin, ethnicity, polymorphisms, source of controls, genotyping method, number of cases and controls, genotype frequency in cases and controls, minor allele frequencies (MAFs) in control subjects, and Hardy-Weinberg equilibrium test in control subjects. Diverse ethnicities were categorized as Caucasian, Asian, African and Mixed, which included more than one race. Disagreements were resolved in consultation with the third reviewer.

STATISTICAL ANALYSIS

The strength of associations was assessed by using odds ratios (ORs) and 95% confidence interval (CIs). The significance of the pooled OR was determined by the Z-test; a P value of <0.05 was considered significant. The OR of hMLH1 polymorphisms and CRC risk was estimated for each study. The pooled ORs were performed for allele (B vs. A), homozygote (BB vs. AA), heterozygote (AB vs. AA), dominant (BB+BA vs. AA) and recessive (BB vs. AB+ AA) models. A Chi square-test based Q-statistic test and an I^2 statistics (I^2= 100 %×(Q-df)/Q) were performed to assess the heterogeneity between studies.[47] A significant Q-statistic (P<0.10) indicated heterogeneity across studies. Venice criteria for the I^2 statistics: "I^2<25% represents no heterogeneity, I^2=25–50% represents moderate heterogeneity, I^2=50–75% represents large heterogeneity and I^2>75% represents extreme heterogeneity". Dependent on the results of heterogeneity test among individual studies, the fixed effect model (Mantel–Haenszel method) or random effect model (DerSimonian–Laird method) was utilized to summarize the pooled OR.[1,48] Furthermore, to detect the source of between-study heterogeneity, subgroup analyses (Meta-regression) by ethnicity, genotyping method and source of controls were performed. A Hardy–Weinberg equilibrium (HWE) was assessed for each study using the goodness-of-fit test (Chi square-test or Fisher exact test) only in control groups, and deviation was considered when P<0.01. The one-way sensitivity analyses were performed to survey the stability of the results, namely, a single study in the meta-analysis was omitted each time to reflect the influence of the individual data set to the pooled OR. Publication bias was evaluated by visual inspection of the funnel plot and Egger's linear regression test, and the significance level was set at 0.05 for both.[49,50] If publication bias observed, the Duval and Tweedie non-parametric "trim and fill" method was assessed to adjust for it. All the statistical analyses were performed by Comprehensive Meta-Analysis (CMA) software (Version 2.20; Biostat, USA). P<0.05 (two-tailed) was considered statistically significant.

RESULTS

Study Selection and Characteristics

After deleting of duplicates, 361 articles were excluded from screening the titles and abstracts, as these were unrelated to hMLH1 polymorphisms, or CRC risk. Further, 40 articles was excluded for no genotypic information, reviews, letters, case report, clinical, and animal studies. Totally, 38 case-control studies in 32 publications [15-46] containing 4092 cases and 5909 controls were included in the meta-analysis. The studies were published from 1998 to 2017. The main characteristics of the selected studies and the genotype distribution of the hMLH1 gene polymorphisms are summarized in Table 1. Of the 30 studies, eight were conducted in Asians (Japan, Korea, China, Kazakhstan, Malaysia, and Iran), 5 in Caucasians (Canada, Czech, USA, UK, Spain, Denmark, and Sweden), and one in a mixed population (Mexico). Of them, there were 19 studies with 20,668 CRC cases and 19,533 controls for -93G>A (rs1800734) polymorphism, eleven studies with 5,786 CRC cases and 8,867 controls for hMLH1 655A>G (rs1799977) polymorphism, and eight studies with 1,409 CRC cases and 1,637 controls for hMLH1 1151T>A (rs63750447) polymorphism. For the ethnicities, 12 studies of Caucasians and six studies of Asians were included on the hMLH1 -93G>A (rs1800734). As to hMLH1 655A>G (rs1799977) polymorphism, six studies of Caucasians and four studies of Asians were included. The eight studies on hMLH1 1151T>A (rs63750447) polymorphism were all based on the Asians. The distribution of the genotypes in the control subjects was in agreement with HWE except three studies.

Quantitative Synthesis

hMLH1 -93G>A (rs1800734) Polymorphism

The main results of the meta-analysis for all 19 case-control studies [15-33] on hMLH1 -93G>A polymorphism are presented in Table 3. The results of pooling all studies showed that there was no statistically

TABLE 1: MAIN CHARACTERISTICS OF STUDIES INCLUDED IN THIS META-ANALYSIS.

First Author	Country (Ethnicity)	SOC	Genotyping Technique	Case/Control	Cases					Controls					MAFs	HWE
					Genotypes			Allele		Genotypes			Allele			
-93G>A (rs1800734)					GG	AG	AA	G	A	GG	AG	AA	G	A		
Ito 1999 15	Japan (Asian)	PB	PCR-SSCP	27/84	8	10	9	26	28	22	46	16	90	78	0.464	0.355
Shin 2002 16	Korea (Asian)	HB	PCR-SSCP	139/157	33	61	45	127	151	42	74	41	158	156	0.496	0.472
Raptis 2007 17	Canada (Caucasian)	PB	TaqMan	929/1098	554	331	44	1439	419	687	352	59	1726	470	0.214	0.118
Chen 2007 18	USA(Caucasian)	NA	Pyroseq	99/286	44	47	8	135	63	169	99	18	437	135	0.236	0.497
Tulupova 2008 19	Czech (Caucasian)	HB	TaqMan	619/611	359	216	44	934	304	365	209	37	939	283	0.231	0.336
Samowitz 2008 20	USA(Caucasian)	PB	DS	1006/1963	610	344	52	1564	448	1170	688	105	3028	898	0.228	0.768
Koessler 2008 21	UK(Caucasian)	PB	TaqMan	2288/2276	1407	778	103	3592	984	1392	777	107	3561	991	0.217	0.914
Allan 2008 22	UK(Caucasian)	NA	PCR-RFLP	1518/589	878	566	74	2322	714	369	196	24	934	244	0.207	0.750
Campbell 2009 23	USA(Caucasian)	PB	PCR-RFLP	1600/1963	952	553	95	2457	743	1170	688	105	3028	898	0.228	0.768
van Roon 2010 24	Netherland(Caucasian)	NA	DS	39/920	12	20	7	44	34	554	331	44	1425	415	0.225	0.542
Whiffin 2011 25	UK(Caucasian)	PB	KASPae	10409/6965	6408	3504	497	16320	4498	4395	2261	309	11051	2879	0.206	0.401
Savio 2012 26	Canada (Caucasian)	PB	PCR-RFLP	252/845	150	96	6	396	108	528	264	53	1320	370	0.218	0.011
Muniz-Mendoza 2012 27	Mexico (Mixed)	HB	PCR-RFLP	100/115	47	44	9	138	62	39	55	21	133	97	0.421	0.834
Nizam 2013 28	Malaysia (Asian)	HB	PCR-RFLP	104/104	22	50	32	94	114	33	33	38	99	109	0.524	0.520
Martinez-Uruena 2013 29	Spain (Caucasian)	HB	PCR-RFLP	383/236	233	131	19	597	169	129	102	5	360	112	0.237	0.002
Djansugurova 2015 30	Kazakhstan (Asian)	HB	PCR-RFLP	249/244	126	94	29	346	152	101	115	28	317	171	0.350	0.581
Li 2015 31	China(Asian)	NA	PCR-RFLP	451/629	88	198	165	374	528	218	301	110	737	521	0.414	0.728
Zhang 2016 32	China(Asian)	HB	TaqMan	312/300	66	139	107	271	353	52	154	94	258	342	0.570	0.413
Mik 2017 33	Poland (Caucasian)	NA	PCR-RFLP	144/151	74	45	25	193	95	53	61	37	167	135	0.447	0.024
655A>G(rs1799977)				20668/19533	AA	AG	GG	A	G	AA	AG	GG	A	G		
Kim 2004 34	Korea (Asian)	PB	TaqMan	107/330	100	7	0	207	7	311	18	1	640	20	0.030	0.191
Mei 2006 35	China (Asian)	HB	PCR	160/150	144	14	2	302	18	141	9	0	291	9	0.030	0.704
Raptis 2007 17	Canada (Caucasian)	PB	TaqMan	929/1098	451	391	87	1293	565	514	485	99	1513	683	0.311	0.309
Berndt 2007 36	USA(Caucasian)	PB	TaqMan	211/2090	100	94	17	294	128	968	896	226	2832	1348	0.322	0.386
Christensen 2008 37	Denmark(Caucasian)	PB	SBE-tags	380/770	172	170	38	514	246	364	327	79	1055	485	0.314	0.660
Nejda 2009 38	Spain (Caucasian)	HB	PCR-RFLP	140/125	41	72	27	154	126	64	44	17	172	78	0.312	0.044
Campbell 2009 23	USA(Caucasian)	PB	PCR-RFLP	1601/1944	764	678	159	2206	996	937	848	159	2722	1166	0.299	0.087
Picelli 2010 39	Sweden (Caucasian)	PB	DS	1781/1701	819	781	181	2419	114	832	708	161	2372	1030	0.302	0.636
Muniz-Mendoza 2012 27	Mexico (Mixed)	HB	PCR-RFLP	102/100	71	26	5	168	36	81	19	0	181	19	0.095	0.293
Milanizadeh 2013 40	Iran (Asian)	HB	PCR-RFLP	219/248	25	62	132	112	326	248	54	119	227	269	0.346	≤0.001
Peng 2016 41	China (Asian)	PB	PCR-HRM	156/311	151	5	0	151	5	307	4	0	618	4	0.006	0.909
1151T>A(rs63750447)				5786/8867	TT	AT	AA	T	A	TT	AT	AA	T	A		
Wang 1998 42	China (Asian)	NA	PCR-SSCP	26/80	22	4	0	48	4	77	3	0	157	3	0.018	0.864
Wang 2000 43	China (Asian)	HB	PCR-SSCP	101/100	88	13	0	189	13	94	6	0	194	6	0.030	0.757
Kim 2004 34	Korea (Asian)	PB	TaqMan	107/330	100	7	0	207	7	313	17	0	643	17	0.025	0.631
Zhang 2005 44	China (Asian)	PB	DHPLC	90/268	82	8	0	172	8	251	17	0	519	17	0.031	0.591
Mei 2006 35	China (Asian)	HB	PCR	160/150	142	18	0	302	18	141	9	0	291	9	0.030	0.474
Ohsawa 2009 45	Japan (Asian)	NA	PCR-RFLP	670/332	630	39	1	1299	41	327	5	0	659	5	0.007	0.890
Wang 2010 46	China (Asian)	NA	DHPLC	99/66	83	16	0	182	16	63	3	0	129	3	0.022	0.850
Peng 2016 41	China (Asian)	PB	PCR-HRM	156/311	142	13	1	297	15	310	1	0	621	1	0.001	0.977

SOP, source of population; HB, Hospital-based study; PB, Population-based study; RT-PCR, Real-Time PCR; PCR-RFLP, PCR-restriction fragment length polymorphism; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MAFs, Minor Allele Frequencies; HWE, Hardy–Weinberg equilibrium; SBE-tags: Single base extension; HRM : High Resolution Melting.

TABLE 2. THE META-ANALYSIS OF HMLH1 -93G>A (RS1800734) POLYMORPHISMS AND CRC RISK.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I2 (%)	PH	OR	95% CI	Ztest	POR	PBeggs	PEggers
Overall (n=19)	A vs. G	Random	99.00	≤0.001	0.946	0.650-1.379	-0.287	0.774	0.161	0.936
	AA vs. GG	Random	80.83	≤0.001	1.220	0.950-1.566	1.555	0.120	0.363	0.769
	AG vs. GG	Random	98.69	≤0.001	0.914	0.546-1.532	-0.341	0.733	0.141	0.625
	AA+AG vs. GG	Random	75.86	≤0.001	1.066	0.954-1.191	1.127	0.260	1.000	0.838
	AA vs. AG+GG	Random	80.60	≤0.001	1.191	0.954-1.486	1.547	0.122	0.401	0.715
By ethnicity										
Caucasians (n=12)	A vs. G	Random	99.35	≤0.001	0.882	0.535-1.453	-0.492	0.623	0.303	0.875
	AA vs. GG	Random	66.00	0.001	1.061	0.859-1.310	0.549	0.583	0.192	0.716
	AG vs. GG	Random	99.16	≤0.001	0.826	0.422-1.619	-0.556	0.578	0.064	0.807
	AA+AG vs. GG	Random	68.02	≤0.001	1.056	0.958-1.164	1.089	0.276	0.631	0.703
	AA vs. AG+GG	Random	53.03	0.019	1.090	0.911-1.304	0.943	0.346	0.119	0.516
Asians (n=6)	A vs. G	Random	88.14	≤0.001	1.179	0.840-1.655	0.951	0.342	1.000	0.355
	AA vs. GG	Random	80.19	≤0.001	1.759	1.054-2.934	2.163	0.031	0.452	0.102
	AG vs. GG	Random	88.07	≤0.001	1.168	0.644-2.119	0.513	0.608	0.707	0.795
	AA+AG vs. GG	Random	84.60	≤0.001	1.139	0.708-1.833	0.535	0.592	1.000	0.593
	AA vs. AG+GG	Random	81.46	≤0.001	1.381	0.885-2.155	1.421	0.155	0.707	0.330
By Population-Based										
PB (n=7)	A vs. G	Random	99.63	≤0.001	0.720	0.349-1.486	-0.888	0.375	0.071	0.640
	AA vs. GG	Fixed	18.16	0.291	1.020	0.917-1.136	0.368	0.713	0.548	0.168
	AG vs. GG	Random	99.51	≤0.001	0.600	0.221-1.628	-1.004	0.316	0.071	0.863
	AA+AG vs. GG	Fixed	0.00	0.656	1.042	0.995-1.092	1.753	0.080	1.000	0.578
	AA vs. AG+GG	Fixed	37.36	0.143	1.030	0.927-1.145	0.558	0.577	0.548	0.446
HB (n=7)	A vs. G	Fixed	41.77	0.112	0.971	0.878-1.074	-0.573	0.566	0.548	0.392
	AA vs. GG	Random	78.04	≤0.001	1.526	0.963-2.418	1.800	0.072	0.229	0.101
	AG vs. GG	Random	81.22	≤0.001	0.943	0.650-1.368	-0.308	0.758	0.367	0.477
	AA+AG vs. GG	Random	55.17	0.037	0.890	0.711-1.114	-1.017	0.309	1.000	0.882
	AA vs. AG+GG	Fixed	35.45	0.158	1.087	0.895-1.321	0.840	0.401	0.763	0.763
By Genotyping Technique										
PCR-RFLP (n=9)	T vs. C	Random	89.42	≤0.001	0.984	0.784-1.234	-0.141	0.888	0.251	0.306
	AA vs. GG	Random	87.93	≤0.001	0.999	0.592-1.686	-0.004	0.997	0.602	0.232
	AG vs. GG	Random	85.77	≤0.001	1.031	0.782-1.360	0.217	0.828	0.916	0.950
	AA+AG vs. GG	Random	84.94	≤0.001	0.997	0.775-1.284	-0.022	0.983	0.251	0.624
	AA vs. AG+GG	Random	88.95	≤0.001	1.146	0.693-1.896	0.531	0.595	0.117	0.384
TaqMan (n=4)	A vs. G	Fixed	0.00	0.720	1.017	0.947-1.093	0.467	0.641	1.000	0.513
	AA vs. GG	Random	84.67	≤0.001	1.291	0.795-2.097	1.034	0.301	0.734	0.585
	AG vs. GG	Fixed	39.21	0.177	1.024	0.934-1.124	0.506	0.613	0.734	0.635
	AA+AG vs. GG	Fixed	13.49	0.325	1.022	0.935-1.117	0.475	0.635	0.734	0.762
	AA vs. AG+GG	Fixed	0.00	0.649	1.018	0.855-1.212	0.203	0.839	1.000	0.680

PCR-RFLP, PCR-restriction fragment length polymorphism; HWE, Hardy–Weinberg equilibrium.

significant association between hMLH1 -93G>A polymorphism and the risk of CRC.

To evaluate the potential effects of specific study characteristics on the association between hMLH1 -93G>A polymorphism and CRC risk; we pooled the ORs and 95% CIs by the subgroups analysis of ethnicity, control source, and genotyping technique. When stratified by ethnicity, significant association between hMLH1 -93G>A polymorphism and CRC risk was detected among the Asian population under the homozygote model (OR = 2.283, 95% CI 1.810-2.880, P < 0.001), but not among Caucasians. Furthermore, no significant associations were detected when the studies were stratified based on the source of control subjects and genotyping method (Table 2).

hMLH1 655A>G (rs1799977) Polymorphism

The main results of the meta-analysis for all eleven case-control studies [17,23,27,34-41] on hMLH1 655A>G polymorphism are presented in Table 4. The results of pooling all studies showed that there was a significant association between hMLH1 655A>G polymorphism and the risk of CRC under the heterozygote (OR = 1.493, 95% CI 1.147-1.944, P = 0.865, P = 0.003), dominant (OR = 1.298, 95% CI 1.085-1.553, P = 0.004) and recessive (OR = 1.150, 95% CI 1.020-1.297, P = 0.022) models.

In the subgroup analysis by ethnicity, we found a significant association between the hMLH1 655A>G polymorphism and the risk of CRC in Asians under the allele (OR = 2.251, 95% CI 1.758-2.884, P < 0.001), homozygote (OR = 10.262, 95% CI 6.419-16.405, P < 0.001), dominant (OR = 2.411, 95% CI 1.663-3.495, P < 0.001) and recessive (OR = 1.660, 95% CI 1.155-2.385, P < 0.001) models with a fixed effect, whereas there was no significant association in any of the genetic models with a random effect models in Caucasians. When stratified by source of controls, significant association between hMLH1 655A>G polymorphism and CRC risk was observed in hospital-based controls in the allele (OR = 2.153, 95% CI 1.763-2.628, P≤0.001), homozygote (OR = 5.873, 95% CI 1.911-18.04, P=0.036), heterozygote (OR = 2.955, 95% CI 1.111-7.859, P=0.036), dominant (OR = 2.513, 95% CI 1.876-3.367, P≤0.001), and recessive (OR = 1.671, 95% CI 1.216-2.297, P=0.036) models, but not in the in population-based controls. Furthermore, hMLH1 655A>G polymorphism was significantly associated with increased CRC risk in the subgroup of PCR-RFLP genotyping method in the allele model (OR = 1.725, 95% CI 1.038-2.866, P=0.036), dominant (OR = 1.961, 95% CI 0.999-3.847, P=0.05) and recessive (OR = 1.366, 95% CI 1.133-1.647, P=0.001) models. In contrast, no significant association was observed in TaqMan genotyping subgroup (Table 3).

hMLH1 1151T>A (rs63750447) Polymorphism

The main results of the meta-analysis for all case-control eight studies [34,35,41-46] on hMLH1 1151T>A polymorphism are presented in Table 5. Significant association between hMLH1 1151T>A polymorphism and CRC was observed in the allele (OR = 2.462, 95% CI 1.763-2.628, P≤0.001), homozygote (OR = 2.501, 95% CI 1.593-3.806, P≤0.001) and dominant (OR = 2.526, 95% CI 1.622-3.934, P≤0.001) models (Table 3).

Sensitivity analysis

Sensitivity analysis was conducted by deleting each study in turn from the pooled analysis to examine the stability of the results. However, no individual study changed the pooled OR qualitatively, indicating that the pooled results were statistically robust.

Publication bias

We have assessed publication bias qualitatively by Begg's funnel plot and quantitatively by Egger's test. The shapes of the funnel plot did not indicate any evidence of obvious asymmetry in all genotypes in overall population. However, the results of Egger's test statistically confirmed the evidence of publication bias in the dominant model for hMLH1 655A>G polymorphism (PBeggs= 0.146, PEggers=0.021). Therefore, we have used the Duval and Tweedie non-parametric "trim and fill" method to adjust for publication bias. However, meta-analysis with and without "trim and fill" did not draw different conclusion, indicating that our results were statistically robust. Moreover, neither Begg's funnel plot nor Egger's test detected obvious evidence of publication bias in subgroup analysis based on ethnicity, source of controls and genotyping methods by using of Begg's and Egger's test.

DISCUSSION/CONCLUSION

An increasing number of studies on genetic association studies, genome-wide association studies (GWASs), and relate meta-analyses have been published to clarify the association between gene polymorphisms and CRC.[32,33,41] Theoretically, polymorphisms in the hMLH1 gene could change the function of this gene, disturb the DNA repair and increase risk of CRC.[32,33] The role of hMLH1 polymorphisms in the risk of CRC is controversial. The association between hMLH1 gene polymorphisms and risk of CRC has been a topic of particular interest, but the results from individual studies had been inconsistent and controversial. To better define the possible association, we carried out a comprehensive meta-analysis of hMLH1 polymorphisms.

Overall, our meta-analysis indicates that 655A>G and 1151T>A polymorphisms are associated with increased CRC risk when all eligible studies were pooled into the meta-analysis, whereas -93G>A polymorphism was not significantly associated with CRC risk. In further stratified, significantly increased

TABLE 3. THE META-ANALYSIS OF HMLH1 655A>G AND 1151T>A POLYMORPHISMS AND CRC RISK.

subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I2 (%)	PH	OR	95% CI	Ztest	POR	PBeggs	PEggers
655A>G										
Overall (n=12)	A vs. G	Random	98.36	≤0.001	1.101	0.638-1.901	0.344	0.731	0.303	0.755
	AA vs. GG	Random	90.65	≤0.001	1.562	0.919-2.655	1.647	0.099	0.350	0.547
	AG vs. GG	Random	87.70	≤0.001	1.493	1.147-1.944	2.979	0.003	0.086	0.054
	AA+AG vs. GG	Random	73.65	≤0.001	1.298	1.085-1.553	2.853	0.004	0.146	0.021
	AA vs. AG+GG	Fixed	19.33	0.260	1.150	1.020-1.297	2.289	0.022	0.640	0.414
By ethnicity										
Caucasians (n=6)	A vs. G	Random	99.15	≤0.001	0.713	0.335-1.518	-0.878	0.380	0.452	0.645
	AA vs. GG	Random	75.85	0.001	1.036	0.770-1.394	0.235	0.814	1.000	0.690
	AG vs. GG	Random	64.92	0.014	1.079	0.931-1.251	1.015	0.310	0.452	0.191
	AA+AG vs. GG	Random	66.83	0.010	1.086	0.940-1.255	1.177	0.264	0.707	0.280
	AA vs. AG+GG	Fixed	0.00	0.420	1.095	0.964-1.243	1.397	0.162	0.452	0.573
Asians (n=4)	A vs. G	Fixed	7.33	0.356	2.251	1.758-2.884	6.425	0.00	0.734	0.381
	AA vs. GG	Fixed	12.08	0.321	10.262	6.419-16.405	9.727	0.00	0.296	0.282
	AG vs. GG	Random	88.34	≤0.001	2.793	0.794-9.818	1.601	0.109	0.734	0.226
	AA+AG vs. GG	Fixed	39.39	0.175	2.411	1.663-3.495	4.644	0.00	0.734	0.352
	AA vs. AG+GG	Fixed	0.00	0.760	1.660	1.155-2.385	2.736	0.006	1.000	0.762
By Population-Based										
PB (n=7)	A vs. G	Random	98.79	≤0.001	0.807	0.411-1.586	-0.622	0.534	0.107	0.797
	AA vs. GG	Random	61.27	0.017	0.960	0.738-1.248	-0.306	0.760	0.548	0.392
	AG vs. GG	Fixed	0.799	0.423	1.037	0.959-1.121	0.901	0.367	0.265	0.150
	AA+AG vs. GG	Fixed	0.00	0.479	1.047	0.971-1.127	1.198	0.231	0.386	0.265
	AA vs. AG+GG	Fixed	0.00	0.677	1.081	0.950-1.230	1.182	0.237	0.367	0.365
HB (n=4)	A vs. G	Fixed	0.00	0.591	2.153	1.763-2.628	7.536	≤0.001	0.734	0.530
	AA vs. GG	Random	74.01	0.009	5.873	1.911-18.04	3.090	0.002	0.734	0.794
	AG vs. GG	Random	89.30	≤0.001	2.955	1.111-7.859	2.171	0.030	0.734	0.414
	AA+AG vs. GG	Fixed	0.00	0.415	2.513	1.876-3.367	6.176	≤0.001	0.308	0.166
	AA vs. AG+GG	Fixed	0.00	0.531	1.671	1.216-2.297	3.168	0.002	0.734	0.145
By Genotyping Technique										
PCR-RFLP (n=4)	A vs. G	Random	92.46	≤0.001	1.725	1.038-2.866	2.103	0.036	1.000	0.158
	AA vs. GG	Random	95.47	≤0.001	3.821	0.963-15.152	1.907	0.057	0.734	0.454
	AG vs. GG	Random	96.27	≤0.001	2.556	0.828-7.892	1.632	0.103	0.734	0.213
	AA+AG vs. GG	Random	90.74	≤0.001	1.961	0.999-3.847	1.958	0.050	1.000	0.083
	AA vs. AG+GG	Fixed	21.29	0.283	1.366	1.133-1.647	3.273	0.001	0.308	0.127
TaqMan (n=3)	A vs. G	Fixed	0.00	0.874	0.955	0.853-1.069	-0.797	0.425	1.000	0.876
	AA vs. GG	Random	77.78	0.011	0.660	0.285-1.530	-0.968	0.333	1.000	0.789
	AG vs. GG	Fixed	0.00	0.742	0.952	0.816-1.110	-0.633	0.527	0.296	0.261
	AA+AG vs. GG	Fixed	0.00	0.902	0.945	0.816-1.094	-0.760	0.447	0.296	0.037
	AA vs. AG+GG	Fixed	0.00	0.484	0.950	0.732-1.231	-0.390	0.696	1.000	0.780
1151T>A										
Overall (n=8)	A vs. G	Fixed	41.92	0.099	2.462	2.350-1.635	3.378	≤0.001	0.063	0.013
	AA vs. GG	Fixed	0.00	0.535	3.189	0.1-30.756	1.003	0.316	NA	NA
	AG vs. GG	Fixed	38.80	0.121	2.416	1.669-3.496	4.678	≤0.001	0.063	0.011
	AA+AG vs. GG	Fixed	41.31	0.103	2.654	1.610-4.375	3.828	≤0.001	0.220	0.055
	AA vs. AG+GG	Fixed	0.00	0.546	2.990	0.310-28.834	0.947	0.343	NA	NA

CRC risk was observed in Asians for -93G>A and 655A>G polymorphisms, but not in Caucasians. It should be considered that the apparent inconsistency of these results may underlie differences in population background, source of controls, lifestyle, disease prevalence, sample size, and also by chance as well as possible limitations due to the relatively small sample size. The current available data support the multifactorial nature of CRC, and both genetic and environmental factors play an important role in development of CRC. Thus, it is unlikely that the same gene polymorphisms may play different roles in cancer susceptibility, because cancer is a complicated multi-genetic disease, and different genetic backgrounds may contribute to the discrepancy.

Present meta-analysis results were not consistent with a previous meta-analysis on MLH1 -93G>A and 655A>G polymorphisms with CRC risk.[51,52] In 2012, Wang et al. included six case–control studies with 17,791 cases and 13,782 controls on MLH1 -93G>A polymorphism. Their results suggested that MLH1 -93G>A polymorphism was associated with increased risk of CRC under the heterozygote (OR= 1.06, 95% CI =1.01–1.11), and the dominant (OR = 1.06, 95% CI = 1.01–1.11) models.[51] In the more recently meta-analysis, Chen et al. included 13 case–control studies on hMLH1 -93G>A, nine studies on 655A>G, and seven studies on 1151T>A. They have reported that there is a significant association between hMLH1 1151T>A polymorphism and CRC risk, but not with hMLH1 655A>G and -93G>A polymorphisms. Additionally, they have found similar results by subgroup analyses according to quality score and genotyping methods.[52] However, their meta-analysis might be generated conflicting results, which had insufficient power in the meta-analysis because the number of studies was considerably smaller than that needed for the achievement of robust conclusions. In addition, due to small size meta-analysis, they could not rule out the possibility of publication bias. With more studies about hMLH1 polymorphisms and CRC have available recently, our updated meta-analysis, which has the largest sample size thus reported, we found that the 655A>G and 1151T>A polymorphisms were associated with risk of CRC. Moreover, we found that they wrongly calculated HWE test for both cases and controls in their meta-analysis. Therefore, cumulative meta-analyses have suggested that no significant association was observed between hMLH1 polymorphisms and CRC, as evidence accumulated by time.

Heterogeneity is a potential problem when interpreting the results for most meta-analyses, and finding out the sources of heterogeneity is one of important goals of meta-analyses.[53-55] In the present meta-analysis there was obvious between-study heterogeneity.[54,55] Three subgroup analyses were conducted by ethnicity, control source, and smoking status and the heterogeneity still existed. Despite some diversity in the studies about designs, sample sizes, inclusion criteria, and ethnicity, significant heterogeneity between studies was only observed for the -93G>A and 655A>G polymorphism. Thus, we performed subgroup analyses by ethnicities, genotyping methods and source of controls to explore the sources of heterogeneity. The results showed that the heterogeneity disappeared or decreased in several subgroups but remained in other subgroups, suggesting that other covariates might confound the association.

The limitations of this meta-analysis should not be ignored when interpreting the results. First, the meta-analysis was limited by the relatively small number of eligible studies for 1151T>A polymorphism, which may fail to provide enough statistical power to detect a possible or weak effect of the polymorphism on CRC and limited our ability to perform subgroup analyses. Second, only articles published in English or Chinese were selected, potentially causing a language bias. Third, our analysis was limited to Asian and Caucasian ethnicities, and it is uncertain whether these results are generalizable to other ethnicities. Forth, there was significant between-study heterogeneity for two genes in the overall and Caucasians. In addition, the unknown factors including lifestyles and environments may account for the heterogeneity in study results and the lack of significant findings in the overall and Caucasian populations. Fifth, some studies were hospital-based, while others were population-based. Thus, selection bias might exist. Finally, CRC is a multifactorial disease that results from complex interactions between various genes and environmental factors. Our results were based on unadjusted estimates; data were not stratified by other main confounding variables such as age, gender, lifestyle, diet, major systemic illness etc., because sufficient information was not available from those studies.

In summary, the study inconsistent with the previous meta-analyses suggested that hMLH1 -93G>A and 1151T>A polymorphisms may be associated with

the risk of CRC. Moreover, the -93G>A polymorphism is associated with the susceptibility of CRC in Asian population. However, to ascertain a definitive conclusion on hMLH1 1151T>A polymorphism, well-designed epidemiologic studies with larger sample size and more ethnic groups are suggested to further clarify the association. Moreover, gene-gene and gene-environment interactions studies should also be considered in future studies.

Conflict of interest

The authors declare no conflict of interest.

RESUMO

OBJETIVO: *Tem havido crescente interesse no estudo da associação entre polimorfismos do gene mutL homólogo 1 humano (hMLH1) e risco de câncer colorretal (CRC). No entanto, os resultados de estudos anteriores não são conclusivos. Assim, uma meta-análise foi conduzida para obter uma estimativa mais precisa dos efeitos desse gene.*

MÉTODOS: *Uma pesquisa abrangente foi realizada nas bases de dados PubMed, Embase, Chinese Biomedical Literature até 1º de janeiro de 2018. Odds ratio (OR) com 95% de intervalo de confiança (IC) foi utilizado para avaliar a força da associação.*

RESULTADOS: *Finalmente, foram identificados 38 estudos de casos e controles em 32 publicações, atendendo aos nossos critérios de inclusão. Houve 14 estudos com 20.668 casos e 19.533 controles em hMLH1 -93G>A, 11 estudos com 5.786 casos e 8.867 controles em 655A>G e cinco estudos com 1.409 casos e 1.637 controles em 1151T>Um polimorfismo. Os resultados combinados mostraram que os polimorfismos 655A>G e 1151T>A estavam significativamente associados ao risco de CRC, enquanto que o polimorfismo -93G>A não estava significativamente associado ao risco de CRC. Quanto à etnia, os polimorfismos de -93G>A e 655A>G foram associados ao risco aumentado de CRC entre os asiáticos, mas não entre os caucasianos. Mais interessante, a análise de subgrupos indicou que 655A>G pode aumentar o risco de CRC em subgrupos PCR-RFLP e HB.*

CONCLUSÃO: *Inconsistente com a meta-análise anterior, esta meta-análise mostra que os polimorfismos hMLH1 655A>G e 1151T>A podem ser fatores de risco para CRC. Além disso, o polimorfismo -93G>A está associado à susceptibilidade do CRC na população asiática.*

PALAVRAS-CHAVE: *Câncer colorretal. hMLH1. Polimorfismo. Meta-análise.*

REFERENCES

1. Khoram-Abadi KM, Forat-Yazdi M, Kheirandish S, Saeidi N, Zarezade Z, Mehrabi N, et al. Asian Pac J Cancer Prev. 2016;17(6):3015-20.
2. Forat-Yazdi M, Gholi-Nataj M, Neamatzadeh H, Nourbakhsh P, Shaker-Ardakani H. Association of XRCC1 Arg399Gln Polymorphism with Colorectal Cancer Risk: A Huge Meta-Analysis of 35 Studies. Asian Pac J Cancer Prev. 2015;16(8):3285-91.
3. Namazi A, Abedinzadeh M, Nourbaksh P, Neamatzadeh H. Association between the XRCC3 Thr241Met polymorphism and risk of colorectal cancer: a meta-analysis of 5,193 cases and 6,645 controls. Asian Pac J Cancer Prev. 2015;16(6):2263-8.
4. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics. CA Cancer J Clin. 2010;60(5):277–300.
5. Xie M, Zhao F, Zou X, Jin S, Xiong S. The association between CCND1 G870A polymorphism and colorectal cancer risk: A meta-analysis. Medicine (Baltimore). 2017;96(42):e8269.
6. Wang L, Qian J, Ying C, Zhuang Y, Shang X, Xu F. X-ray cross-complementing groups 1 rs1799782 C>T polymorphisms and colorectal cancer susceptibility: A meta-analysis based on Chinese Han population. J Cancer Res Ther. 2016;12(Supplement):C264-C267.
7. Kheirelseid EA, Miller N, Chang KH, Curran C, Hennessey E, Sheehan M, et al. Mismatch repair protein expression in colorectal cancer. J Gastrointest Oncol. 2013;4(4):397-408.
8. Hsieh P, Yamane K. DNA mismatch repair: Molecular mechanism, cancer, and ageing. Mech Ageing Dev. 2008;129(7-8):391-407.
9. Broustas CG, Lieberman HB. DNA Damage Response Genes and the Development of Cancer Metastasis. Radiat Res. 2014;181(2):111-130.
10. Ricker CN, Hanna DL, Peng C, Nguyen NT, Stern MC, Schmit SL, et al. DNA mismatch repair deficiency and hereditary syndromes in Latino patients with colorectal cancer. Cancer. 2017;123(19):3732-3743.
11. Ellison AR, Lofing J, Bitter GA. Human MutL homolog (MLH1) function in DNA mismatch repair: a prospective screen for missense mutations in the ATPase domain. Nucleic Acids Res. 2004;32(18):5321-5338.
12. Peltomäki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. J Clin Oncol. 2003;21(6):1174-9.
13. Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la Chapelle A, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer. 1999; 81(2):214-218.
14. Millar AL, Pal T, Madlensky L, Sherman C, Temple L, Mitri A, et al. Mismatch repair gene defects contribute to the genetic basis of double primary cancers of the colorectum and endometrium. Hum Mol Genet. 1999; 8(5):823-829.
15. Ito E, Yanagisawa Y, Iwahashi Y, Suzuki Y, Nagasaki H, Akiyama Y, et al. A core promoter and a frequent single-nucleotide polymorphism of the mismatch repair gene hMLH1. BiochemBiophys Res Commun. 1999;256(3):488-494.
16. Shin KH, Shin JH, Kim JH, Park JG. Mutational analysis of promoters of mismatch repair genes hMSH2 and hMLH1 in hereditary nonpolyposis colorectal cancer and early onset colorectal cancer patients: identification of three novel germ-line mutations in promoter of the hMSH2 gene. Cancer Res. 2002;62(1):38-42.
17. Raptis S, Mrkonjic M, Green RC, Pethe VV, Monga N, Chan YM, et al. MLH1 -93G>A promoter polymorphism and the risk of microsatellite-unstable colorectal cancer. J Natl Cancer Inst. 2007;99(6):463-474.
18. Chen H, Taylor NP, Sotamaa KM, Mutch DG, Powell MA, Schmidt AP, et al. Evidence for heritable predisposition to epigenetic silencing of MLH1. Int J Cancer. 2007;120(8):1684-1688.
19. Tulupova E, Kumar R, Hanova M, Slyskova J, Pardini B, Polakova V, et al. Do polymorphisms and haplotypes of mismatch repair genes modulate risk of sporadic colorectal cancer? Mutat Resch. 2008;648(1-2):40-45.
20. Samowitz WS, Curtin K, Wolff RK, Albertsen H, Sweeney C, Caan BJ, et al. The MLH1 -93 G>A promoter polymorphism and genetic and epigenetic alterations in colon cancer. Genes Chromosomes Cancer. 2008; 47(10):835-844.
21. Koessler T, Oestergaard MZ, Song H, Tyrer J, Perkins B, Dunning AM, et al. Common variants in mismatch repair genes and risk of colorectal cancer. Gut. 2008; 57(8):1097-1101.

22. Allan JM, Shorto J, Adlard J, Bury J, Coggins R, George R, et al. UK NCRI Colorectal Clinical Studies Group; Colorectal Cancer Study Group. MLH1 -93G>A promoter polymorphism and risk of mismatch repair deficient colorectal cancer. Int J Cancer. 2008;123(10):2456-9.

23. Campbell PT, Curtin K, Ulrich CM, Samowitz WS, Bigler J, Velicer CM, et al. Mismatch repair polymorphisms and risk of colon cancer, tumour microsatellite instability and interactions with lifestyle factors. Gut. 2009;58(5):661-667.

24. van Roon EH, van Puijenbroek M, Middeldorp A, van Eijk R, de Meijer EJ, Erasmus D, et al. Early onset MSI-H colon cancer with MLH1 promoter methylation, is there a genetic predisposition? BMC Cancer. 2010;10:180.

25. Whiffin N, Broderick P, Lubbe SJ, Pittman AM, Penegar S, Chandler I, et al. MLH1-93G > A is a risk factor for MSI colorectal cancer. Carcinogenesis. 2011;32(8):1157-1161.

26. Savio AJ, Lemire M, Mrkonjic M, Gallinger S, Zanke BW, Hudson TJ, et al. MLH1 region polymorphisms show a significant association with CpG island shore methylation in a large cohort of healthy individuals. PLoS One. 2012;7(12):e51531.

27. Muniz-Mendoza R, Ayala-Madrigal ML, Partida-Perez M, Peregrina-Sandoval J, Leal-Ugarte E, Macias-Gomez N, et al. MLH1 and XRCC1 polymorphisms in Mexican patients with colorectal cancer. Genet Mol Res. 2012; 11(3):2315-2320.

28. Nizam ZM, Abdul Aziz AA, Kaur G, Abu Hassan MR, Mohd Sidek AS, Lee YY, et al. Contribution of the MLH1 -93G>a promoter polymorphism in modulating susceptibility risk in Malaysian colorectal cancer patients. Asian Pac J Cancer Prev. 2013;14(2):619-624.

29. Martinez-Uruena N, Macias L, Perez-Cabornero L, Infante M, Lastra E, Cruz JJ, et al. Incidence of -93 MLH1 promoter polymorphism in familial and sporadic colorectal cancer. Colorectal Dis. 2013; 15(3):e118-123.

30. Djansugurova L, Zhunussova G, Khussainova E, Iksan O, Afonin G, Kaidarova D, et al. Association of DCC, MLH1, GSTT1, GSTM1, and TP53 gene polymorphisms with colorectal cancer in Kazakhstan. Tumour Biol. 2015;36(1):279-289.

31. Li G, Hu F, Yuan F, Fan J, Yu Z, Wu Z, et al. Intronic and promoter polymorphisms of hMLH1/hMSH2 and colorectal cancer risk in Heilongjiang Province of China. J Cancer Res Clin Oncol. 2015;141(8):1393-404.

32. Zhang LL, Tang XJ, Wang XY, Zhu YW, Peng XB, Gong L. A promoter polymorphism in the hMLH1 gene (-93G/A) associated with sporadic colorectal cancer. Oncol Lett. 2016; 12(5):4035-4040.

33. Mik M, Dziki L, Malinowska K, Trzcinski R, Majsterek I, Dziki A. Polymorphism of MSH2 Gly322Asp and MLH1 -93G>A in non-familial colon cancer - a case-controlled study. Arch Med Sci. 2017;13(6):1295-1302.

34. Kim JC, Roh SA, Koo KH, Ka IH, Kim HC, Yu CS, et al. Genotyping possible polymorphic variants of human mismatch repair genes in healthy Korean individuals and sporadic colorectal cancer patients. Fam Cancer. 2004;3(2):129-137.

35. Mei Q, Yan HL, Ding FX, Xue G, Huang JJ, Wang YZ, et al. Single-nucleotide polymorphisms of mismatch repair genes in healthy Chinese individuals and sporadic colorectal cancer patients. Cancer Genet Cytogenet. 2006; 171(1):17-23.

36. Berndt SI, Platz EA, Fallin MD, Thuita LW, Hoffman SC, Helzlsouer KJ. Mismatch repair polymorphisms and the risk of colorectal cancer. Int J Cancer. 2007;120(7):1548-1554.

37. Christensen LL, Madsen BE, Wikman FP, Wiuf C, Koed K, Tjonneland A, et al. The association between genetic variants in hMLH1 and hMSH2 and the development of sporadic colorectal cancer in the Danish population. BMC Med Genet. 2008;9:52.

38. Nejda N, Iglesias D, Moreno Azcoita M, Medina Arana V, Gonzalez-Aguilera JJ, et al. A MLH1 polymorphism that increases cancer risk is associated with better outcome in sporadic colorectal cancer. Cancer Genet Cytogenet. 2009;193(2):71-77.

39. Picelli S, Zajac P, Zhou XL, Edler D, Lenander C, Dalen J, et al. Common variants in human CRC genes as low-risk alleles. Eur J Cancer. 2010;46(6):1041-1048.

40. Milanizadeh S, Khanyaghma M, Haghighi MM, Mohebbi S, Damavand B, Almasi S, et al. Molecular analysis of imperative polymorphisms of MLH1 gene in sporadic colorectal cancer. Cancer Biomark. 2013;13(6):427-432.

41. Peng HX, Xu X, Yang R, Chu YM, Yang DM, Xu Y, et al.Molecular analysis of MLH1 variants in Chinese sporadic colorectal cancer patients. Genet Mol Res. 2016;15(2).

42. Wang Y, Friedl W, Propping P, Li J, Li Z, Wang J. Val384Asp in hMLH1 gene in Chinese, Japanese and German and its etiological role in colorectal cancer. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 1998;15(5):263-6.

43. Wang Y, Zhou J, Li Z, Wang J, Li J, Gao C, et al. One of the etiological factors of digestive tract cancers in Chinese: the missense mutation Val- 384Asp in the hMLH1 gene. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2000;17(2):82–86.

44. Zhang XM, Li JT, Zhu M, Wu XL, Gao P, Zhou P, et al. Study on the relationship between genetic polymorphism Val384Asp in hMLH1 gene and the risk of four different carcinomas. [Article in Chinese]. Zhonghua Liu Xing Bing Xue Za Zhi. 2004;25(11):978-981.

45. Ohsawa T, Sahara T, Muramatsu S, Nishimura Y, Yathuoka T, Tanaka Y, et al. Colorectal cancer susceptibility associated with the hMLH1 V384D variant. Mol Med Rep. 2009;2(6):887-891.

46. Wang D, Song L, Zhang X. Etiological role of Val384Asp in hMLH1 gene in familial colorectal cancer. Acta Univ Med Nanjing. 2010; 1:1–6.

47. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539-58.

48. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177-188.

49. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629-634.

50. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50(4):1088-1101.

51. Wang T, Liu Y, Sima L, Shi L, Wang Z, Ni C, et al. Association between MLH1 -93G>a polymorphism and risk of colorectal cancer. PLoS One. 2012;7(11):e50449.

52. Chen H, Shen Z, Hu Y, Xiao Q, Bei D, Shen X, et al. Association between MutL homolog 1 polymorphisms and the risk of colorectal cancer: a meta-analysis. J Cancer Res Clin Oncol. 2015 Dec;141(12):2147-58.

53. Sobhan MR, Mehdinejad M, Jamaladini MH, Mazaheri M, Zare-Shehneh M, Neamatzadeh H. Association between aspartic acid repeat polymorphism of the asporin gene and risk of knee osteoarthritis: A systematic review and meta-analysis. Acta Orthop Traumatol Turc. 2017;51(5):409-415.

54. Mehdinejad M, Sobhan MR, Mazaheri M, Zare-Shehneh M, Neamatzadeh H, et al. Genetic Association between ERCC2, NBN, RAD51 Gene Variants and Osteosarcoma Risk: a Systematic Review and Meta-Analysis. Asian Pac J Cancer Prev. 2017;18(5):1315-1321.

55. Sobhan MR, Forat-Yazdi M, Mazaheri M, Zare-Shehneh M, Neamatzadeh H. Association between the DNA Repair Gene XRCC3 rs861539 Polymorphism and Risk of Osteosarcoma: a Systematic Review and Meta-Analysis. Asian Pac J Cancer Prev. 2017;18(2):549-555.