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Meta-analysis of CHEK2 1100delC variant and colorectal cancer susceptibility

He-ping Xiang [a], *Xiao-ping Geng* [b,*], *Wei-wei Ge* [a], *He Li* [a]

[a] *Emergency Department of The Second Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China*
[b] *Organ Transplantation Center of The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China*

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ABSTRACT

Cell cycle checkpoint kinase 2 (CHEK2) gene has been inconsistently associated with colorectal cancer (CRC), particularly the 1100delC variant. To generate large-scale evidence on whether the CHEK2 1100delC variant is associated with CRC susceptibility we have conducted a meta-analysis. Data were collected from the following electronic databases: PubMed, Excerpta Medica Database and Chinese Biomedical Literature Database, with the last report up to November 2010. The odds ratio (OR) and its 95% confidence interval (95% CI) were used to assess the strength of association. We evaluated the contrast of carriers versus non-carriers. Meta-analysis was performed in a fixed/random effect model by using the software Review Manager 4.2. A total of six studies including 4194 cases and 10,010 controls based on the search criteria were involved in this meta-analysis. A significant association of the CHEK2 1100delC variant with unselected CRC was found (OR = 2.11, 95% CI = 1.41–3.16, $P = 0.0003$). We also found an association of the CHEK2 1100delC variant with familial CRC (OR = 2.80, 95% CI = 1.74–4.51, $P < 0.0001$). However, the association was not established for sporadic CRC (OR = 1.45, 95% CI = 0.49–4.30, $P = 0.50$). This meta-analysis demonstrates that the CHEK2 1100delC variant may be an important CRC-predisposing gene, which increases CRC risk.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer in the world, and there are approximately 1,000,000 new cases of CRC and 500,000 deaths associated with CRC each year.[1] In Europe and the United States, CRC represents one of the primary causes of cancer deaths.[1,2] In Asia, CRC is the fourth leading cause of mortality by cancer, and its incidence is increasing.[3] CRC is a serious problem for public health in many countries. Unfortunately, to date, mechanisms that lead to its development remain largely unknown. However, twin study has suggested that susceptibility to CRC is influenced by genetic factors.[4] The genetic contribution in CRC has been estimated to be ~35%. Studying the genetics of CRC has never been more exciting.

Cell cycle checkpoint kinase 2 (CHEK2) is a cell cycle checkpoint kinase involved in deoxyribonucleic acid (DNA) repair, cell death and cell cycle control by stabilising the p53 protein.[5] In 1999, Bell et al.[6] first described the possible association of the CHEK2 gene with Li-Fraumeni syndrome. Subsequently, multiple lines of evidence indicate that it may be a cancer susceptibility gene.[7] The protein truncating variant CHEK2 1100delC, present in exon 10 of the functional gene on chromosome 22q, abolishes the kinase function of CHEK2.[8] The role of the CHEK2 1100delC variant and other germline variants has been well studied in breast cancer. The frequency

* *Corresponding author:* Tel.: +86 105513869670; fax: +86 105515588328.
E-mail address: xp-geng@163.net (X.-p. Geng).
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of CHEK2 1100delC allele varies among different populations.[9] The 1100delC allele has been reported at frequencies of 1.2–1.9% among individuals with breast cancer.[10] A recent meta-analysis indicates that the 1100delC allele was a moderate penetrance susceptibility allele for breast cancer and carriers appear to have a 2–5-fold increase in breast cancer risk.[10] CHEK2 protein is abrogated or reduced to a large extent in breast tumours of heterozygous CHEK2 1100delC variant carriers.[11,12] Recently, considerable epidemiological studies have suggested a role for the CHEK2 1100delC variant in CRC susceptibility.[13–22] However, the association between this variant and CRC susceptibility is controversial. The inconsistent results might have resulted from relatively small sample sizes and differences in patient populations.

Meta-analysis is a statistical procedure for combining the results of several studies to produce a single estimate of the major effect with enhanced precision.[23] In the current study, we performed a meta-analysis to examine the association between the CHEK2 1100delC variant and CRC susceptibility.

2. Materials and methods

2.1. Identification of eligible studies

Case–control studies of the CHEK2 1100delC variant and CRC susceptibility published before November 2010 were identified through computer-based searches of PubMed, Excerpta Medica Database (Embase), and Chinese Biomedical Literature Database (CBM) by using the keywords 'CHEK2', 'CHK2', 'colorectal', 'colon' and 'rectum'. Additional studies were identified by a hand search of references of original studies and review articles on the association of CHEK2 variants with CRC susceptibility. A study was included in the current meta-analysis if it was a case–control study, and explored the association between the CHEK2 1100delC variant and CRC susceptibility. No language restrictions were applied. When there were multiple studies from the same population, only the largest study was included.

2.2. Search methods

Two investigators independently searched the electronic databases. An independent PubMed search was done (by Xiang HP and Geng XP) with the same method. An independent Embase search was done (by Ge WW and Li H) with the same method. An independent CBM search was done (by Ge WW and Li H) with the same method. The abstracts were reviewed independently by two investigators (Xiang HP and Geng XP) to determine if they met eligibility criteria for inclusion. References in the studies were reviewed (by Ge WW and Li H) to identify additional studies. Where discrepancies occurred, a third investigator (Geng XP) did additional assessment.

2.3. Data extraction

Information was carefully extracted from all eligible publications independently by two of the authors (Xiang HP and Ge WW) according to the inclusion criteria, and the result was reviewed by a third investigator (Geng XP). Discrepancies were resolved by discussion with our research team. From each study, we extracted the first author's name, year of publication, source of publication, racial ancestry of subjects, types of CRC, the number of cases and controls, and the frequencies of genotypes. If original data were unavailable in articles, a request for original data was sent to the corresponding author.

2.4. Meta-analysis methods

We classified CRC as unselected CRC (cases were unselected for family history of CRC), familial CRC (two or more first degree relatives are diagnosed with CRC in the same family), and sporadic CRC (non-familial CRC). Meta-analysis was performed for unselected CRC, familial CRC, and sporadic CRC. We evaluated the contrast of carriers versus non-carriers. Odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of the association. The heterogeneity between the studies was assessed by the Chi square-test based Q-statistic.[24] A significant Q-statistic ($P < 0.10$) indicated heterogeneity across studies. Meanwhile, we measured the effect of heterogeneity by another measure, $I^2 = 100\% \times (Q - df)/Q$.[25] The pooled OR was calculated by a fixed effect model (using the Mantel–Haenszel method) or a random effect model (using the DerSimonian–Laird method) according to the heterogeneity among studies.[26,27] The significance of the pooled OR was determined by the Z-test. Publication bias was observed with the funnel plot. Funnel plot asymmetry was further assessed by the method of Egger's linear regression test.[28]

Statistical analyses were performed using the software Review Manager (v.4.2; Cochrane Collaboration, Oxford, England) and Stata (v.10.1; Stata Corporation, College Station, TX, USA). A P value less than 0.05 was considered statistically significant, and all the P values were two sided.

3. Results

3.1. Characteristics of eligible studies

Characteristics of studies investigating the association of the CHEK2 1100delC variant with CRC susceptibility are presented in Table 1.[13–18] The study selection process is shown in Fig. 1. The search identified 228 articles (Pubmed: 81; Embase: 101; CBM: 46). Of these, 14 case–control studies examined the association of the CHEK2 variants with CRC susceptibility.[13–22,29–32] However, eight studies were excluded (four was duplicate report,[19–22] five did not explore the CHEK2 1100delC variant[29–32]). Finally, six studies[13–18] were included in the current meta-analysis. In these studies, we identified six studies of unselected CRC,[13–18] four of familial CRC[15–18] and two of sporadic CRC.[17,18]

3.2. Meta-analysis

The results of the meta-analysis and the heterogeneity test are shown in Table 2.

3.2.1. Unselected CRC

Six studies (4194 cases and 10,010 controls) examining the association between the CHEK2 1100delC variant and unselected CRC were included. No significant heterogeneity was

Table 1 – Characteristics of studies on CHEK2 1100delC variant and CRC susceptibility.[a]

ID	Study	Year	Ethnic group	Case ascertainment	Overall sample size		Carriers *n* (frequency of carriers, %)	
					Case	Control	Case	Control
Unselected cases								
1	Suchy et al.[13]	2010	Caucasian	Hospital-based	1085	5496	5(0.46)	12(0.22)
2	Kleibl et al.[14]	2009	Caucasian	Hospital-based	631	730	4(0.64)	2(0.27)
3	Wasielewski et al.[15]	2008	Caucasian	Population-based	369	909	10(2.71)	9(0.99)
4	Djureinovic et al.[16]	2006	Caucasian	Population-based/ hospital-based	818	760	8(0.98)	5(0.66)
5	de Jong et al.[17]	2005	Caucasian	Population-based	629	230	10(1.59)	1(0.43)
6	Kilpivaara et al.[18]	2003	Caucasian	Hospital-based	662	1885	17(2.57)	26(1.38)
Familial cases								
1	Wasielewski et al.[15]	2008	Caucasian	Population-based	237	909	10(4.22)	9(0.99)
2	Djureinovic et al.[16]	2006	Caucasian	Hospital-based	174	760	2(1.15)	5(0.66)
3	de Jong et al.[17]	2005	Caucasian	Population-based	126	230	4(3.17)	1(0.43)
4	Kilpivaara et al.[18]	2003	Caucasian	Hospital-based	149	1885	2(1.34)	26(1.38)
Sporadic cases								
1	de Jong et al.[17]	2005	Caucasian	Population-based	503	230	6(1.19)	1(0.43)
2	Kilpivaara et al.[18]	2003	Caucasian	Hospital-based	513	1885	15(2.92)	26(1.38)

[a] CRC: colorectal cancer; CHEK2: cell cycle checkpoint kinase 2; and *n*: number.

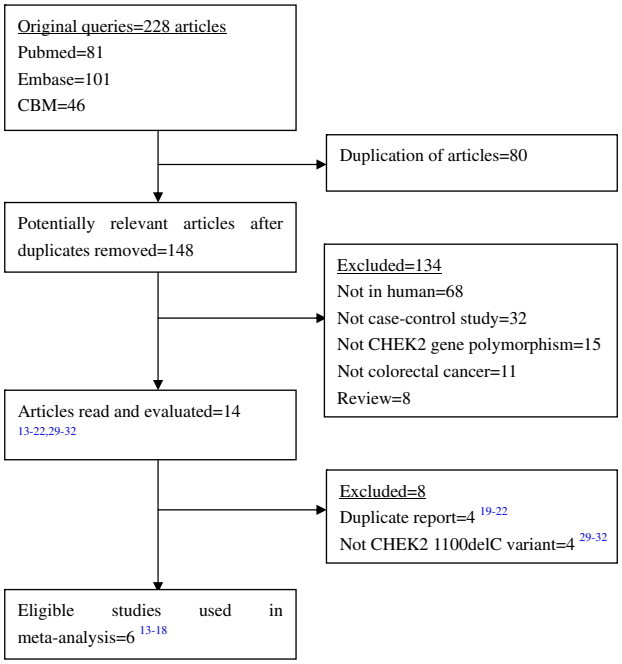

Fig. 1 – Flow diagram of the study selection process.

observed and the original data were combined by means of the fixed effect model. We found an association of the CHEK2 1100delC variant with unselected CRC (OR = 2.11, 95% CI = 1.41–3.16, $P = 0.0003$). The forest plot is shown in Fig. 2A. Fig. 3A showed that the distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot. Similarly, the Egger's test (Fig. 3B) provided no evidence of publication bias in six reviewed studies ($t = 0.93$, $P = 0.404$).

3.2.2. *Familial CRC*

Four studies (1050 cases and 3784 controls) examining the association between the CHEK2 1100delC variant and familial CRC were included. The Q-test of heterogeneity was not significant. By using the fixed effect model, we found an association of the CHEK2 1100delC variant with familial CRC (OR = 2.80, 95% CI = 1.74–4.51, $P < 0.0001$). The forest plot is shown in Fig. 2B. Fig. 3C showed that the distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot. Similarly, the Egger's test (Fig. 3D) provided no evidence of publication bias in four reviewed studies ($t = 0.68$, $P = 0.567$).

3.2.3. *Sporadic CRC*

Two studies (652 cases and 2115 controls) examining the association between the CHEK2 1100delC variant and sporadic

Table 2 – Meta-analysis of the risk of CRC for CHEK2 1100delC carriers versus non-carriers[a].

Diseases	Number of studies	Sample size		Test of heterogeneity			Test of association		
		Case	Control	χ^2	P-value	I^2 (%)	OR	95% CI	P-value
Unselected CRC	6	4194	10,010	1.15	0.95	0.0	2.11	1.41–3.16	0.0003
Familial CRC	4	1050	3784	2.67	0.45	0.0	2.80	1.74–4.51	<0.0001
Sporadic CRC	2	652	2115	0.65	0.42	0.0	1.45	0.49–4.30	0.50

[a] CRC: colorectal cancer; CHEK2: cell cycle checkpoint kinase 2; OR: odds ratio; and CI: confidence interval.

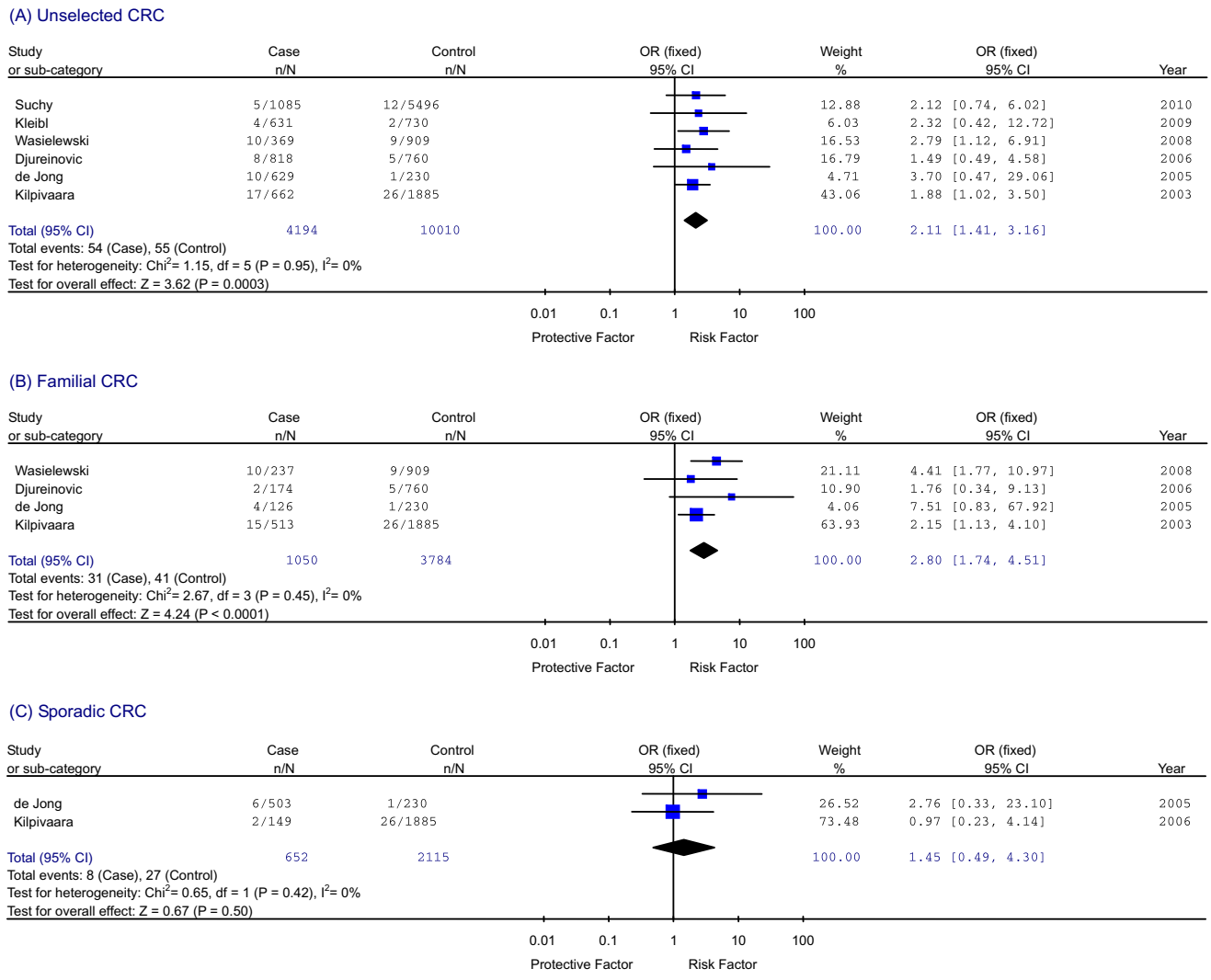

Fig. 2 – Meta-analysis of the risk of colorectal cancer (CRC) for cell cycle checkpoint kinase 2 (CHEK2) 1100delC carriers versus non-carriers.

CRC were included. The Q-test of heterogeneity was not significant and we conducted analysis using the fixed effect model. We did not detect an association of the CHEK2 1100delC variant with sporadic CRC (OR = 1.45, 95% CI = 0.49–4.30, $P = 0.50$). The forest plot is shown in Fig. 2C. However, publication bias was not assessed due to the small number of studies.

4. Discussion

The exact aetiology of CRC remains unclear. However, evidences have suggested an important role for genetics in determining risk for CRC.[4] Association studies are appropriate for searching susceptibility genes involved in CRC.[33] Unfortunately, analysing the results of association studies about the CHEK2 1100delC variant and CRC susceptibility, definite conclusions cannot be drawn. Some studies reported an increased risk of CRC,[15,18] whereas others reported no associations.[13,14,16,17] Therefore, we did a meta-analysis to estimate the association between the CHEK2 1100delC variant and susceptibility to CRC. To the best of our knowledge, this is the first meta-analysis that investigated the association. The present meta-analysis of six case–control studies including 4194 cases and 10,010 controls provides the most comprehensive assessment for the association between the CHEK2 1100delC variant and the risk of CRC. A significant association of the CHEK2 1100delC variant with unselected CRC was found, and carriers have 2.11-fold increase in CRC risk. We also found an association of the CHEK2 1100delC variant with familial CRC, and carriers have 2.80-fold increase in CRC risk.

Our results suggest that the CHEK2 1100delC variant could be a risk factor for CRC. CHEK2 encodes a serine/threonine-protein kinase which plays a critical role in DNA damage signalling pathways.[7] DNA damage results in activation of cell-cycle checkpoints that block cell proliferation and initiate DNA repair.[7] In early precursor lesions of some cancers (before genomic instability and malignant conversion), activated CHEK2 has been found, suggesting that DNA damage checkpoints are activated in the early stages of tumourigenesis.[34] Variants in CHEK2 may allow tumourigenic cells to evade normal cell cycle checkpoints, leading to aberrant cell proliferation and survival, increased genomic instability and

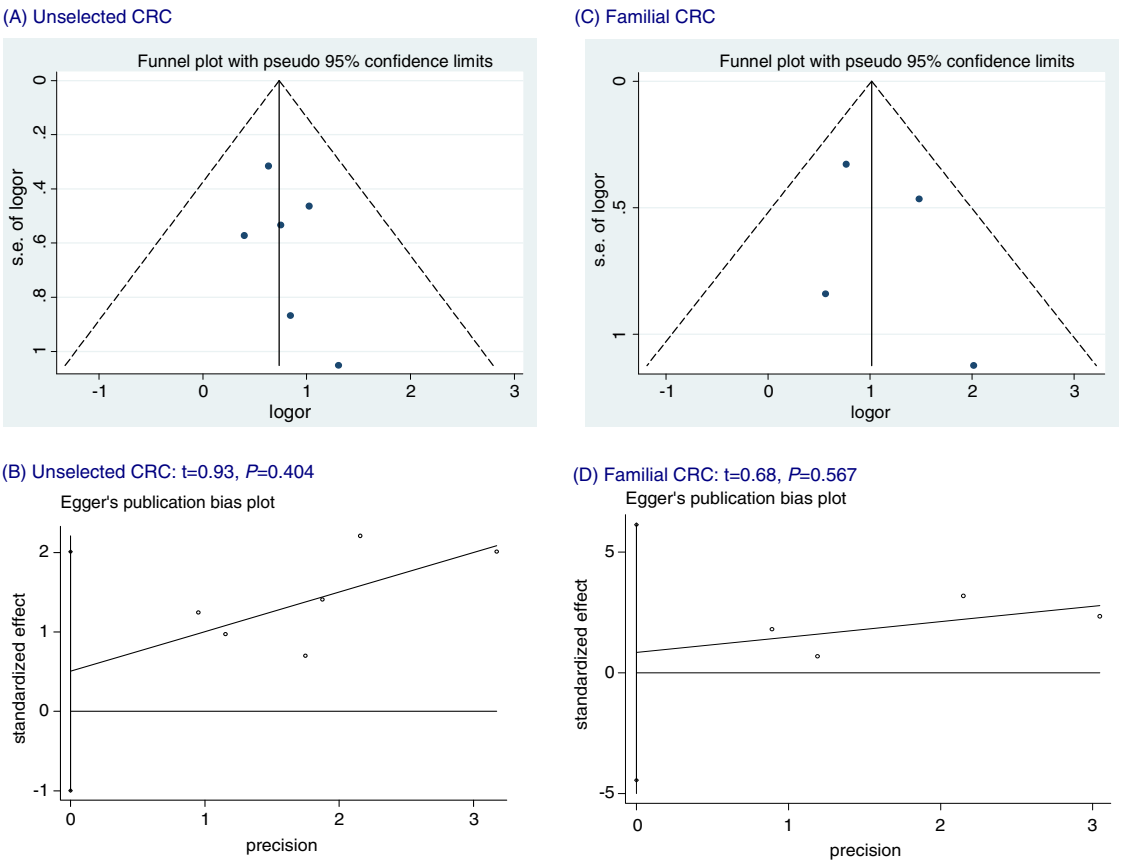

Fig. 3 – Funnel plots and Egger's publication bias plots for the associations.

ultimately tumour progression.[35] The first evidence suggesting that CHEK2 plays a critical role in cancer development was the germline variant CHEK2 1100delC found in several Li-Fraumeni and Li-Fraumeni-like families.[6,36] The CHEK2 1100delC variant is located in exon 10 of the functional gene on chromosome 22q.[8] It is caused by a deletion of a single cytosine at position 1100, resulting in the introduction of a stop codon after amino acid 380 and abolishes the kinase function of CHEK2.[6] Recently, the CHEK2 1100delC variant has also been associated with breast and prostate cancer.[11,37] These evidences seem to support our conclusion that the CHEK2 1100delC variant could be a risk factor for CRC. Of course, the association may also result from linkage disequilibrium with another functional polymorphism in the structural part of the gene or other genes. However, the association was not established for sporadic CRC in subgroup analysis. Only two studies (652 cases and 2115 controls) examining the association between the CHEK2 1100delC variant and sporadic CRC were included in the subgroup analysis. Association studies on variants conferring modest risks require large sample sizes. Meanwhile, the results of association studies are also influenced by the variant frequency. In the subgroup analysis, the number of CHEK2 1100delC heterozygotes is limited. Further case–control studies based on larger sample size are still needed in future research.

Some limitations of this study should be discussed. Firstly, we could not construct funnel plot and Egger's test for each meta-analysis due to small numbers of studies. Thus, publication bias may be present. Secondly, in this meta-analysis, six studies were all conducted in Caucasian population, and the results of the meta-analysis suggest that there is an association between the CHEK2 1100delC variant and CRC susceptibility, mainly in Caucasian population. Thirdly, our results were based on unadjusted estimates, while a more precise analysis stratified by age, different lifestyle habits, and different grades/stages of CRC could be performed if individual data were available. Finally, meta-analysis remains retrospective research that is subject to the methodological deficiencies of the included studies. If considering these limitations, our results should be interpreted with caution.

In conclusion, our meta-analysis demonstrates that the CHEK2 1100delC variant may be a potential risk factor for CRC. However, to reach a definitive conclusion, further gene–gene and gene–environment interactions studies based on larger sample size are still needed, especially in non-Caucasian population.

Conflict of interest statement

None declared.

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