

## RESEARCH COMMUNICATION

# The CHEK2 I157T Variant and Colorectal Cancer Susceptibility: A Systematic Review and Meta-analysis

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### Abstract

**Background:** The cell cycle checkpoint kinase 2 (CHEK2) gene I157T variant may be associated with an increased risk of colorectal cancer, but it is unclear whether the evidence is sufficient to recommend testing for the mutation in clinical practice. **Materials and Methods:** We systematically searched PubMed, EMBASES, Elsevier and Springer for relevant articles before Apr 2012. Summary odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using a fixed-effects or random-effects models with Review Manager 5.0 software. **Results:** A total of seven studies including 4,029 cases and 13,844 controls based on the search criteria were included for analysis. A significant association of the CHEK2 I157T C variant with unselected CRC was found (OR = 1.61, 95% CI = 1.40–1.87, P < 0.001). We also found a significant association with sporadic CRC (OR = 1.48, 95% CI = 1.23–1.77, P < 0.001) and separately with familial CRC (OR = 1.97, 95% CI = 1.41–2.74, P < 0.001). **Conclusion:** This meta-analysis demonstrates that the CHEK2 I157T variant may be another important CRC-predisposing gene, which increases CRC risk, especially in familial CRC.

**Keywords:** Meta-analysis - colorectal cancer - CHEK2 I157T

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### Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females, with over 1.2 million new cancer cases and 608,700 deaths estimated to have occurred in 2008 (Ahmedin et al., 2011). The highest incidence rates is found in Australia and New Zealand, Europe, and North America, whereas the lowest rates are found in Africa and South-Central Asia, CRC is a serious problem for public health in the world. However, the exact mechanisms of the etiology that lead to its CRC remain unknown. Modifiable risk factors for colorectal cancer include smoking, physical inactivity, overweight and obesity, red and processed meat consumption, and excessive alcohol consumption (Barone et al., 2012; Hutter et al., 2012; Kuchiba et al., 2012; zur Hausen, 2012). The genetic factors that susceptibility to CRC has been estimated to be about 35% (Lichtenstein et al., 2000). So the study on the relationship between genetics and CRC is necessary.

Cell-cycle-checkpoint kinase 2 (CHEK2, CHK2) is a key protein that plays an important role in the maintenance of genome integrity and in the regulation of the G2/M cell cycle checkpoint (Chaturvedi et al., 1999; Bartek et al., 2001). Mutation analyses indicate that CHEK2 acts as the multiorgan cancer susceptibility gene contributing to the development of numerous cancers, including breast, colorectal, prostate, ovarian, thyroid and kidney cancer

(Brennan et al., 2007; Tischkowitz et al., 2008; Weischer et al., 2008; Lizi-Kolus et al., 2010; Kuusisto et al., 2011; Tomlinson et al., 2012). Four CHEK2 mutations are found in Poland, three of these are protein-truncating (del5395, IVS2+1G→A, 1100delC) and the other is a common missense variant I157T (Cybulski et al., 2004). Previously, a meta-analysis has been proved that the 1100delC variant may be an important CRC-predisposing Gene (Xiang et al., 2011). Over the last decade, considerable epidemiological studies have suggested a role for the CHEK2 I157T variant in CRC susceptibility (Cybulski et al., 2004; Irmejs et al., 2006; Kilpivaara et al., 2006; Cybulski et al., 2007; Kleibl Z, et al., 2009; Konstantinova et al., 2010; Suchy et al., 2010). 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. The aim of this meta-analysis is to assess the CHEK2 I157T variants susceptible to CRC.

### Materials and Methods

#### *Study identification and selection*

Prospective cohort and case-control studies on CHEK2 I157T and the risk of colorectal cancer published before Apr 2012 were identified through computer-based searches of PubMed, EMBASES, Elsevier and Springer by using the keywords 'CHEK2', 'CHK2', 'I157T', and

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**Table 1. Characteristics of Studies on CHEK2 I157T Variant and CRC Susceptibility**

study	country	year	Genotyping method	Study design	overall n Carriers n (frequency of carriers, %)			
					case	control	case	control
Unselected cases								
Cybulski C [2004]	Szczecin, Poland	2004	RFLP-PCR <sup>a</sup>	CC <sup>b</sup>	300	4000	28(9.33)	193(4.83)
Irmejs A [2006]	Latvia	2006	RFLP-PCR	CC	235	978	24(10.21)	63(6.44)
Kilpivaara O [2006]	Finnish	2006	RFLP	CC/PR <sup>c</sup>	972	1885	76(7.82)	100(5.31)
Cybulski C [2007]	Poland	2007	ASO <sup>d</sup> /RFLP-PCR	CC	1085	5496	77(7.09)	264(4.80)
Kleibl Z [2009]	Czech	2009	DHPLC <sup>e</sup>	CC	631	683	30(4.75)	17(4.39)
Suchy J [2010]	Poland	2009	ASO/RFLP-PCR	CC	463	5496	36(7.78)	264(4.80)
Konstantinova D [2010]	Bulgaria	2010	PCR-RFLP	CC	343	802	9(2.62)	21(2.62)
Sporadic cases								
Kilpivaara O [2006]	Finnish	2006	RFLP	CC/PR	837	1885	62(7.41)	100(5.31)
Cybulski C [2007]	Poland	2007	ASO/RFLP-PCR	CC	975	5496	66(6.77)	264(4.80)
Kleibl Z [2009]	Czech	2009	DHPLC	CC	531	683	25(4.71)	17(2.49)
Suchy J [2010]	Poland	2009	ASO/RFLP-PCR	CC	346	5496	26(7.51)	264(4.80)
Konstantinova D [2010]	Bulgaria	2010	PCR-RFLP	CC	219	802	6(2.74)	21(2.62)
Familial cases								
Kilpivaara O [2006]	Finnish	2006	RFLP	CC/PR	135	1885	14(10.37)	100(5.31)
Cybulski C [2007]	Poland	2007	ASO/RFLP-PCR	CC	110	5496	11(10.00)	264(4.80)
Kleibl Z [2009]	Czech	2009	DHPLC	CC	100	683	5(5.00)	17(2.49)
Suchy J [2010]	Poland	2009	ASO/RFLP-PCR	CC	117	5496	10(8.55)	264(4.80)
Konstantinova D [2010]	Bulgaria	2010	PCR-RFLP	CC	118	802	3(2.54)	21(2.62)

<sup>a</sup>Reaction fragment length polymorphism-polymerase chain reaction; <sup>b</sup>Case control study; <sup>c</sup>Prospective study; <sup>d</sup>Allelo specific oligonucleotide; <sup>e</sup>Denaturing high performance liquid chromatography

'CHEK2 I157T' alone and in combination with colorectal cancer. 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. Inclusion criteria was defined as follows: (1) The articles evaluating the association between colorectal cancer and CHEK2 I157T; (2) The studies are designed as case-control; (3) The sufficient datas are available to estimate an odds ratio (OR) with its 95% CI.

The retrieved literatures were then read in their entirety to assess their appropriateness for the inclusion in this meta-analysis by the two authors (Liu and Qing) independently. The reference lists of reviews and retrieved articles were searched simultaneously to find additional eligible studies. If there was a disagreement, it was resolved through discussion by all the authors. Two studies (Cybulski et al., 2007; Suchy et al., 2010) used the same controls, we used the data once when we calculate the total controls.

#### Data abstraction

From each study, we extracted the first author's name, country or region, year of publication, source of publication, genotyping method of CRC, the number of cases and controls, and the frequencies of genotypes in cases and controls. If original data were unavailable in articles, a request for original data was sent to the corresponding author.

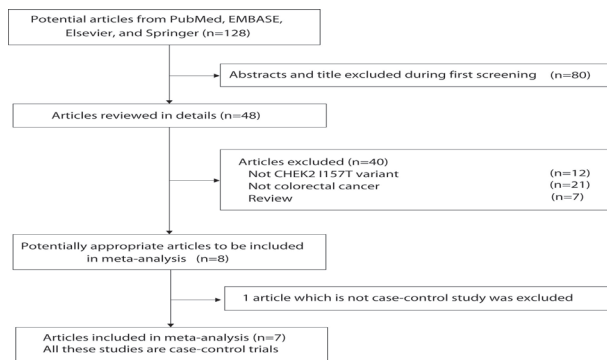
#### Statistical analysis

Before analysis, We classified CRC into three subgroups: unselected CRC (cases were unselected for family history of CRC), sporadic CRC (non-familial CRC) and familial CRC (two or more first degree relatives are diagnosed with CRC in the same family). Meta-analysis was performed for the three subgroups. Heterogeneity

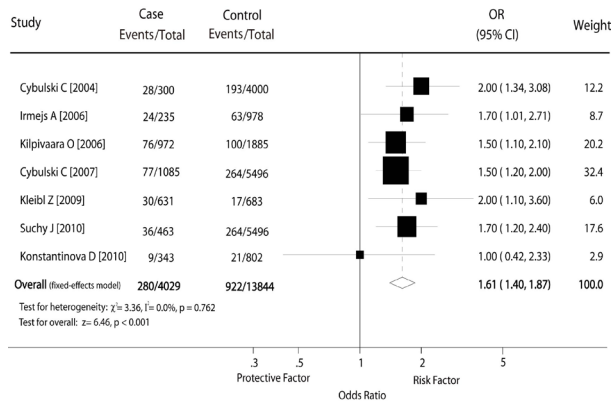
among studies was checked by the Chi square-test based Q-statistic. A significant Q-statistic ( $P < 0.10$ ) indicated heterogeneity across studies (Cochran, 1954). Meanwhile, we measured the effect of heterogeneity by another measure,  $I^2 = 100\% \times (Q - df) / Q$  (Higgins et al., 2002). When the heterogeneity between the studies was absent in the subgroup, The pooled OR was calculated by a fixed-effects model (the Mantel-Haenszel method) (Mantel et al., 1959). Otherwise, a random-effects model (the DerSimonian and Laird method) (Der Simonian et al., 1986) was selected. The association between CHEK2 I157T and colorectal cancer risk was assessed by odds ratio (OR) with the corresponding 95% CI. Meanwhile, Publication bias was observed with the funnel plot and Egger's linear regression test (Egger et al., 1997). Statistical analyses were performed by using the software Review Manager 5.0. A P value less than 0.05 was considered statistically significant, and all the P values were two sided.

## Results

Characteristics of studies investigating the association of the CHEK2 I157T variant with CRC susceptibility are presented in Table 1. The study selection process is shown in Figure 1. The search identified 128 articles (Pubmed: 42; Embase:25; Elsevier=47; Springer=14). Of these, we exclude the articles which the CHEK2 I157T was not variant, not colorectal cancer. Review 8 studies (Cybulski et al., 2004; Irmejs et al., 2006; Kilpivaara et al., 2006; Cybulski et al., 2007; Kleibl et al., 2009; Konstantinova et al., 2010; Scharrer et al., 2010; Suchy et al., 2010) examined the association of the CHEK2 variants with CRC susceptibility, and exclude one article (Scharrer et al., 2010) which is not the case-control study. Finally, seven studies (Cybulski et al., 2004; Irmejs et al., 2006;



**Figure 1. Study Selection Process for Meta-analysis**



**Figure 2. Meta-analysis of the Risk of Unselected CRC for CHEK2 I157T Carriers Versus Non-carriers**

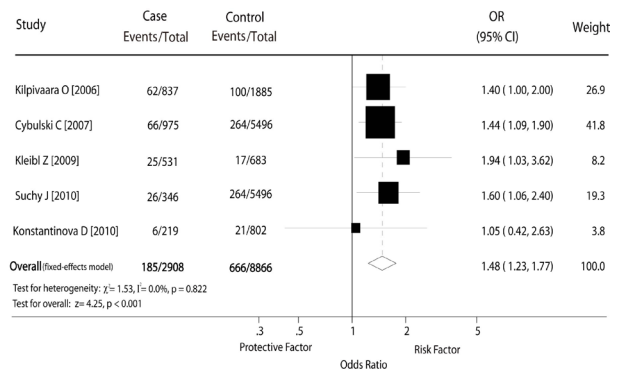
Kilpivaara et al., 2006; Cybulski et al., 2007; Kleibl et al., 2009; Konstantinova et al., 2010; Suchy et al., 2010) were included in this meta-analysis. In these studies, we identified seven studies of unselected CRC (Cybulski et al., 2004; Irmejs et al., 2006; Kilpivaara et al., 2006; Cybulski et al., 2007; Kleibl et al., 2009; Konstantinova et al., 2010; Suchy et al., 2010), five of sporadic CRC (Kilpivaara et al., 2006; Cybulski et al., 2007; Kleibl et al., 2009; Konstantinova et al., 2010; Suchy et al., 2010) and five of familial CRC (Konstantinova et al., 2010; Kilpivaara et al., 2006; Cybulski et al., 2007; Kleibl et al., 2009; Suchy et al., 2010).

#### Unselected CRC

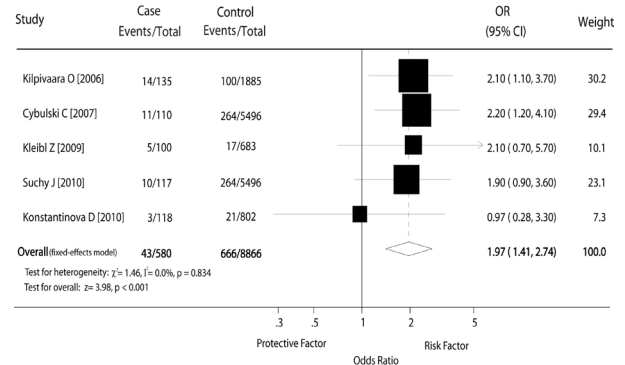
A total of seven studies (Cybulski et al., 2004; Irmejs et al., 2006; Kilpivaara et al., 2006; Cybulski et al., 2007; Kleibl et al., 2009; Konstantinova et al., 2010; Suchy et al., 2010) (4,029 cases and 13,844 controls) evaluating the association between the CHEK2 I157T variant and unselected CRC were included. Heterogeneity between studies by the Chi square-test based Q-statistic was not significant ( $P = 0.762$ ), so the pooled OR was calculated by a fixed-effects model. The result was shown in Figure 2. We found an association of the CHEK2 I157T variant with unselected CRC (OR = 1.61, 95% CI = 1.40–1.87,  $P < 0.001$ ). The distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot (Figure 5A). Similarly, the Egger's test provided no evidence of publication bias in six reviewed studies ( $t = 1.51, P = 0.192$ ).

#### Sporadic CRC

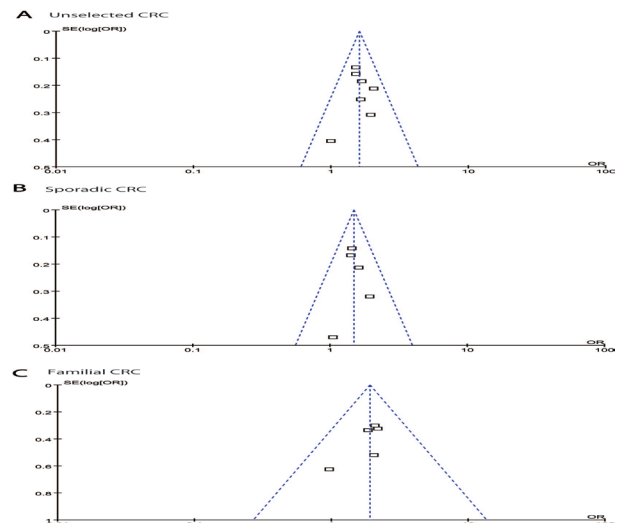
A total of five studies (Kilpivaara et al., 2006;



**Figure 3. Meta-analysis of the Risk of Sporadic CRC for CHEK2 I157T Carriers Versus Non-carriers**



**Figure 4. Meta-analysis of the Risk of Familial CRC for CHEK2 I157T Carriers Versus Non-carriers**



**Figure 5. Funnel Plots of CHEK2 I157T Variants and Colorectal Cancer Risk for Publication Bias**

Cybulski et al., 2007; Kleibl et al., 2009; Konstantinova et al., 2010; Suchy et al., 2010) (2,908 cases and 8,866 controls) evaluating the association between the CHEK2 I157T variant and sporadic CRC were included. The Q-test of heterogeneity was not significant ( $P = 0.822$ ), so we conducted the analysis by using the fixed-effect model. The result was shown in Figure 3. We found an association of the CHEK2 I157T variant with sporadic CRC (OR = 1.48, 95% CI = 1.23–1.77,  $P < 0.001$ ). The distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot (Figure 5B). Similarly, the Egger's test provided no evidence of publication bias in four reviewed studies ( $t = 0.82, P = 0.474$ ).

### Familial CRC

A total of five studies (Kilpivaara et al., 2006; Cybulski et al., 2007; Kleibl et al., 2009; Konstantinova et al., 2010; Suchy et al., 2010) (580 cases and 8,866 controls) evaluating the association between the CHEK2 I157T variant and familial CRC were included. The Q-test of heterogeneity was not significant ( $P = 0.834$ ). So we conducted the analysis by using the fixed-effect model. The result was showed in Figure 4. We found an association of the CHEK2 I157T variant with familial CRC (OR = 1.97, 95% CI = 1.41–2.74,  $P < 0.001$ ). The distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot (Figure 5C). Similarly, the Egger's test provided no evidence of publication bias in four reviewed studies ( $t = -1.79$ ,  $P = 0.171$ ).

### Discussion

Cell cycle checkpoint kinase 2 (CHEK2) participates in the DNA damage response in several cell types and is a key component of the DNA damage pathway. The mutation of I157T in CHEK2 gene have been found which was associated with many kinds of cancers, particularly of the breast and prostate cancers (Vahteristo et al., 2002; Cybulski et al., 2004; Kilpivaara et al., 2004). Moreover, there are also many trials reported that the I157T variant in CHEK2 gene increase the risk of CRC, whereas others reported the opposite results. The conclusion is not confirmed. Therefore, seven case-control trials included in this meta-analysis to reveal the association between the CHEK2 I15T variant and the CRC susceptibility.

The meta-analyses shows that CHEK2 I157T variant increases the risk of CRC one to two fold, which supports the conclusion of the patients with unselected, sporadic and familial CRC in some previous studies. From this research we observe a significantly increased risk of unselected colorectal cancer associated with the I157T variant (OR=1.61,  $P < 0.0001$ ) as well as the sporadic (OR=1.48,  $P < 0.0001$ ) and familial CRC (OR=1.97,  $P < 0.0001$ ). Moreover, it is interested that the mutation of CHEK2 has a closer association with the familial CRC. The odds ratio of patients with familial CRC is obviously higher than the sporadic CRC (1.97 vs 1.48).

It is not surprised of this result, CHEK2 is an important component of major functional networks in controlling cell cycle and DNA repair, and it is also one of the susceptible genes in hereditary cancer syndromes. Since 1999, the family Li-Fraumeni syndrome (Bell et al., 1999) was first reported associated with the protein-truncating mutation of CHEK2 which abolishes the kinase function of CHEK2, has also been found in families with breast cancer (Domagala et al., 2012).

From the 'two-hit' model, we know inherited predisposition to cancer entails a germline mutation dominantly, while tumorigenesis requires a second, somatic, mutation (Knudson et al., 2001). APC gene mutations occur at a high frequency in familial CRC especially in familial adenomatous polyposis (FAP). The patients with the allele of APC in familial CRC are more susceptible to tumor generation by receiving a 'second hit'

than the sporadic CRC (Rowan et al., 2000).

CHEK2 is a part of the P53 pathway which can lead to cell-cycle arrest at G1 by encodes a protein kinase that is post-translationally modified after DNA damage (Chehab et al., 2000). CHEK2 is a stable, long-lived, predominantly nuclear protein that remains expressed and can become activated upon DNA damage in all phases of the mammalian cell cycle cells. CHEK2 is a stable protein expressed throughout the cell cycle (Bartek et al., 2001), it is activated mainly by ATM in response to double-strand DNA breaks, and its activation involves dimerization and autophosphorylation (Bartek et al., 2003; Turnbull et al., 2012). So the presence of the allele of I157T may effect the expression of the CHEK2 and involve in the development of the malignant tumor. From this meta-analysis, we can conclude that the CHEK2 I157T variant could be a risk factor for CRC especially familial CRC.

Of course, there are some limitations of this study. Firstly, all population of the seven trials were from Europe, they were lack of the decent from other ethnic groups, so the result of the I157T variant in CHEK2 gene increase the risk of CRC heterogeneity. Secondly, we ignored the clinic background of the every individual in this studies, like age, sexual, stages, pathological types of CRC, habits of the life. Thirdly, as a common limitation in a meta-analyses, the publication bias must be present. However, as showed in this article, as to the small numbers of the studies, the funnel plot provided no evidence of publication bias.

In conclusion, the study demonstrates that the CHEK2 I157T variant may be a potential risk factor for CRC. The CHEK2 I157T maybe an effective target for treating CRC. However, to reach a definitive conclusion, the studies should be based on larger sample size, especially in non-European population.

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