RESEARCH COMMUNICATION

The CHEK2 I157T Variant and Breast 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 breast 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, Embase, Elsevier and Springer for relevant articles published before Nov 2011. Summary odds ratio (OR) and 95% confidence interval (95% CI) incidence rates were calculated using a random-effects model with STATA (version 10.0) software. Results: A total of fifteen case-control studies, including 19,621 cases and 27,001 controls based on the search criteria, were included for analysis. A significant association was found between carrying the CHEK2 I157T variant and increased risk of unselected breast cancer (OR = 1.48, 95% CI = 1.31–1.66, P < 0.0001), familial breast cancer (OR = 1.48, 95% CI = 1.16–1.89, P < 0.0001), and early-onset breast cancer (OR = 1.47, 95% CI = 1.29–1.66, P < 0.0001). We found an even stronger significant association between the CHEK2 I157T C variant and increased risk of lobular type breast tumors (OR = 4.17, 95% CI = 2.89–6.03, P < 0.0001). Conclusion: Our research indicates that the CHEK2 I157T variant may be another important genetic mutation which increases risk of breast cancer, especially the lobular type.

Keywords: Meta-analysis - breast cancer - CHEK2 I157T

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Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide, accounting for 23% (1.38 million) of total new cancer cases and 14% (458,400) of total cancer deaths in 2008 (Ahmedin et al., 2011). Breast cancer has long been known to have a significant genetic component, and females with an affected first-degree relative have an approximately 1.8-fold increased relative risk compared with the general population (Lancet, 2001). As such, determining the genetic causes underlying familial and sporadic cancers will have an important impact on breast cancer screening and prevention (Desjardins et al., 2008). Since BRCA1 was identified in 1994 and BRCA2 was identified in 1995 (Miki et al., 1994; Wooster et al., 1995), tests for breast cancer susceptibility due to the two genes are widely available in North America and Europe (Narod et al., 1998). However, the two genes do not explain all breast cancer families and it is expected that additional susceptibility genes will be discovered.

The checkpoint kinase 2 [CHEK2, Chk2, (OMIM 604373)] gene is located at chromosome 22q12.1 and codes for a 60- kDa protein consisting of 546 amino acid residues (Matsuoka et al., 1998). It is an important mediator for a DNA damage signaling pathway, defects in which have been found to contribute to the development of breast and other cancers (Falck et al., 1998). A previous meta-analysis found that the 1100delC variant may predispose females to breast cancer (Weischer et al., 2008). The I157T (470 T>C) mutation in the FHA domain has been previously detected in families with classical or variant Li-Fraumeni syndrome (LFS) (Bell et al., 1999; Lee et al., 2001), in breast cancer families and patients, as well as in the normal Finnish population (Vahteristo et al., 2001). Over the last decade, epidemiological studies have suggested a role for the CHEK2 I157T variant in breast cancer susceptibility (Allinen et al., 2001; Schutte et al., 2003; Cybulski et al., 2004; Dufault et al., 2004; Kilpivaara et al., 2004; Bogdanova et al., 2005; Górski et al., 2005; Cybulski et al., 2005; Huzarski et al., 2005; Cybulski et al., 2006; Nevanlinna et al., 2006; Meyer et al., 2007; Cybulski et al., 2008; Falchetti et al., 2008; Novak et al., 2008; Kaufman et al., 2008; Kleib et al., 2008; Serrano-Fernandez et al., 2009; Scharrer et al., 2010; Cybulski et al., 2011; Domagala et al., 2011), but the association is controversial. The inconsistent results from various studies may have resulted from relatively small sample sizes and differences in patient populations. The aim of this meta-analysis is to assess the association between the CHEK2 I157T variant and female breast cancer susceptibility.

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Materials and Methods

Study identification and selection

Case–control studies of the CHEK2 I157T variant and breast cancer susceptibility published before November 2011 were included through computer-based searches of PubMed, Embase, Elsevier and Springer using the keywords 'CHEK2', 'CHK2' , 'I157T', and 'CHEK2 I157T' alone and in combination with 'breast cancer.' Additional studies were identified by a hand search of references from original studies and review articles on the association between CHEK2 variants and breast cancer susceptibility.

Inclusion criteria were defined as: (1) Articles evaluating the association between breast cancer and CHEK2 I157T; (2) Studies designed as case-control; (3) Studies with sufficient data available to estimate an odds ratio (OR) with 95% CI.

Cases from selected studies were classified as unselected (cases were unselected for family history of breast cancer), early-onset (age<=51 year at diagnosis), familial (two or more first degree relatives diagnosed with breast cancer in the same family) and lobular breast cancer (confirmed by pathology).

Data abstraction

From each study, information on the first author's name, country or region, year of publication, source of publication, genotyping method of breast cancer, the number of cases and controls, and the frequencies of genotypes in cases and controls were extracted. Cases with both truncating and missense (I157T) variants were available in two studies (Cybulski et al., 2007; Domagala et al., 2011).

We assessed the methodological quality of included studies using the Newcastle-Ottawa Scale (Wells et al., 1997) (NOS) for quality of case control and cohort studies, based on following three subscales: the selection of the study groups (4 items), the comparability of the groups (1 item), and the ascertainment of the exposure or outcome of interest for case-control or cohort studies, respectively (3 items). A 'star system' (ranging from 0 to 9) was developed for assessment. In our research, we considered a study awarded 7 or more stars as a high-quality study, since standard criteria have not been established.

Statistical analysis

The association between carrying the CHEK2 I157T variant and breast cancer risk was assessed by odds ratio (OR) with the corresponding 95% CI. Although a fixed-effect model and a random-effects model yielded similar conclusions, we chose to use the random-effects model with Mantel-Haenszel statistics (DerSimonian et al., 1986; Ades et al., 2005), which assumed that the true underlying effect varied among included individuals. Many investigators also consider the random effects model to be a more natural choice than the fixed effects model in medical decision-making contexts. We performed subgroup analyses for unselected, early-onset, familial and lobular breast cancer cases. Heterogeneity among studies was checked by the chi-square test based Q-statistic. A significant Q-statistic ($P < 0.05$) indicated heterogeneity across studies (Cochran et al., 1954). Meanwhile, we measured the effect of heterogeneity by another measure, $I^2 = 100\% \times (Q - df)/Q$ (Higgins et al., 2002). Publication bias was analyzed with the funnel plot and Egger's linear regression test (Egger et al., 1997).

If two or more studies used the same data as a control group, we merged the data from both studies by using the single sample estimated method CMA software. Statistical analyses were performed using the STATA (version 10.0) software. A P value less than 0.05 was considered statistically significant, and all P values were two-sided.

Results

The study selection process is shown in Figure 1. The search identified 192 articles. After screening, we excluded articles in which CHEK2 I157T was not a studied variant or in which CHEK2 I157T was studied but not in breast cancer. 21 studies (Allinen et al., 2001; Schutte et al., 2003; Cybulski et al., 2004; Dufault et al., 2004; Kilpivaara et al., 2004; Bogdanova et al., 2005; Górski et al., 2005; Cybulski et al., 2005; Huzarski et al., 2005; Cybulski et al., 2006; Nevanlinna et al., 2006; Meyer et al., 2007; Cybulski et al., 2008; Falchetti et al., 2008; Novak et al., 2008; Kaufman et al., 2008; Kleib et al., 2008; Serrano-Fernandez et al., 2009; Scharrer et al., 2010; Cybulski et al., 2011; Domagala et al., 2011) examined the association between CHEK2 variants and breast cancer susceptibility. We then excluded non-case-controlled, data duplicating, review, new gene variant, and male breast cancer studies. Finally, fifteen studies (Allinen et al., 2001; Schutte et al., 2003; Cybulski et al., 2004; Dufault et al., 2004; Kilpivaara et al., 2004; Bogdanova et al., 2005; Górski et al., 2005; Cybulski et al., 2005; Huzarski et al., 2005; Cybulski et al., 2006; Nevanlinna et al., 2006; Meyer et al., 2007; Cybulski et al., 2008; Falchetti et al., 2008; Novak et al., 2008; Kaufman et al., 2008; Kleib et al., 2008; Serrano-Fernandez et al., 2009; Scharrer et al., 2010; Cybulski et al., 2011; Domagala et al., 2011) containing 19,621 cases and 27,001 controls were included in this meta-analysis.

In these studies, we identified thirteen studies of unselected breast cancer (Allinen et al., 2001; Schutte et al., 2003; Cybulski et al., 2004; Kilpivaara et al., 2004; Bogdanova et al., 2005; Cybulski et al., 2005; Górski et al., 2005; Huzarski et al., 2005; Cybulski et al., 2008; Kleib et al., 2008; Serrano-Fernandez et al., 2009; Scharrer

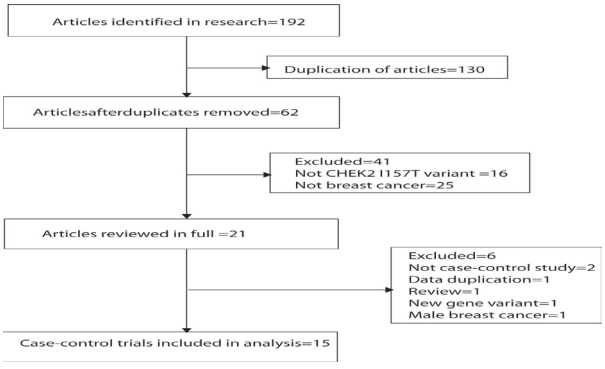

Figure 1. Study Selection Process for Meta-analysis

Table 1. Characteristics of studies of the CHEK2 I157T variant and breast cancer susceptibility

Study	Country	NOS score	Year	Overall size n case	control	Carriers n (frequency of carriers, %) case	control	Genotyping method
Unseleced								
Allinen M [2001]	Finnish	8	2001	259	200	10(3.86)	13(6.50)	CSGE
Schutte M [2003]	UK, NA, Netherland	8	2003	459	723	0(0)	1(0.14)	ASO,PCR
Kilpvaara O [2004]	Finnish	8	2004	1035	1885	77(7.44)	100(5.31)	CSGE
Cybulski C [2004]	Szczecin,Poland	7	2004	1017	4000	68(6.69)	193(4.83)	RFLP-PCR
Huzarski T [2005]	Szczecin, Poland	7	2005	505	4000	33(6.53)	193(4.83)	G-A-3100A
Bogdanova N [2005]	German,Byelorussia	8	2005	1420	793	46(3.24)	7(0.88)	G-A-3100A
Gorski B [2005]	Poland	8	2005	2012	4000	132(6.56)	193(4.83)	RFLP-PCR
Cybulski C [2007]	Poland	7	2007	1978	5496	134(6.77)	264(4.80)	ASO/RFLP-PCR
Kleibl Z [2008]	Czech	8	2007	673	683	19(2.82)	17(2.49)	DHPLC
Cybulski C [2009]	Poland	7	2008	7782	6233	614(7.89)	323(5.18)	RFLP-PCR
S-Fernandez P [2009]	Poland	8	2008	2778	2041	170(6.12)	73(3.58)	RFLP PCR
Scharrer U [2010]	German, Saxony,	8	2010	150	101	3(2.00)	5(4.95)	DHPLC
Cybulski C [2011]	Szczecin,Poland	8	2011	7496	4346	535(7.14)	215(4.95)	RFLP-PCR
Familiar								
Allinen M [2001]	Finnish	8	2001	79	200	7(8.86)	13(6.50)	CSGE
Schutte M [2003]	UK, NA,Netherland	8	2003	737	723	2(0.27)	1(0.14)	ASO,PCR
Dufault MR [2004]	German	7	2004	516	500	10(1.94)	8(1.60)	DHPLC
Kilpvaara O [2004]	Finnish	8	2004	507	1885	28(5.52)	100(5.31)	CSGE
Bogdanova N [2005]	German,Byelorussian	8	2005	252	793	7(2.78)	7(0.88)	G-A-3100A
Cybulski C [2011]	Szczecin,Poland	8	2011	1451	4346	115(7.93)	215(4.95)	RFLP-PCR
Early-onset								
Cybulski C [2007]	Poland	7	2007	3228	5496	207(6.41)	264(4.80)	ASO/ RFLP-PCR
Domagala P [2011]	Poland	7	2011	350	5496	31(8.86)	264(4.80)	RFLP-PCR
Cybulski C [2011]	Szczecin,Poland	8	2011	5152	4346	349(6.77)	215(4.95)	RFLP-PCR
Lobular								
Huzarski T [2005]	Szczecin, Poland	7	2005	52	4000	13(25.00)	193(4.83)	G-A-3100A
Domagala P [2011]	Poland	7	2011	186	5496	24(12.90)	322(5.86)	RFLP-PCR
Cybulski C [2011]	Szczecin,Poland	8	2011	479	4346	88(18.37)	215(4.95)	RFLP-PCR

UK, United Kingdom; NA, North American; CSGE, conformation sensitive gel electrophoresis; ASO, allele-specific oligonucleotide; RFLP-PCR, restriction fragment length polymorphism polymerase chain reaction; G-A-3100A, Genetic Analyzer 3100 Avant; DHPLC, denaturant high-performance liquid chromatography

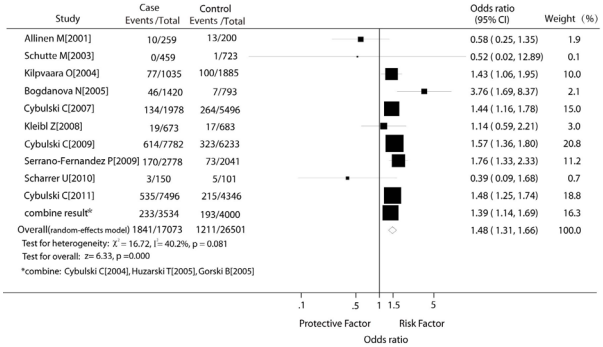

Figure 2. Meta-analysis of the Risk of Unselected Breast Cancer for CHEK2 I157T Variant Versus Non-carriers

et al., 2010; Cybulski et al., 2011), six of familial breast cancer (Allinen et al., 2001; Schutte et al., 2003; Dufault et al., 2004; Kilpivaara et al., 2004; Bogdanova et al., 2005; Cybulski et al., 2011), three of early-onset breast cancer (Cybulski et al., 2005; Cybulski et al., 2011; Domagala et al., 2011) and three of lobular breast cancer (Cybulski et al., 2005; Cybulski et al., 2011; Domagala et al., 2011). Characteristics of included studies are summarized in Table 1.

We used Egger's test to check for potential publication bias, which showed no evidence of publication bias for the outcomes of CHEK2 I157T variant and breast cancer susceptibility association (P =0.083) and the conclusions were not changed after adjustment for publication bias by the trim and fill method (Duvall et al., 2000).

Unselected breast cancer

A total of thirteen studies (17,073 cases and 26,501 controls) evaluating the association between the CHEK2 I157T variant and unselected breast cancer were included. Because 3 studies (Cybulski et al., 2004; Górski et al., 2005; Huzarski et al., 2005) used the same control cases, we merged the data from these by using the single sample estimated method with CMA software. Heterogeneity between studies was not significant (P = 0.081) by the chi-square test based Q-statistic. Results obtained using the random-effect model are shown in Figure 2. We found an association between carrying the CHEK2 I157T variant and increased risk of unselected breast cancer (OR = 1.48, 95% CI = 1.31–1.66, P < 0.0001). The distribution of the ORs from individual studies in relation to their respective standard deviation was symmetrical in a funnel plot.

Familial breast cancer

A total of six studies (3,542 cases and 8,447 controls) evaluating the association between the CHEK2 I157T variant and familial breast cancer were included. Heterogeneity between studies was not significant (P = 0.343) by the chi-square test based Q-statistic. Results obtained using the random-effect model are shown in

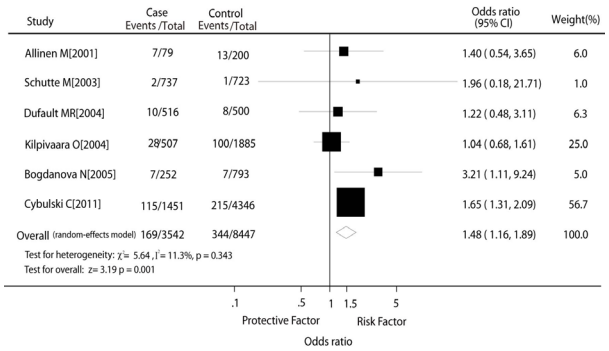

Figure 3. Meta-analysis of the Risk of Familial Breast Cancer for CHEK2 I157T Variant Versus Non-carriers

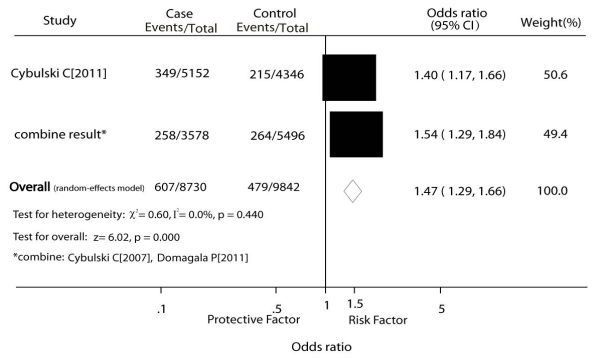

Figure 4. Meta-analysis of the Risk of Early-onset Breast Cancer for CHEK2 I157T Variant Versus Non-carriers

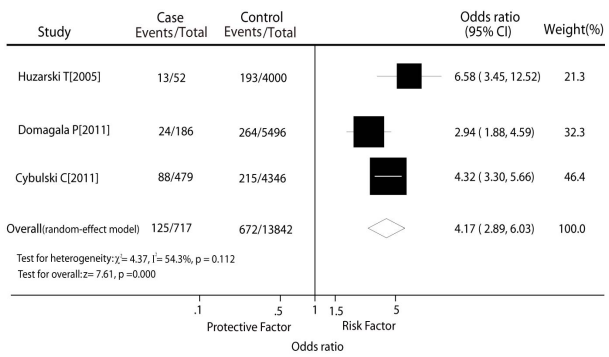

Figure 5. Meta-analysis of the Risk of Lobular Breast Cancer for CHEK2 I157T Variant Versus Non-carriers

Figure 3. We found an association between carrying the CHEK2 I157T variant and increased risk of familial breast cancer (OR = 1.48, 95% CI = 1.16–1.89, P < 0.0001). The distribution of the ORs from individual studies in relation to their respective standard deviation was symmetrical in a funnel plot.

Early-onset breast cancer

A total of three studies (8,730 cases and 9,842 controls) evaluating the association between the CHEK2 I157T variant and early-onset breast cancer were included. Because 2 studies (Cybulski et al., 2005; Cybulski et al., 2011) used the same control cases, we merged the control cases from these studies using the single sample estimated method with CMA software. Heterogeneity between studies was not significant (P = 0.440) by the chi-square test based Q-statistic. Results obtained using the random-effect model are shown in Figure 4. We found an association between carrying the CHEK2 I157T variant and increased risk of early-onset breast cancer (OR = 1.47, 95% CI = 1.29–1.66, P < 0.0001). The distribution of the ORs from individual studies in relation to their respective standard deviation was symmetrical in a funnel plot.

Lobular breast cancer

A total of three studies (717 cases and 13,842 controls) evaluating the association between the CHEK2 I157T variant and lobular breast cancer were included. Heterogeneity between studies was significant (P =0.112) by the chi-square test based Q-statistic. Results obtained using the random-effect model are shown in Figure 5. We found an association between carrying the CHEK2 I157T variant and increased risk of lobular breast cancer (OR = 4.17, 95% CI = 2.89–6.03, P < 0.0001). The distribution of the ORs from individual studies in relation to their respective standard deviation was symmetrical in a funnel plot.

Discussion

Breast cancer has long been known to have a significant genetic component. CHEK2 is the most extensively studied of the breast cancer genes after the initially identified BRCA1 and BRCA2 (McInerney et al., 2010). Four CHEK2 mutations have been identified in a cohort of Polish patients, three of which are protein-truncating (del5395, IVS2+1G→A, 1100delC) while the fourth is a common missense variant (I157T) (Cybulski et al., 2004). Although many mutations in CHEK2 have been described, the most common are CHEK2 1100delC and CHEK2 I157T. A previous meta-analysis (Weischer et al., 2008) has shown that the 1100delC variant may be an key gene imparting increased breast cancer risk. Unfortunately, definite conclusions cannot be drawn by analyzing previous results of association studies of the CHEK2 I157T variant and female breast cancer susceptibility. Some studies have reported an increased risk of breast cancer associated with the variant, whereas others have reported no association. Therefore, we conducted this meta-analysis to further investigate the association between the CHEK2 I15T variant and breast cancer susceptibility.

This is the first meta-analysis to investigate the association, and represents the most comprehensive analysis of the CHEK2 I157T mutation in breast cancer, containing 15 case-control studies. The studies included in this meta-analysis showed no evidence of publication bias. Our meta-analysis shows that the CHEK2 I157T variant increases the risk of breast cancer about 1.5-fold, supporting the previous studies which concluded that the CHEK2 I157T variant was associated with breast cancer susceptibility.

Interestingly, among patients with lobular breast cancer, we found that the CHEK2 I157T variant conferred a 4-fold increased risk of developing breast cancer. We also found that in one included study (Domagala et al., 2011), CHEK2 I157T variant cancers were more likely to express ER and PR, as compared with other tumors. The CHEK2 I157T variant was found predominantly in tumors of the luminal A subtype, whereas tumors

associated with truncating mutations predominantly exhibited luminal B characteristics, though this association needs more investigation to be confirmed. The mutant CHEK2 1100delC protein is unstable and defective in kinase function(Sodha et al., 2006), while the CHEK2 I157T protein impairs the binding of BRCA1, Cdc25A, and p53 (Falck et al., 2001; Falck and Lukas et al., 2001).

This study's conclusions contain some limitations. First, a common limitation of meta-analysis is study heterogeneity. Heterogeneity is often caused by variation in the environmental and genetic background of study participants, which is unavoidable when combining many studies; however, an I^2 of less than 25% in the meta-analysis is regarded as low, according to Higgins (Higgins et al., 2003). We found minimal evidence of study heterogeneity in our study of patients with familiar (I^2=11.3%) and early-onset breast cancer (I^2=0.0%), presumably because the number of included studies was low, the populations studied in this meta-analysis were mainly from Europe, and few patients were descended from non-European ethnic groups. Thus, the applicability of our results associating the I157T variant of CHEK2 with predisposition to breast cancer is limited to Caucasians. Second, because the analysis used pooled data either published or provided by individual study authors, and individual patient data or original data were unavailable, we were unable to do more detailed relevant analysis and obtain more comprehensive results. Third, a common limitation in meta-analyses is the presence of publication bias. However, due to the small number of studies used in this analysis, the funnel plot provided no evidence of publication bias.

After BRCA1 and BRCA2 were discovered in 1994 and 1995 (Miki et al., 1994; Wooster et al., 1995), it was hoped that a third breast cancer susceptibility gene would be identified. To date, this gene remain undiscovered. Arguably, the third most important breast cancer gene discovery has been CHEK2, which is typical of a third category of genes; mutations of genes in this category are rare and associated with modest penetrance. It is difficult to study these genes, because very large sample sizes are needed to identify significant relative risks. More than a decade after the discovery of the CHEK2 gene (Bell et al., 1999), and based on the conclusions of this and a previous meta-analysis, it is now time to accept CHEK2 as a clinically useful third gene (in addition to BRCA1 and BRCA2) imparting predisposition to breast cancer.

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The author(s) declare that they have no competing interests.

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