RESEARCH COMMUNICATION

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

Chuan Liu&, Ying Wang&, Qing-Shui Wang, Ya-Jie Wang*

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

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., 2008; Cybulski et al., 2004; Dufault et al., 2004; Kilpivaara et al., 2004; Bogdanova et al., 2005; Górska 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.
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, F = 100%×(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; Görski 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; Görski et al., 2005; Cybulski et al., 2006; 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; Görski et al., 2005; Huzarski et al., 2005; Cybulski et al., 2006; Nevanlinna et al., 2006; 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.
Table 1. Characteristics of studies of the CHEK2 I157T variant and breast cancer susceptibility

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>NOS score</th>
<th>Year</th>
<th>Overall size</th>
<th>Carriers n (frequency of carriers, %)</th>
<th>Genotyping method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allinen M [2001]</td>
<td>Finnish</td>
<td>8</td>
<td>2001</td>
<td>79</td>
<td>200 (7.86)</td>
<td>CSGE</td>
</tr>
<tr>
<td>Schutte M [2003]</td>
<td>UK, NA, Netherlands</td>
<td>8</td>
<td>2003</td>
<td>737</td>
<td>723 (20.27)</td>
<td>ASO,PCR</td>
</tr>
<tr>
<td>Kilpivaara O [2004]</td>
<td>Finnish</td>
<td>8</td>
<td>2004</td>
<td>1035</td>
<td>1885 (77.44)</td>
<td>CSGE</td>
</tr>
<tr>
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<td>Szczeclin, Poland</td>
<td>7</td>
<td>2004</td>
<td>1017</td>
<td>4000 (68.69)</td>
<td>RFLP-PCR</td>
</tr>
<tr>
<td>Huzarski T [2005]</td>
<td>Szczeclin, Poland</td>
<td>7</td>
<td>2005</td>
<td>505</td>
<td>4000 (33.653)</td>
<td>G-A-3100A</td>
</tr>
<tr>
<td>Bogdanova N [2005]</td>
<td>Germany,Byelorussia</td>
<td>8</td>
<td>2005</td>
<td>1420</td>
<td>793 (46.324)</td>
<td>RFLP-PCR</td>
</tr>
<tr>
<td>Gorski B [2005]</td>
<td>Poland</td>
<td>7</td>
<td>2005</td>
<td>2012</td>
<td>4000 (132.566)</td>
<td>RFLP-PCR</td>
</tr>
<tr>
<td>Cybulski C [2007]</td>
<td>Poland</td>
<td>7</td>
<td>2007</td>
<td>1978</td>
<td>5496 (136.77)</td>
<td>RFLP-PCR</td>
</tr>
<tr>
<td>Kleibl Z [2008]</td>
<td>Czech</td>
<td>8</td>
<td>2007</td>
<td>673</td>
<td>683 (19.28)</td>
<td>DHPLC</td>
</tr>
<tr>
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<td>Poland</td>
<td>7</td>
<td>2009</td>
<td>7782</td>
<td>2041 (614.789)</td>
<td>RFLP-PCR</td>
</tr>
<tr>
<td>S-Fernandez P [2009]</td>
<td>Poland</td>
<td>8</td>
<td>2009</td>
<td>2778</td>
<td>2041 (73.628)</td>
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</tr>
<tr>
<td>Scharrer U [2010]</td>
<td>Germany, Saxony,</td>
<td>8</td>
<td>2010</td>
<td>150</td>
<td>101 (3.00)</td>
<td>DHPLC</td>
</tr>
<tr>
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<td>Szczeclin, Poland</td>
<td>8</td>
<td>2011</td>
<td>7496</td>
<td>4346 (535.714)</td>
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<tr>
<td>Allinen M [2001]</td>
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<td>2001</td>
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<td>CSGE</td>
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<td>Schutte M [2003]</td>
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<td>8</td>
<td>2003</td>
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</tr>
<tr>
<td>Dufault MR [2004]</td>
<td>Germany</td>
<td>7</td>
<td>2004</td>
<td>516</td>
<td>500 (10.194)</td>
<td>DHPLC</td>
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<tr>
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<td>8</td>
<td>2011</td>
<td>1451</td>
<td>4346 (115.793)</td>
<td>RFLP-PCR</td>
</tr>
<tr>
<td>Early-onset</td>
<td></td>
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<tr>
<td>Cybulski C [2007]</td>
<td>Poland</td>
<td>7</td>
<td>2007</td>
<td>3228</td>
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<td>RFLP-PCR</td>
</tr>
<tr>
<td>Domagala P [2011]</td>
<td>Poland</td>
<td>7</td>
<td>2011</td>
<td>350</td>
<td>5496 (31.886)</td>
<td>RFLP-PCR</td>
</tr>
<tr>
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<td>Szczeclin, Poland</td>
<td>8</td>
<td>2011</td>
<td>5152</td>
<td>4346 (115.793)</td>
<td>RFLP-PCR</td>
</tr>
<tr>
<td>Lobular</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Huzarski T [2005]</td>
<td>Szczeclin, Poland</td>
<td>7</td>
<td>2005</td>
<td>52</td>
<td>4000 (135.250)</td>
<td>G-A-3100A</td>
</tr>
<tr>
<td>Domagala P [2011]</td>
<td>Poland</td>
<td>7</td>
<td>2011</td>
<td>186</td>
<td>5496 (24.129)</td>
<td>RFLP-PCR</td>
</tr>
<tr>
<td>Cybulski C [2011]</td>
<td>Szczeclin, Poland</td>
<td>8</td>
<td>2011</td>
<td>479</td>
<td>4346 (88.137)</td>
<td>RFLP-PCR</td>
</tr>
</tbody>
</table>

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., 2011; Domagala et al., 2005). 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 (Duval 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; Görski 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 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...
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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 (717 cases and 13,842 controls) evaluating the association between the CHEK2 I157T variant and early-onset 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 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 = 0.012) 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 a 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 I157T 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.
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