# Evaluation of variants in the CHEK2, BRIP1 and PALB2 genes in an Irish breast cancer cohort

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Conflict of Interest: None

**Abstract**

Background: It has been proposed that rare variants within the double strand break repair genes *CHEK2*, *BRIP1* and *PALB2* predispose to breast cancer. The aim of this study was to evaluate the prevalence of these variants in an Irish breast cancer cohort and determine their contribution to the development of breast cancer in the West of Ireland.

Methods: We evaluated the presence of CHEK2_1100delC variant in 903 breast cancer cases and 1016 controls. Six previously described variants within *BRIP1* and five within *PALB2* were screened in 192 patients with early-onset or familial breast cancer. Where a variant was evident, it was then examined in the remainder of our 711 unselected breast cancer cases.

Results: CHEK2_1100delC was found in 5/903 (0.5%) breast cancer cases compared to 1/1016 (0.1%). One mutation at BRIP1 (2392 C>T) was identified in the early-onset/familial cohort. Examination of this variant in the remainder of our cohort (711 cases) failed to identify any additional cases. None of the previously described PALB2 variants were demonstrated in the early-onset/familial cohort.

Conclusions: We show evidence of CHEK2_1100delC and BRIP1 2392 C>T within the Irish population. CHEK2_1100delC and BRIP1 mutations incidence in Ireland is similar to that found in other unselected breast cancer cohorts from Northern European countries. We found no evidence to suggest that PALB2 mutation is an important breast cancer predisposition gene in this population.

**Introduction**

Breast cancer has long been known to have a significant genetic component; females with an affected first-degree relative carry an approximately 1.8 increased relative risk compared with the general population [1]. Mutations in the high-penetrance predisposition genes *BRCA1* and *BRCA2* account for less than 20% of familial breast cancer [2]. *BRCA1* and *BRCA2* have roles in the double strand break repair (DSBR) pathway and DSBR genes have been screened for mutations in breast cancer cases without *BRCA1* or *BRCA2* mutations. Rare variants in three of these genes – *CHEK2, BRIP1* and *PALB2* – with allele frequencies of 0.4%, 0.1% and less than 0.1% respectively have been associated with breast cancer risk [3]. These variants demonstrate moderate penetrance with regard to breast cancer susceptibility with an increased relative risk of 2-4 fold [4-6].

The most extensively studied of the three breast cancer genes is *CHEK2*. Although many mutations in *CHEK2* have been described the commonest in Northern Europeans countries is *CHEK2_1100delC*, with an incidence of 0.7% in unselected breast cancer and 2.1% in familial breast cancer [4]. *CHEK2_1100delC* is less common in Mediterranean and Asian countries [7-9]. *Pharoah et al* estimated the absolute risk of breast cancer by age 70 in carriers of *CHEK2_1100delC* to be 13% compared to 5.7% in non-carriers, while in first-degree relatives it is responsible for 1.4% of the excess risk [10].

*Seal et al* identified 6 truncating mutations within the Fanconi anaemia J gene *BRIP1* which were estimated to confer a 2 fold increased relative risk of breast cancer in monoallelic carrriers [5]. *BRIP1* mutations were present in 9/1212 breast cancer cases negative for *BRCA1/2* mutations compared with 2/2081 controls ($p = 0.003$) [5]. A nonsense mutation *2392C>T* (R798X) accounted for the majority of mutations in this gene. These findings have not, however, been replicated in more recent studies [11-19].

Study of *BRCA2* revealed an associated gene *PALB2* (also known as FANCN), in which biallelic mutations cause Fanconi anaemia. *Rahman et al* screened *PALB2* in 923 familial breast cancer

patients and identified 5 truncating mutations which were estimated to confer a 2.3-fold increased risk of breast cancer [6]. Additional rare novel truncating mutations have also been discovered by other groups [20, 21]. The Finnish founder mutation identified by *Erkko et al*, *c.1592delT*, has been estimated to confer a lifetime risk of breast cancer equivalent to *BRCA2* mutation carriers [22, 23]. The possibility of specific *PALB2* variants conferring high penetrance in breast cancer was further proposed by *Tischkowitz et al* following their discovery of a truncating mutation *PALB2 229delT* [24]. These finding suggest that the relative risk of developing breast cancer previously attributed to *PALB2* mutations may have been underestimated by *Rahman et al. Gunnarsson et al*, however, found no role for *PALB2* in breast cancer susceptibility in an Icelandic cohort [25].

Although still at an early stage in the investigation of these moderate-penetrance variants, there is evidence of geographical variation in their frequencies. The existence of clinically-relevant founder mutations in some populations is possible. The West of Ireland is geographically isolated from the rest of Europe and, while it shares some of its ancestry with the UK, it has experienced less inward demographic movement [26] and is relatively homogeneous. The aims of this study were to investigate whether known variants in *CHEK2*, *BRIP1* and *PALB2* contribute to breast cancer susceptibility in the West of Ireland.

**Methods**

Breast cancer patients and controls from the West of Ireland were collected with appropriate ethical approval as part of the Breast cancer in Galway Genetics Study (BIGGS). Cases were not selected with regard to family history of breast or ovarian cancer, personal history of ovarian cancer, the presence of a contralateral breast cancer or other second primary cancer. All controls were from a West of Ireland lineage (as were cases) and comprised women over the age of 60 years, with no self-reported personal history of any cancer and no family history of breast or ovarian cancer. From this cohort of unselected breast cancer cases, 192 cases were identified as being at relatively high risk of having an inherited predisposition to breast cancer (cases presenting at <42 years old or <55 years old with a history of breast cancer in one or more first or second degree relative).

DNA was purified from 10ml samples of blood using the Chemagic Magnetic Separation Module (Chemagen, Baesweiler, Germany) using the manufacturer's reagents. Primers (Supplementary Data, Table 1) were designed to amplify a DNA fragment of 167 base pairs across *CHEK2_1100delC* and Genotyper software (ABI) was used to size the fragment. Genotyping was performed in 96-well format and optimised using a known *CHEK2_1100delC* heterozygous control on each plate. Samples with 2 bands and any equivocal results were sequenced to confirm the result using the ABI 3100 automated sequencer and sequence analysis software (Applied Biosystems, CA), Figure 1.

Truncating mutations in *BRIP1* and *PALB2* previously identified in a UK cohort by Seal *et al* and Rahman *et al*, Table 1, were analysed by direct sequencing in the early onset/familial subset. Primers are listed in Supplementary Data, Table 1. Where a variant was identified, it was subsequently genotyped in the remaining 711 breast cancer patient samples using the KASPar SNP genotyping system (KBiosciences).

Meta-analysis data were combined using the Mantel-Haenszel method. All statistical analyses were undertaken using STATA 9.2 (Stata Corp, College Station, TX).

**Results**

The pathological characteristics of the whole cohort are described elsewhere [27]. From this cohort of unselected breast cancer cases, 192 cases were identified as being at relatively high risk of having an inherited predisposition to breast cancer (cases presenting at <42 years old or <55 years old with a history of breast cancer in one or more first or second degree relative). Histological information was available in all of these cases and is summarized in Table 2.

*Analysis of CHEK2*

5/903 (0.5%) *CHEK2_1100delC* mutations were identified in the cases and 1/1016 (0.1%) controls (OR 5.65, 95%CI 0.66-48.46,p=0.09 1-tailed, p= 0.11 2-tailed Fisher's exact test). None of these mutations occurred in the early onset/familial subgroup. The clinical features of these individuals are summarized in Table 3. One *CHEK2_1100delC* mutation carrier was diagnosed at 60 and had a family history of breast cancer on her paternal side, with an aunt, two first cousins and grandmother being affected.The remaining four *CHEK2_1100delC* mutation carriers had no family history of breast cancer but two had a family history of leukaemia. The control carrier was an 82 year old female with no personal or family history of cancer.

Population variation is evident in the prevalence of *CHEK2_1100delC*, with the highest incidence reported in Dutch cohorts (3.8% cases and 1.6% controls) and a negative association with breast cancer susceptibility demonstrated in a Spanish cohort [4, 7]. We have combined our data with that of previous meta- analyses carried out by the *CHEK2 Breast Cancer Case-Control Consortium* and *Weischer et al* [4, 28], excluding any none European studies and any familial breast cancer studies, Figure 3 and Supplementary Data Table 2. This gives an overall odds ratio is 2.1 (95 CI 1.7-2.7) similar to that found by *Weischer et al (*OR 2.4, 95% CI 1.8-3.2) and shows that the relative risk associated with *CHEK2_1100delC* in the Irish population is similar to other Northern / Central European populations. The incidence of *CHEK2_1100delC* in West Ireland is similar ($\chi^2$ test p=0.13) to that in the UK (1.2% in cases and 0.53% controls, ABC study) but significantly less ($\chi^2$ test p<0.0001) than unselected cases series from the Netherlands (RMOTC study - cases 3.8%, controls 1.6%, Prospect study - cases 3.3%, controls 0%), Supplementary Data Table 2, [4].

*BRIP1 Analysis*

1/192 (0.5%) of the early-onset/familial patients was found to have a *BRIP1* mutation (2392 C>T , Figure 2). This patient was diagnosed with ductal breast cancer at age 53 and had a sister affected at 55 years of age. We then examined this variant, *BRIP1 2392 C>T*, in the remainder of our cohort of 711 cases, but none was found. None of the *other BRIP1* mutations previously found by *Rahman et al* were present in the early-onset/familial series and were therefore not screened for in the

remaining cases. Table 4 summarizes the findings from other studies. Very few studies have looked for *BRIP1* mutations and due to the small numbers a meta-analysis is not possible. However there was no evidence of heterogeneity between studies (p=0.145, heterogeneity $\chi^2$ test) suggesting BRIP1 is a rare breast cancer predisposition gene. The finding of one *BRIP1* mutation in our subset of early-onset/familial breast cancer and no other mutations in the whole cohort is consistent with the population frequency of 0.1% previously estimated.

*PALB2 Analysis*

None of the PALB2 variants previously described *Rahman et al* were present in the early-onset/familial series. The findings of other studies are summarized in Table 5. We performed a meta-analysis of these studies ( studies with the same controls were considered together) which showed an overall odds ratio of 8.92 (95 CI 3.4-23.4). There was no evidence of heterogeneity between studies (p=0.11, heterogeneity $\chi^2$ test).

**Discussion**

We have shown an increased risk of breast cancer with *CHEK2_1100delC* mutations in the West of Ireland, similar to that found in other Northern and Central European countries. The recent meta-analysis of *CHEK2_1100delC* by *Weischer et al,* [28], demonstrated that unselected and early onset cases had a similar relative risk of breast cancer (OR 2.7, 95% CI 2.1-3.4; OR 2.6, 95% CI 1.3-5.5 respectively), however the familial cases (defined as at least one first and one second degree relative with breast cancer, a male relative with breast cancer, or at least one patient case of female breast cancer and one patient case of ovarian cancer among relatives) had a much higher relative risk (OR 4.8, 95% CI 3.3-7.2, p<0.0001)). We did not see this in our series, in contrast our subset of familial/early onset cases showed no *CHEK2_1100delC* mutation. However this is likely to be due to the small numbers and the inclusion of early onset breast cancers in our 'high risk' group.

To our knowledge this is the first study to replicate the identification by *Seal et al* of the *BRIP1 2392 C>T* mutation. We analysed those variants in *BRIP1* identified by *Seal et al* due to the relative

homogeneity between the Irish and UK populations [5]. The *BRIP1 2392 C>T* mutation was the most common mutation found by Seal et al, accounting for 5 of the 9 mutations they detected. It has not been demonstrated in any population outside Ireland or the UK, suggesting it may be specific to these populations.

Like *CHEK2_1100delC* there appears to be considerable population variation in the incidence of PALB2 mutations, with a particularly high incidence in Finland (1592delT) and very few in the Icelandic and Irish populations. However the Icelandic study [25] and our study did not screen the whole gene for novel mutations, it is therefore possible that these populations contain as yet unidentified mutations.

In conclusion this is the first study of proposed moderate-penetrance breast cancer susceptibility variants in an Irish cohort of breast cancer patients. 0.5% of cases carried CHEK2_1100delC, compared with 0.1% of controls, a similar prevalence to that found in the UK. BRIP and PALB2 mutation appear to be rare in the Irish breast cancer population as they are in other populations, but mutation screening of these genes in a large familial Irish cohort will be required to fully establish what possible role mutations in these genes play in familial breast cancer in Ireland.

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**Table 1: Mutations analysed in CHEK2, BRIP1 and PALB2**

| Gene | Mutation | Alteration | Galway Early Onset/ Familial | Galway Unselected | Controls |
|---|---|---|---|---|---|
| CHEK2 | 1100delC | | 0/192 | 5/711 | 1/1016 |
| BRIP | 2392 C>T | R798X | 1/192 | 0/711 | - |
| BRIP | 141 del C | Premature Truncation | 0/192 | - | - |
| BRIP | IVS17+2insT | Exon 17 or 18 skipped | 0/192 | - | - |
| BRIP | 2255delAA | Premature Truncation | 0/192 | - | - |
| BRIP | 2108delAinsTCC | Premature Truncation | 0/192 | - | - |
| BRIP | 2008insT | Premature Truncation | 0/192 | - | - |
| PALB2 | 2386 G>T | G796X | 0/192 | - | - |
| PALB2 | 2982 insT | A995 fs | 0/192 | - | - |
| PALB2 | 3113 G >A | W1038X | 0/192 | - | - |
| PALB2 | 3116 delA | N1039 fs | 0/192 | - | - |
| PALB2 | 3549 C >G | Y1183X | 0/192 | - | - |

**Table 2: Histological Characteristics in 192 patients identified with early onset/familial breast cancer. Estrogen receptor status is denoted by ER.**

| Histological Characteristics | % of Cases |
|---|---|
| Average Age | 42 (25-55) |
| Ductal | 77 |
| Lobular | 7 |
| Unknown | 16 |
| Grade 1 | 9 |
| Grade 2 | 30 |
| Grade 3 | 36 |
| Unknown grade | 25 |
| ER positive | 51 |
| ER Negative | 25 |
| ER unknown | 24 |
| Node positive | 39 |
| Node negative | 46 |
| Node Unknown | 28 |
| Family History of 1st degree relative with breast cancer | 18 |
| 2 1st degree relatives affected | 3 |
| 3 1st degree relatives affected | 1 |
| Family History of 2nd degree relative with breast cancer | 36 |
| 2 x 2nd degree relatives affected | 10 |
| 3 x 2nd degree relatives affected | 2 |
| Both 1st and 2nd degree relatives affected | 5 |
| Bilateral | 10 |

**Table 3: Clinicopathological details of CHEK2_1100delC mutation carriers;**

| Clinical Details | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Age at Diagnosis | 67 | 71 | 56 | 55 | 60 |
| Histological Type | Ductal | UNK | Ductal | Ductal | Lobular |
| Grade | UNK | UNK | 3 | UNK | UNK |
| ER<br>PR<br>HER2 | NEG<br>NEG<br>NEG | UNK<br>UNK<br>UNK | POS<br>POS<br>NEG | POS<br>POS<br>NEG | POS<br>POS<br>NEG |
| Family History of breast cancer | No | No | No | No | Aunt, 2 First Cousins, Grandmother. |
| Family History of any cancer | Sister-Colon<br>Mother-Leukaemia | No | No | Brother- Testicular<br>Mother- Leukaemia | No |

**Table 4: Summary of studies looking at BRIP1 mutations in breast cancer**

| | **BRIP1** | | | | | |
|---|---|---|---|---|---|---|
| **Country** | **Author** | **Whole gene screened** | **Truncating Mutations Detected** | **Cases** | **Controls** | |
| UK | Seal[5] | Yes | 141del C<br>2392 C>~~T~~><br>IVS17+INST<br>2008insT<br>2255del AA<br>2108delAinsTCC | 9/1,212 | 2/2,081 | Familial |
| China | Cao[11] | Yes | | 0/357 | 0/864 | Familial |
| Canada | Guenard[12] | | | 0/96 | 0/73 | Familial |
| USA | Rutter[13] | Yes | | 0/58 | 0/30 | Familial |
| Italy | De Nicolo[29] | Yes | 2992-2995delAAGA | 1/49 | | Familial |
| Australia | Lewis[16] | Yes | 3401delC<br>(did not segregate with breast cancer) | 1/75<br>(did not segregate with breast cancer) | 0/93 | Familial |
| Finland | Vahteristo et al [17] | Yes | | 0/43 | | Familial |
| Ireland | BIGGS | No only<br>141del C<br>*2392 C>T*<br>IVS17+INST<br>2008insT<br>2255del AA<br>2108delAinsTCC | *2392 C>T* | 1/192 | | Familial/ early onset |

**Table 5: Summary of studies looking at PALB2 mutations in breast cancer**

| Country | Author | Whole gene screened | Truncating Mutations Detected | Cases | Controls | |
|---|---|---|---|---|---|---|
| UK | Rahman [6] | Yes | 2386G >T<br>2982insT<br>3113G >A<br>3116delA<br>3549C >G | 10/923 | 0/1,084 | Familial |
| China | Cao[30] | Yes | 751C>T<br>1050_1051del AAinsTCT | 3/360 | 0/864 | Familial |
| Canada | Foulkes[21] | Yes | 2323 C>T | 1/50<br>2/356 | 0/6440 | Familial<br>Early onset |
| Spain | Garcia[20] | Yes | 1056_1057del GA | 1/95 | | Familial |
| USA | Tishkowitz[24] | Yes | 229delT | 1/68 | | Familial |
| South Africa | Sluiter[31] | Yes | 697delG | 1/48 | | Early onset |
| Iceland | Gunnarsson[25] | No<br>1592delT only screened | | 0/61<br>0/638 | | Familial<br>Unselected |
| Finland | Erkko[22] | Yes | 1592delT | 3/113<br>18/1918 | 6/2501 | Familial<br>Unselected |
| Finland | Heikkinen[32] | No<br>1592delT only screened | 1592delT | 19/947<br>8/1274 | 2/1079 | Familial<br>Unselected |
| Ireland | BIGGS | No<br>2386G >T<br>2982insT<br>3113G >A<br>3116delA<br>3549C >G<br>Sreened | | 0/192 | | Familial/ early onset |

**Figure Legends:**

**Figure 1**

(A): Normal nucleotide sequence at nucleotide 1100 in the CHEK2 gene as indicated by the presence of a cytosine at position 1100.

(B): Single base cytosine deletion at nucleotide 1100 resulting in abrogated protein function in CHEK2_1100delC mutation carriers.

(C): CHEK2_1100delC mutation detected by PCR - 167bp fragments in normal individuals and additional peaks at 166bp indicating deletion of 1bp in mutation carrier.

**Figure 2**

(A): Nucleotide sequence at position 2392 in the BRIP1 gene in the DNA of normal individual.

(B): BRIP1 2392 mutation demonstrating the C-T transition at nucleotide 2392 resulting in the generation of a stop codon in the encoded protein.

**Figure 3:** Meta-analysis of CHEK2 European studies (excluding familial studies)

**Figure 4:** Meta-analysis of PALB2 studies

**Figure 1**

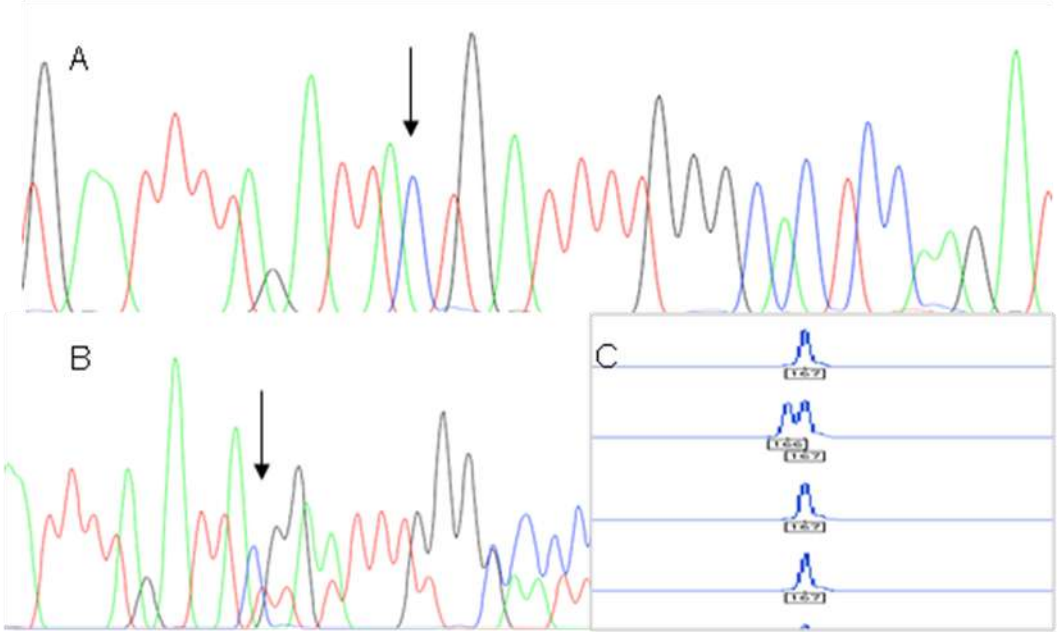


**Figure 2**

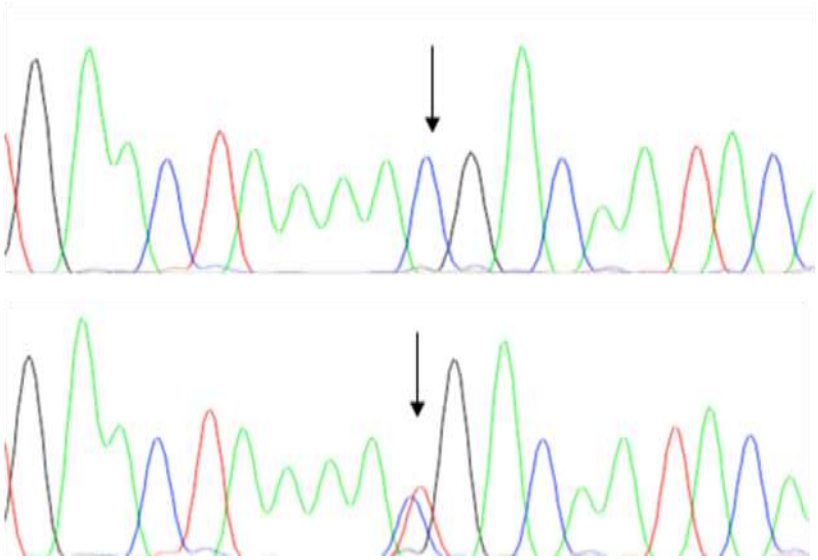


Figure 3: Meta-analysis of CHEK2 European studies (excluding familial studies).

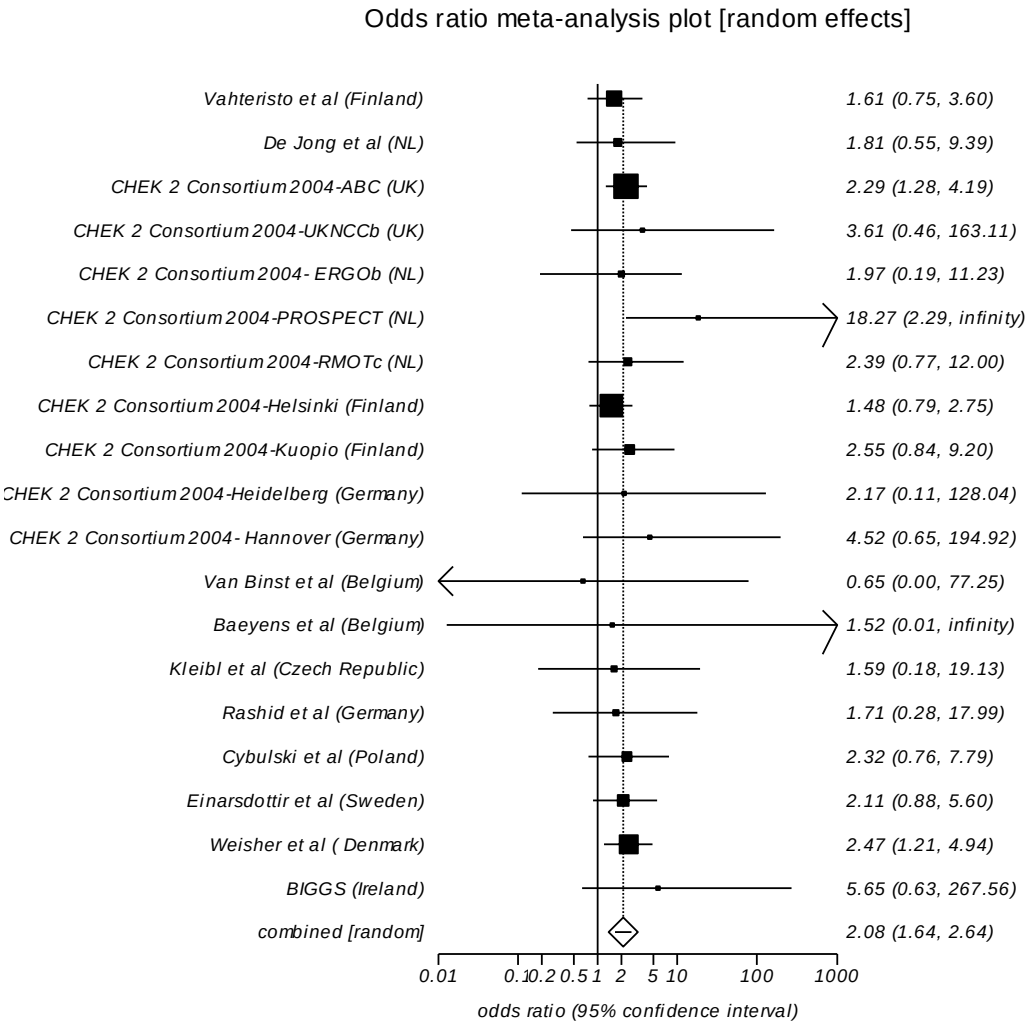
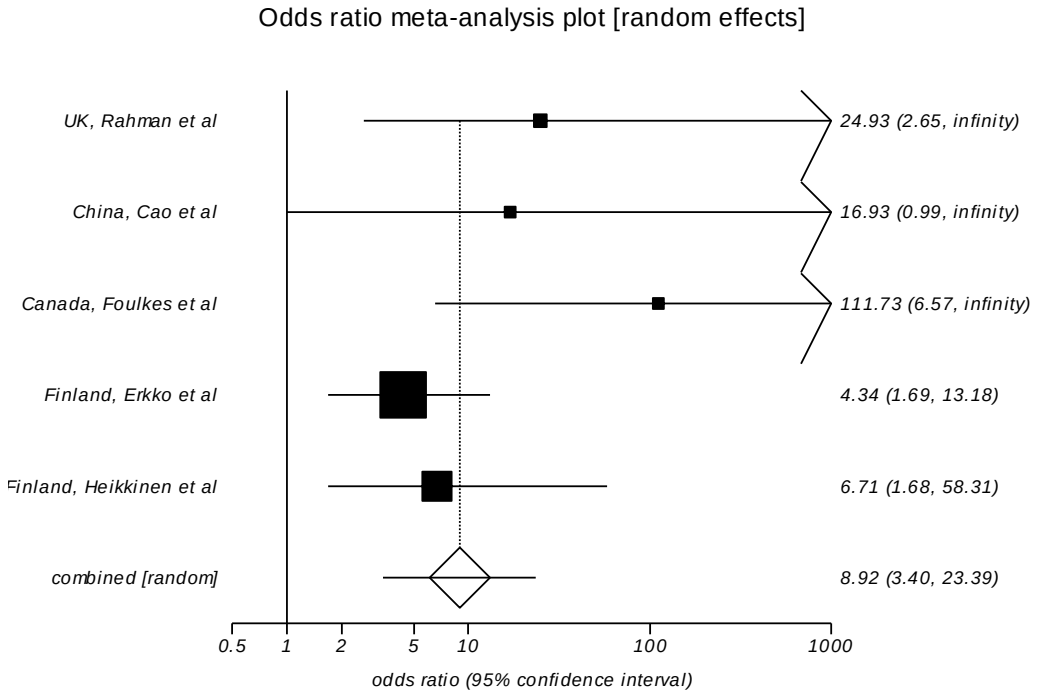


Figure 4: Meta-analysis of PALB2 studies



**Supplementary Table 1: Primers used for CHEK2_1100delC, BRIP1 and PALB2 mutations**

| | |
|---|---|
| CHEK2-1100delC-f | TGTCTTCTTGGACTGGCAGA |
| CHEK2-1100delC-r | TTCAGGCGCCAAGTAGGTGG |
| BRIP1-2392 C>T-f | TGAAAAACAAAATAAAATCTCTACCC |
| BRIP1-2392 C>T-r | ATACCACTGACGGCCAGGTA |
| BRIP1-141 del C-f | TCAGAGGATTAAACAGCAAGCA |
| BRIP1-141 del C-r | CACTAAGAGATTGTTGCCATGC |
| BRIP1-IVS17+2-146-f | TTAAACCAGGCCCTTGGTAG |
| BRIP1-IVS17+2-146-r | CCAGTTCCTATGGTTCCAGTT |
| BRIP1-2008insT-99-f | GGTTTGGGTTGGTACCATTG |
| BRIP1-2008insT-99-r | GCTCCCACTTCATCTTGGAA |
| BRIP1-2255delAA-114-f | GAACCACAGGGAGGAGAAAA |
| BRIP1-2255delAA-114-r | TTTTCACCGACCATGAAATAA |
| BRIP1-2108delA-124-f | GCCGTAGTCACATTGGCTTA |
| BRIP1-2108delA-124-r | CCAGTAGAGAGCCAACGTTCTT |
| PALB2-2386-f | AGCCCAGCAAAACCACATAC |
| PALB2-2386-r | TCGACGGAATGTTTATGCAG |
| PALB2-3113-f | AAGGGATGCAAGAAGCTCTG |
| PALB2-3113-r | CACCTGGGTGATAGGAGGAG |
| PALB2-3549-f | GGCTGGACAAAAAGATGGAA |
| PALB2-3549-r | CATCCAAGATCAGTGGTGCT |
| PALB2-2982-f | CTTGGCCTGACAAAGAGGAG |
| PALB2-2982-r | CCCAACTTTCTCTGAAACCTGT |
| BRIPR798X_ALT (KASPar) | GAAGGTGACCAAGTTCATGCTAATTTTGAATGGTGGTCATTGTATTGTCA |
| BRIPR798X_ALC (KASPar) | GAAGGTCGGAGTCAACGGATTTTGAATGGTGGTCATTGTATTGTCG |
| BRIPR798X_C1 (KASPar) | ATATTTTAAAATTATTAGGTTGAACTAAAA |

**Supplementary Table 2:** Summary of Northern/Central European *CHEK2_1100delC* studies ( excluding all familial and early onset studies)

| Study | Country | Cases | total | % | Controls | total | % |
|---|---|---|---|---|---|---|---|
| Vahteristo et al[33] | Finland | 21 | 1035 | 2.0 | 12 | 943 | 1.3 |
| De Jong et al[34] | NL | 28 | 962 | 2.9 | 3 | 184 | 1.6 |
| CHEK 2 Consortium 2004-ABC[4] | UK | 35 | 2886 | 1.2 | 20 | 3749 | 0.5 |
| CHEK 2 Consortium 2004-UKNCCb[35] | UK | 7 | 564 | 1.2 | 1 | 288 | 0.3 |
| CHEK 2 Consortium 2004-ERGO[35] | NL | 2 | 79 | 2.5 | 6 | 460 | 1.3 |
| CHEK 2 Consortium 2004-PROSPECT[4] | NL | 35 | 1066 | 3.3 | 0 | 265 | 0.0 |
| CHEK 2 Consortium 2004-RMOTc[4, 33] | NL | 65 | 1706 | 3.8 | 3 | 184 | 1.6 |
| CHEK 2 Consortium 2004-Helsinki[33] | Finland | 21 | 1035 | 2.0 | 26 | 1885 | 1.4 |
| CHEK 2 Consortium 2004-Kuopio[4] | Finland | 13 | 464 | 2.8 | 5 | 447 | 1.1 |
| CHEK 2 Consortium 2004-Heidelberg[4] | Germany | 2 | 601 | 0.3 | 1 | 650 | 0.2 |
| CHEK 2 Consortium 2004-Hannover[4] | Germany | 11 | 985 | 1.1 | 1 | 401 | 0.2 |
| Van Binst et al[36] | Belgium | 0 | 52 | 0.0 | 1 | 103 | 1.0 |
| Baeyens et al[37] | Belgium | 1 | 100 | 1.0 | 0 | 50 | 0.0 |
| Kleibl et al[38] | Czech republic | 3 | 688 | 0.4 | 2 | 730 | 0.3 |
| Rashid et al[39] | Germany | 5 | 613 | 0.8 | 2 | 417 | 0.5 |
| Cybulski et al[40] | Poland | 10 | 1978 | 0.5 | 6 | 2748 | 0.2 |
| Einarsdottir et al[41] | Sweden | 19 | 1509 | 1.3 | 8 | 1334 | 0.6 |
| Weisher et al[42] | Denmark | 16 | 1374 | 1.2 | 22 | 4633 | 0.5 |
| BIGGS | Ireland | 5 | 903 | 0.6 | 1 | 1016 | 0.1 |