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

N. M. Mcinerney, N. Miller, A. Rowan, G. Colleran, E. Barclay, C. Curran, M. J. Kerin, I. P. Tomlinson, E. Sawyer

► To cite this version:

N. M. Mcinerney, N. Miller, A. Rowan, G. Colleran, E. Barclay, et al.. Evaluation of variants in the CHEK2, BRIP1 and PALB2 genes in an Irish breast cancer cohort. Breast Cancer Research and Treatment, 2009, 121 (1), pp.203-210. 10.1007/s10549-009-0540-9 . hal-00485069

HAL Id: hal-00485069

<https://hal.science/hal-00485069>

Submitted on 20 May 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

McInerney NM^{1,2}, Miller N¹, Rowan A², Collieran G^{1,2}, Barclay E², Curran C¹, Kerin MJ¹, Tomlinson IP², Sawyer E^{2,3}.

¹Department of Surgery, Clinical Science Institute, University College Hospital, Galway, Ireland

²Molecular and Population Genetics Laboratory, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN

³Guy's, King's, St Thomas' Cancer Centre, Guy's Hospital, St Thomas's Street, London SE1 9RT, UK

Corresponding Author: Niall McInerney, Department of Surgery, Clinical Science Institute, University College Hospital, Galway, Ireland Fax 00 353 91, Tel 00 353 86 4011740
e-mail address: nmac@ymail.com

Key Words: breast cancer, genetic susceptibility, West Ireland, CHEK2, BRIP1, PALB2

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 European 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 carriers [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 findings 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 non 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 (χ^2 test $p=0.13$) to that in the UK (1.2% in cases and 0.53% controls, ABC study) but significantly less (χ^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 χ^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 χ^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.

Acknowledgements

We are grateful to the patients in the study and colleagues involved in patient ascertainment and sample collection. Help was also kindly provided by the Equipment Park, London Research Institute, Cancer Research UK. NM and GC were funded by the National Breast Cancer Research Institute, Ireland. ES is funded by a Department of Health Clinician Award and via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust

References

1. Collaborative Group on Hormonal Factors in Breast Cancer: **Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease.** *Lancet* 2001, **358**(9291):1389-1399.
2. Anglian Breast Cancer Study Group: **Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases.** *Br J Cancer* 2000, **83**(10):1301-1308.
3. Turnbull C, Rahman N: **Genetic predisposition to breast cancer: past, present, and future.** *Annu Rev Genomics Hum Genet* 2008, **9**:321-345.
4. CHEK2 Breast Cancer Case-Control Consortium: **CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies.** *Am J Hum Genet* 2004, **74**(6):1175-1182.
5. Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, Chagtai T, Jayatilake H, Ahmed M, Spanova K *et al*: **Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles.** *Nat Genet* 2006, **38**(11):1239-1241.
6. Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T *et al*: **PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene.** *Nat Genet* 2007, **39**(2):165-167.
7. Osorio A, Rodriguez-Lopez R, Diez O, de la Hoya M, Ignacio Martinez J, Vega A, Esteban-Cardenosa E, Alonso C, Caldes T, Benitez J: **The breast cancer low-penetrance allele 1100delC in the CHEK2 gene is not present in Spanish familial breast cancer population.** *Int J Cancer* 2004, **108**(1):54-56.
8. Bellosillo B, Tusquets I, Longaron R, Perez-Lezaun A, Bellet M, Fabregat X, Serrano S, Sole F: **Absence of CHEK2 mutations in Spanish families with hereditary breast cancer.** *Cancer Genet Cytogenet* 2005, **161**(1):93-95.
9. Chen W, Yurong S, Liansheng N: **Breast cancer low-penetrance allele 1100delC in the CHEK2 gene: Not present in the Chinese familial breast cancer population.** *Adv Ther* 2008, **25**(5):496-501.
10. Pharoah PD, Antoniou AC, Easton DF, Ponder BA: **Polygenes, risk prediction, and targeted prevention of breast cancer.** *N Engl J Med* 2008, **358**(26):2796-2803.
11. Cao AY, Huang J, Hu Z, Li WF, Ma ZL, Tang LL, Zhang B, Su FX, Zhou J, Di GH *et al*: **Mutation analysis of BRIP1/BACH1 in BRCA1/BRCA2 negative Chinese women with early onset breast cancer or affected relatives.** *Breast Cancer Res Treat* 2008.
12. Guenard F, Labrie Y, Ouellette G, Joly Beauparlant C, Simard J, Durocher F: **Mutational analysis of the breast cancer susceptibility gene BRIP1 /BACH1/FANCI in high-risk non-BRCA1/BRCA2 breast cancer families.** *J Hum Genet* 2008, **53**(7):579-591.

13. Rutter JL, Smith AM, Davila MR, Sigurdson AJ, Giusti RM, Pineda MA, Doody MM, Tucker MA, Greene MH, Zhang J *et al*: **Mutational analysis of the BRCA1-interacting genes ZNF350/ZBRK1 and BRIP1/BACH1 among BRCA1 and BRCA2-negative probands from breast-ovarian cancer families and among early-onset breast cancer cases and reference individuals.** *Hum Mutat* 2003, **22**(2):121-128.
14. Sigurdson AJ, Hauptmann M, Chatterjee N, Alexander BH, Doody MM, Rutter JL, Struewing JP: **Kin-cohort estimates for familial breast cancer risk in relation to variants in DNA base excision repair, BRCA1 interacting and growth factor genes.** *BMC Cancer* 2004, **4**:9.
15. Song H, Ramus SJ, Kjaer SK, Hogdall E, Dicioccio RA, Whittemore AS, McGuire V, Hogdall C, Jacobs IJ, Easton DF *et al*: **Tagging single nucleotide polymorphisms in the BRIP1 gene and susceptibility to breast and ovarian cancer.** *PLoS ONE* 2007, **2**(3):e268.
16. Lewis AG, Flanagan J, Marsh A, Pupo GM, Mann G, Spurdle AB, Lindeman GJ, Visvader JE, Brown MA, Chenevix-Trench G: **Mutation analysis of FANCD2, BRIP1/BACH1, LMO4 and SFN in familial breast cancer.** *Breast Cancer Res* 2005, **7**(6):R1005-1016.
17. Vahteristo P, Yliannala K, Tamminen A, Eerola H, Blomqvist C, Nevanlinna H: **BACH1 Ser919Pro variant and breast cancer risk.** *BMC Cancer* 2006, **6**:19.
18. Frank B, Hemminki K, Meindl A, Wappenschmidt B, Sutter C, Kiechle M, Bugert P, Schmutzler RK, Bartram CR, Burwinkel B: **BRIP1 (BACH1) variants and familial breast cancer risk: a case-control study.** *BMC Cancer* 2007, **7**:83.
19. Garcia-Closas M, Egan KM, Newcomb PA, Brinton LA, Titus-Ernstoff L, Chanock S, Welch R, Lissowska J, Peplonska B, Szeszenia-Dabrowska N *et al*: **Polymorphisms in DNA double-strand break repair genes and risk of breast cancer: two population-based studies in USA and Poland, and meta-analyses.** *Hum Genet* 2006, **119**(4):376-388.
20. Garcia MJ, Fernandez V, Osorio A, Barroso A, Llorc G, Lazaro C, Blanco I, Caldes T, de la Hoya M, Ramon YCT *et al*: **Analysis of FANCB and FANCN/PALB2 Fanconi Anemia genes in BRCA1/2-negative Spanish breast cancer families.** *Breast Cancer Res Treat* 2008.
21. Foulkes WD, Ghadirian P, Akbari MR, Hamel N, Giroux S, Sabbaghian N, Darnel A, Royer R, Poll A, Fafard E *et al*: **Identification of a novel truncating PALB2 mutation and analysis of its contribution to early-onset breast cancer in French-Canadian women.** *Breast Cancer Res* 2007, **9**(6):R83.
22. Erkko H, Xia B, Nikkila J, Schleutker J, Syrjakoski K, Mannermaa A, Kallioniemi A, Pylkas K, Karppinen SM, Rapakko K *et al*: **A recurrent mutation in PALB2 in Finnish cancer families.** *Nature* 2007, **446**(7133):316-319.
23. Erkko H, Dowty JG, Nikkila J, Syrjakoski K, Mannermaa A, Pylkas K, Southey MC, Holli K, Kallioniemi A, Jukkola-Vuorinen A *et al*: **Penetrance Analysis of the PALB2 c.1592delT Founder Mutation.** *Clin Cancer Res* 2008, **14**(14):4667-4671.
24. Tischkowitz M, Xia B, Sabbaghian N, Reis-Filho JS, Hamel N, Li G, van Beers EH, Li L, Khalil T, Quenneville LA *et al*: **Analysis of PALB2/FANCN-**

- associated breast cancer families.** *Proc Natl Acad Sci U S A* 2007, **104**(16):6788-6793.
25. Gunnarsson H, Arason A, Gillanders EM, Agnarsson BA, Johannesdottir G, Johannsson OT, Barkardottir RB: **Evidence against PALB2 involvement in Icelandic breast cancer susceptibility.** *J Negat Results Biomed* 2008, **7**:5.
 26. Hill EW, Jobling MA, Bradley DG: **Y-chromosome variation and Irish origins.** *Nature* 2000, **404**(6776):351-352.
 27. McInerney N, Collieran G, Rowan A, Walther A, Barclay E, Spain S, Jones AM, Tuohy S, Curran C, Miller N *et al*: **Low penetrance breast cancer predisposition SNPs are site specific.** *Breast Cancer Res Treat* 2008.
 28. Weischer M, Bojesen SE, Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG: **CHEK2*1100delC genotyping for clinical assessment of breast cancer risk: meta-analyses of 26,000 patient cases and 27,000 controls.** *J Clin Oncol* 2008, **26**(4):542-548.
 29. De Nicolo A, Tancredi M, Lombardi G, Flemma CC, Barbuti S, Di Cristofano C, Sobhian B, Bevilacqua G, Drapkin R, Caligo MA: **A novel breast cancer-associated BRIP1 (FANCI/BACH1) germ-line mutation impairs protein stability and function.** *Clin Cancer Res* 2008, **14**(14):4672-4680.
 30. Cao AY, Huang J, Hu Z, Li WF, Ma ZL, Tang LL, Zhang B, Su FX, Zhou J, Di GH *et al*: **The prevalence of PALB2 germline mutations in BRCA1/BRCA2 negative Chinese women with early onset breast cancer or affected relatives.** *Breast Cancer Res Treat* 2008.
 31. Sluiter M, Mew S, van Rensburg EJ: **PALB2 sequence variants in young South African breast cancer patients.** *Fam Cancer* 2009.
 32. Heikkinen T, Karkkainen H, Aaltonen K, Milne RL, Heikkila P, Aittomaki K, Blomqvist C, Nevanlinna H: **The breast cancer susceptibility mutation PALB2 1592delT is associated with an aggressive tumor phenotype.** *Clin Cancer Res* 2009, **15**(9):3214-3222.
 33. Vahteristo P, Bartkova J, Eerola H, Syrjakoski K, Ojala S, Kilpivaara O, Tamminen A, Kononen J, Aittomaki K, Heikkila P *et al*: **A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer.** *Am J Hum Genet* 2002, **71**(2):432-438.
 34. de Jong MM, Nolte IM, Te Meerman GJ, van der Graaf WT, Oosterom E, Bruinenberg M, Steege G, Oosterwijk JC, van der Hout AH, Boezen HM *et al*: **No increased susceptibility to breast cancer from combined CHEK2 1100delC genotype and the HLA class III region risk factors.** *Eur J Cancer* 2005, **41**(12):1819-1823.
 35. Meijers-Heijboer H, van den Ouweland A, Klijn J, Wasielewski M, de Snoo A, Oldenburg R, Hollestelle A, Houben M, Crepin E, van Veghel-Plandsoen M *et al*: **Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations.** *Nat Genet* 2002, **31**(1):55-59.
 36. Van Binst T GG, Sermijn E, Van Hassel G, De Neef K, Bonduelle M, Fontaine C, Teugels E, and De Greve JJ: **The CHEK2*1100delC mutation predisposes for early-onset breast cancer in breast cancer families.** *Breast Cancer Res Treat* 2004, **88**: S151.

37. Baeyens A, Claes K, Willems P, De Ruyck K, Thierens H, Vral A: **Chromosomal radiosensitivity of breast cancer with a CHEK2 mutation.** *Cancer Genet Cytogenet* 2005, **163**(2):106-112.
38. Kleibl Z, Novotny J, Bezdicikova D, Malik R, Kleiblova P, Foretova L, Petruzelka L, Ilencikova D, Cinek P, Pohlreich P: **The CHEK2 c.1100delC germline mutation rarely contributes to breast cancer development in the Czech Republic.** *Breast Cancer Res Treat* 2005, **90**(2):165-167.
39. Rashid MU, Jakubowska A, Justenhoven C, Harth V, Pesch B, Baisch C, Pierl CB, Bruning T, Ko Y, Benner A *et al*: **German populations with infrequent CHEK2*1100delC and minor associations with early-onset and familial breast cancer.** *Eur J Cancer* 2005, **41**(18):2896-2903.
40. Cybulski C, Wokolorczyk D, Huzarski T, Byrski T, Gronwald J, Gorski B, Debniak T, Masojc B, Jakubowska A, van de Wetering T *et al*: **A deletion in CHEK2 of 5,395 bp predisposes to breast cancer in Poland.** *Breast Cancer Res Treat* 2007, **102**(1):119-122.
41. Einarsdottir K, Humphreys K, Bonnard C, Palmgren J, Iles MM, Sjolander A, Li Y, Chia KS, Liu ET, Hall P *et al*: **Linkage disequilibrium mapping of CHEK2: common variation and breast cancer risk.** *PLoS Med* 2006, **3**(6):e168.
42. Weischer M, Bojesen SE, Tybjaerg-Hansen A, Axelsson CK, Nordestgaard BG: **Increased risk of breast cancer associated with CHEK2*1100delC.** *J Clin Oncol* 2007, **25**(1):57-63.

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 1 st degree relative with breast cancer	18
2 1 st degree relatives affected	3
3 1 st degree relatives affected	1
Family History of 2 nd degree relative with breast cancer	36
2 x 2 nd degree relatives affected	10
3 x 2 nd degree relatives affected	2
Both 1 st and 2 nd 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	NEG	UNK	POS	POS	POS
PR	NEG	UNK	POS	POS	POS
HER2	NEG	UNK	NEG	NEG	NEG
Family History of breast cancer	No	No	No	No	Aunt, 2 First Cousins, Grandmother.
Family History of any cancer	Sister-Colon Mother-Leukaemia	No	No	Brother- Testicular 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 2392 C>T IVS17+INST 2008insT 2255del AA 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 (did not segregate with breast cancer)	1/75 (did not segregate with breast cancer)	0/93	Familial
Finland	Vahteristo et al [17]	Yes		0/43		Familial
Ireland	BIGGS	No only 141del C 2392 C>T IVS17+INST 2008insT 2255del AA 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 2982insT 3113G >A 3116delA 3549C >G	10/923	0/1,084	Familial
China	Cao[30]	Yes	751C>T 1050_1051del AAinsTCT	3/360	0/864	Familial
Canada	Foulkes[21]	Yes	2323 C>T	1/50 2/356	0/6440	Familial 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 1592delT only screened		0/61 0/638		Familial Unselected
Finland	Erkko[22]	Yes	1592delT	3/113 18/1918	6/2501	Familial Unselected
Finland	Heikkinen[32]	No 1592delT only screened	1592delT	19/947 8/1274	2/1079	Familial Unselected
Ireland	BIGGS	No 2386G >T 2982insT 3113G >A 3116delA 3549C >G Screened		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	CCAGTTCCTATGGTCCAGTT
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)	GAAGGTGACCAAGTTCATGCTAATTTGAATGGTGGTCATTGTATTGTCA
BRIPR798X_ALC (KASPar)	GAAGGTGCGAGTCAACGGATTTTGAATGGTGGTCATTGTATTGTGCG
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