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Breast Cancer Research 2011, **13**:R20 doi:10.1186/bcr2832

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ISSN 1465-5411

Article type Research article

Submission date 9 August 2010

Acceptance date 28 February 2011

Publication date 28 February 2011

Article URL <http://breast-cancer-research.com/content/13/1/R20>

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Screening for *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *BRIP1*, *RAD50*, and *CDH1* mutations in high-risk Finnish *BRCA1/2*-founder mutation-negative breast and/or ovarian cancer individuals

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Abstract

Introduction: Two major high-penetrance breast cancer genes, *BRCA1* and *BRCA2*, are responsible for approximately 20% of hereditary breast cancer (HBC) cases in Finland. Additionally, rare mutations in several other genes that interact with *BRCA1* and *BRCA2* increase the risk of HBC. Still, a majority of HBC cases remain unexplained which is challenging for genetic counseling. We aimed to analyze additional mutations in HBC-associated genes and to define the sensitivity of our current *BRCA1/2* mutation analysis protocol used in genetic counseling.

Methods: Eighty-two well-characterized, high-risk hereditary breast and/or ovarian cancer (HBOC) *BRCA1/2*-founder mutation-negative Finnish individuals, were screened for germline alterations in seven breast cancer susceptibility genes, *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *BRIP1*, *RAD50*, and *CDH1*. *BRCA1/2* were analyzed by multiplex ligation-dependent probe amplification (MLPA) and direct sequencing. *CHEK2* was analyzed by the high resolution melt (HRM) method and *PALB2*, *RAD50*, *BRIP1* and *CDH1* were analyzed by direct sequencing. Carrier frequencies between 82 (HBOC) *BRCA1/2*-founder mutation-negative Finnish individuals and 384 healthy Finnish population controls were compared by using Fisher's exact test. *In silico* prediction for novel missense variants effects was carried out by using Pathogenic-Or-Not –Pipeline (PON-P).

Results: Three previously reported breast cancer-associated variants, *BRCA1* c.5095C>T, *CHEK2* c.470T>C, and *CHEK2* c.1100delC, were observed in eleven (13.4%) individuals. Ten of these individuals (12.2%) had *CHEK2* variants, c.470T>C and/or c.1100delC. Fourteen novel sequence alterations and nine individuals with more than one non-synonymous variant were identified. One of the novel variants, *BRCA2* c.72A>T

(Leu24Phe) was predicted to be likely pathogenic *in silico*. No large genomic rearrangements were detected in *BRCA1/2* by multiplex ligation-dependent probe amplification (MLPA).

Conclusions: In this study, mutations in previously known breast cancer susceptibility genes can explain 13.4% of the analyzed high-risk *BRCA1/2*-negative HBOC individuals. *CHEK2* mutations, c.470T>C and c.1100delC, make a considerable contribution (12.2%) to these high-risk individuals but further segregation analysis is needed to evaluate the clinical significance of these mutations before applying them in clinical use. Additionally, we identified novel variants that warrant additional studies. Our current genetic testing protocol for 28 Finnish *BRCA1/2*-founder mutations and protein truncation test (PTT) of the largest exons is sensitive enough for clinical use as a primary screening tool.

Introduction

Breast cancer (BrCa) is the most common cancer among women in Finland, with about 4000 diagnosed cases yearly (Finnish Cancer Registry). It has been estimated that a monogenic trait accounts for 5-10 % of all BrCa cases [1]. The two major high-penetrance BrCa genes, *BRCA1* (*breast cancer 1*) and *BRCA2* (*breast cancer 2*), are responsible for 30 % of hereditary breast cancer (HBC) cases worldwide, but only for about 20 % in Finland [2-4]. *BRCA2* mutations have been found to be more common in the Finnish population than *BRCA1* [5]. In addition to *BRCA1* and *BRCA2* mutations, there are certain hereditary cancer syndromes, such as Li-Fraumeni, Cowden, Peutz-Jeghers and diffuse gastric cancer syndromes, associated with a high risk of BrCa [6-9]. However, these syndromes very seldom explain HBC.

BRCA1 and *BRCA2* have many DNA damage response functions in the cell [10]. Therefore, it has been hypothesized that genes coding for proteins that interact with *BRCA1/2* or act in the same DNA repair pathway would be likely candidate genes for HBC susceptibility. As expected, *CHEK2* (*checkpoint kinase 2*), *PALB2* (*partner and localizer of BRCA2*), *BRIP1* (*BRCA1-interacting protein 1*), and *RAD50* (*human homolog of S. cerevisiae RAD50*) have been shown to have rare, moderate-risk BrCa-associated variants, which have also been studied in the Finnish population [11-14]. In addition, BrCa-associated variants have been reported in the *CDH1* (*cadherin-1*) [15].

Although mutations in many genes have been found to predispose an individual to BrCa, approximately 75-80% of HBC cases remain unexplained [16]. It is likely that additional BrCa susceptibility gene mutations remain unidentified, especially in the category of moderate- to low-penetrance gene variants that individually confer only minimal risk but

that through multiplicative and/or cumulative effects, can cause relatively high risk for the carriers [17]. Genome-wide association studies (GWAs) have revealed multiple low-penetrance, single nucleotide polymorphisms (SNPs) in many genes and chromosomal loci with increased risk of BrCa. For example, SNPs in the *fibroblast growth factor receptor 2* (*FGFR2*) gene have shown significant association with increased risk among BrCa cases with strong family history [18].

To address the problem of heterogeneous HBC in genetic counselling, we wanted to investigate possible additional mutations in HBC-associated genes. The aim of this study was to screen seven known BrCa susceptibility genes for additional mutations in 82 well-characterized, Finnish, high-risk hereditary breast and/or ovarian cancer (HBOC) individuals tested to be *BRCA1/2*-founder mutation negative. In addition, the sensitivity of our current *BRCA1/2* mutation analysis protocol was defined for genetic counseling purposes.

Materials and methods

Patients and controls

Index individuals of 82 high-risk Finnish HBOC families were screened for germline alterations in BrCa-associated genes. All individuals had been detected to be founder mutation-negative by minisequencing of the previously known 28 Finnish *BRCA1/2* mutations and by protein truncation test (PTT) of exon 11 for *BRCA1* and exons 10 and 11 for *BRCA2*. Study material had been collected from the individuals, who visited the Tampere University Hospital Genetics Outpatient Clinic between January 1997 and May 2008. The hospital district, in the area of Pirkanmaa, consists of over 20% (1.23 million) of the Finnish population. Individuals were chosen into this study according to the following criteria of high-risk HBC: (a) the individual or her first-degree relative (only female family members were included when defining first-degree relatives) had BrCa or OvCa at <30 years of age; or (b) two first-degree relatives in the family had BrCa and/or OvCa and at least one of the cancers had been diagnosed at <40 years of age; or (c) three first-degree relatives in the family had BrCa and/or OvCa and at least one of the cancers had been diagnosed at <50 years of age; or (d) four or more first-degree relatives had BrCa and/or OvCa at any age; or (e) the same individual had BrCa and OvCa. Patient with bilateral breast cancer was considered to have two separate cancers. According to these criteria, our study material also included 11 non-affected females in addition to 71 BrCa and/or OvCa patients. We were also able to get blood samples from two affected relatives in two out of 11 separate families with healthy index. These relatives with breast cancer were screened for the same variant as that identified in the index. The clinical data of the studied individuals is presented in Table 1. As controls, 384 blood samples from anonymous healthy females, collected from the Finnish Red Cross, were used. All individuals have been informed of the analyses, and they have given a written consent to

use their already existing DNA samples. Permission for the research project has been received from the Ethical Committee of Tampere University Hospital and the National Authority for Medicolegal Affairs.

Mutation detection

DNA samples of the individuals were kindly received from the Tampere University Hospital Genetics Outpatient Clinic. Mutation screening for *BRCA1*, *BRCA2*, *PALB2*, *BRIP1*, *RAD50*, and *CDH1* was done by direct sequencing. Whole coding regions and exon-intron boundaries were analyzed. Primer sequences for *PALB2*, *BRIP1*, and *RAD50* have been reported previously [12, 13, 19]. Primers for *BRCA1* and *BRCA2* (excluding previously analyzed exon 11 for *BRCA1* and exons 10 and 11 for *BRCA2*) and *CDH1* were designed by using Primer3 software [20]. *CHEK2* was screened by using high-resolution melt (HRM) analysis on a Bio-Rad platform (Bio-Rad Laboratories Headquarters, Hercules, CA, USA). Sequencing was carried out using the Big Dye Terminator v.3.1 Cycle Sequencing Kit and ABIPRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were analyzed with Sequencher v.4.7 software (Gene Codes Corporation, Ann Arbor, MI). Primer sequences, detailed HRM and PCR reaction conditions are available upon request.

Control frequencies were determined for 18 variants by HRM (*CHEK2* variants), direct sequencing (*BRCA1* c.4883T>C and *RAD50* c.1544A>G) and TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA, USA) and with an ABI7900 instrument (Applied Biosystems). Assays were already designed and functionally tested for the following SNPs: c.8182G>A (rs28897749), c.9976A>T (rs11571833), c.10234A>G (rs1801426), and c.1676A>G (rs152451). As for the c.72A>T, c.814G>A, c.1000T>G, and

c.2993G>A (rs45551636) variants, assays were designed by Custom TaqMan® Assay Design Tool (Applied Biosystems) according to manufacturer's instructions.

The multiplex ligation-dependent probe amplification (MLPA) analysis was performed for *BRCA1* and *BRCA2* (SALSA MLPA kit P002-B1 for *BRCA1* (lot 0508) and kit P090-A2 for *BRCA2* (lot 0808), MRC-Holland, Amsterdam, the Netherlands) according to manufacturer's instructions and analyzed with ABIPRISM 3130xl Genetic Analyzer and Genemapper® v.4.0 software (Applied Biosystems, Foster City, CA, USA).

Statistical analyses

Carrier frequencies between 82 studied individuals and 384 population controls were compared by using Fisher's exact test [21]. All P-values were two sided. Odds ratios (OR) were generated by two-by-two table.

***In silico* prediction of novel missense variants effects**

The effects of five novel coding missense variants, *BRCA2* c.72A>T (Leu24Phe), *CHEK2* c.1363G>A (Val455Ile), *PALB2* c.814G>A (Glu272Lys), *PALB2* c.1000T>G (Tyr334Asp), and *RAD50* c.1544A>G (Asp515Gly), were predicted with a number of tools by using Pathogenic-Or-Not –Pipeline (PON-P) [22]. The predictions included those for amino acid tolerance (programs PolyPhen version 2, Sift, PhD-SNP, SNAP) and protein stability (I-Mutant version 3). PON-P allows simultaneous submission of a number of variations and proteins to selected predictors. PON-P utilizes machine learning to combine results from several individual predictions.

MicroRNA database and BLAST search for novel variants

MicroRNA (miRNA) target site search was done for the novel variant genomic positions from the microRNA database (miRBase) [23]. Also BLAST search [24] was performed for the novel human variant genomic positions to see if these sites are conserved among different organisms including mouse, rat, cow and chicken.

Results

Index individuals of 82 high-risk HBOC families were screened for germline alterations in *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *BRIP1*, *RAD50*, and *CDH1* genes. Detailed clinical information of analyzed individuals is shown in Table 1. All of the identified 54 sequence variants with their observed genotype frequencies and rs-numbers are presented in Supplementary Table S1 in Additional file 1. All of the identified non-synonymous and novel sequence alterations are summarized in Table 2. Table 2 variants are presented in Table 3 with index individual and family cancer history. In addition, since our study material included also healthy index individuals from 11 families, we made an effort to get blood samples from two affected relatives in two out of 11 separate families. These relatives with breast cancer were screened for the same variant as that identified in the healthy index. Analysis was performed for the new cases in family 112 (*CHEK2* c.470T>C and *PALB2* c.1676A>G variants) and family 231 (*BRCA1* c.4883T>C variant) (Table 3). In family 112, the case proved to have the same *PALB2* c.1676A>G variant as the index individual but in family 231, the affected relative did not carry the *BRCA1* c.4883T>C variant (data not shown). To further evaluate the impact of these 11 healthy index cases, we recalculated the frequencies without these 11 individuals for those variants accepted to be meaningful for breast cancer risk. Supplementary Table S2 in Additional file 2 shows these recalculated frequencies for *BRCA1* c.5095C>T, *CHEK2* c.470T>C, and *CHEK2* c.1100delC variants. No statistically significant effect was seen for exclusion of the 11 cases.

***BRCA1* and *BRCA2* mutation analysis**

Analysis of *BRCA1* and *BRCA2* revealed altogether 16 different sequence variants, seven in *BRCA1* and nine in *BRCA2* (Supplementary Table S1 in Additional file 1). All but two of the identified variants in *BRCA1*, c.4883T>C and c.5095C>T, have been reported to be

neutral in the databases. Heterozygous c.4883T>C variant was observed in 4/82 (4.9%) women of which three had BrCa and one had a family history of breast, cervix and skin cancers (Tables 2 and 3). In population controls, the frequency of the c.4883T>C variant was 6/367 (1.6%). The c.5095C>T variant has been classified as a deleterious mutation in the Breast Cancer Information Core (BIC) database. The heterozygous c.5095C>T mutation was observed in 1/82 (1.2%) women. The mutation carrying woman had BrCa diagnosed at the age of 42 years and a strong family history of cancer (Tables 2 and 3, Figure 1, Family 249). Additional mutation analysis revealed also two other affected women carrying the c.5095C>T mutation in the same family. In *BRCA2*, three of the nine identified variants were novel, c.68-80insT, c.72A>T, and c.793+34T>G (Tables 2 and 3). The heterozygous missense variant c.72A>T (Leu24Phe), was observed in 1/82 (1.2%) women but not in population controls. The c.72A>T variant carrying woman had BrCa diagnosed at the age of 53 years. She had also two affected first-degree relatives (mother and sister). Protein predictions by PON-P suggested that substitution of leucine by phenylalanine in position 24 changes significantly the properties of the side chain and the substitution would not be tolerated. All the other identified variants in *BRCA2* have been reported previously and they are either neutral or the clinical significance of the variants is yet uncertain especially with the three missense variants, c.8182G>A, c.9976A>T and c.10234A>G (Tables 2 and 3). No deletions or duplication were identified either in *BRCA1* or *BRCA2* by MLPA.

CHEK2 mutation analysis

In *CHEK2*, two previously reported BrCa associated variants in the Finnish population, c.470T>C and c.1100delC, were identified in 10/82 (12.1%) individuals (Tables 2 and 3). The heterozygous c.470T>C variant was observed in eight women of which three were

healthy. Two of the c.470T>C variant carriers had bilateral BrCa and they carried also *PALB2* missense variants (an example of the family pedigree of the index individual carrying the both variants is presented in Figure 2, Family 129). The heterozygous c.1100delC variant was detected in three women (Tables 2 and 3). One woman carrying c.1100delC with an early-onset disease of 26 years of age also carried the c.470T>C and the novel c.792+39C>T *CHEK2* variants as well as the *RAD50*, c.2398-32A>G variant (Figure 3, Family 110). A second patient with the c.1100delC variant had bilateral BrCa at the age of 44 years and two other affected individuals in her family (mother and father's sister) (Figure 4, Family 264). A third patient with the c.1100delC variant had BrCa diagnosed at the age of 45 years and one affected individual (mother) in her family. This woman carried also the *PALB2* c.1676A>G variant (Figure 5, Family 265). In addition to c.470T>C and c.1100delC, five novel variants (Table 2) and one common polymorphism (Supplementary Table S1 in Additional file 1) were identified in *CHEK2*. The novel non-synonymous variant, c.1363G>A (Val455Ile), is based on the computational predictions, likely benign.

***PALB2* mutation analysis**

In *PALB2*, altogether nine different variants, including three novel ones, were identified (Supplementary Table S1 in Additional file 1). Only one of the identified variants reported previously, c.2586+58C>T, has been associated with a 36% increase of breast cancer risk (OR: 1.36; 95% confidence intervals, 1.13-1.64; $P = 0.001$) in a Chinese population [25]. We identified the c.2586+58C>T variant in 36/82 (43.9%) women. A novel heterozygous c.814G>A variant was identified in 1/82 (1.2%) women but not in population controls (Tables 2 and 3). The c.814G>A variant carrying woman had BrCa diagnosed at the age of 28 years, but no other affected individuals were seen in her family. The c.814G>A

variant results in aminoacid substitution of Glutamic acid to Lysine at position 272, which causes a significant change to side chain properties including size and change of the charge to opposite. However, protein predictions by PON-P suggest that variation is neutral. The second novel heterozygous variant, c.1000T>G (Tyr334Asp), was observed in 1/82 (1.2%) women and in 4/380 (1.1%) population controls. The c.1000T>G variant carrying woman had bilateral BrCa diagnosed at the ages of 45 and 58 years and a family history of three other cancers (Tables 2 and 3, Figure 6, Family 262). She carried also the *CHEK2* c.470T>C variant. However, the protein predictions for the c.1000T>G (Tyr334Asp) variant suggest it to be neutral. A third novel heterozygous variant, c.2205A>G (Pro735Pro), is silent and likely neutral. It was observed in 1/82 (1.2%) women (Tables 2 and 3). Previously reported *PALB2* missense variants, c.1010T>C, c.1676A>G, c.2794G>A and c.2993G>A were identified here with frequencies from 1.2% to 12.2% in analyzed individuals (Tables 2 and 3) but the variants have not been associated with BrCa risk (an example of the family pedigree of the index individual carrying the c.1676A>G variant in addition to the *BRIP1* c.584T>C variant is presented in Figure 7, Family 131).

***BRIP1*, *RAD50*, and *CDH1* mutation analysis**

In *BRIP1*, two silent (Supplementary Table S1 in Additional file 1) and two missense variants (Tables 2 and 3) were identified. All of the identified variants have been reported previously and they are likely neutral. In *RAD50*, altogether seven sequence alterations were observed (Supplementary Table S1 in Additional file 1) and three of these were novel (Table 2). The novel missense variant, c.1544A>G (Asp515Gly), was observed in 1/82 (1.2%) women and in 4/384 (1.1%) population controls. The c.1544A>G variant carrying woman had BrCa diagnosed at the age of 39 years and one affected first-degree relative (Table 3). According to protein predictions, c.1544A>G variant is likely neutral. Two other

novel variants, c.2398-32A>G and c.3475+33C>G, were both observed with the frequency of 1/82 (1.2%) in analyzed individuals (Tables 2 and 3). In *CDH1*, ten different sequence alterations were identified (Supplementary Table S1 in Additional file 1). All of the variants have been reported previously and they are likely neutral.

MicroRNA database and BLAST search for novel variants

No known miRNA target sites were found in the identified novel variant genomic positions. In BLAST search, *BRCA2* c.72A>T variant position was found to have sequence similarities between rat and cow. *RAD50* c.1544A>G variant position shared similarities with mouse, rat, cow and chicken. Three novel variant positions in *CHEK2* exon 11 and the *RAD50* c.3475+33C>G variant shared sequence similarity between mouse, rat and cow. Variants that occur in the genomic regions that are conserved across species may indicate a pathogenic role.

Discussion

In the present study, we screened BrCa susceptibility genes in 82 Finnish high-risk HBOC individuals with no known Finnish *BRCA1/2*-founder mutations. Because genetic counseling and surveillance is greatly needed for these individuals and their families, we decided to study *BRCA1/2* in more detail and also to analyze five additional genes that had previously been associated with BrCa risk.

A majority of known *BRCA1/2* alterations are small insertions and deletions or point mutations (BIC database). Also, large genomic rearrangements have been reported in both genes with varying frequencies in different populations [26]. In Finland, so far only Pykäs *et al.* have reported a large deletion in *BRCA1* identified in a Finnish OvCa family [27]. In our study, no deletions or duplications were found in either *BRCA1* or *BRCA2* by MLPA, which suggests the existence of more restricted alterations. A total of 16 different sequence variants were identified from these two genes (Supplementary Table S1 in Additional file 1) and only one of the identified variants, c.5095C>T in *BRCA1*, has been classified as a clinically significant mutation in the BIC database. In line with this classification, our BrCa patient carrying this variant had strong family history of cancer (Tables 2 and 3, Figure 1, Family 249) and two other variant carriers with BrCa were also observed in the same family. The c.5095C>T mutation thus can explain a fraction of the BrCa cases also in the Finnish population. The clinical significance of the *BRCA1* c.4883T>C variant in BrCa predisposition is uncertain [28, 29]. Our data supports the idea that it is a low-penetrant risk allele, since the variant was observed to be three times more common in analyzed high-risk individuals than healthy population controls (Tables 2 and 3). Novel variant findings in *BRCA2* (Tables 2 and 3) warrant additional studies, especially the novel missense variant, c.72A>T (Leu24Phe), which was shown not to be tolerated by

protein prediction. Prediction indicated that the substitution decreases the stability of the produced protein and this might be the mechanism behind the disease for this variant. The amino acid position 24 is located near the N-terminal part of BRCA2. Amino acids 1-40 interact with PALB2, and sequence variants in this region have been shown to have effects on the PALB2 and BRCA2 interaction and thus are suspected to have a role in cancer predisposition [30]. The role of the three *BRCA2* missense variants, c.8182G>A, c.9976A>T and c.10234A>G, in HBOC risk, is uncertain [31-33]. All three heterozygous variants were observed in two healthy women with BrCa history, one carrying the c.9976A>T variant and the other both the c.8182G>A, and the c.10234A>G variants (Tables 2 and 3, Figure 8, Family 005). At this stage, because we only have samples from the index individuals, no segregation analyses of the variants have been performed, but these families clearly warrant additional studies. In recent risk models, it has been suggested that multiple low-risk variants within the same individual may actually cause a significantly elevated risk for the carrier [17]. The overall low frequency of new variants identified in *BRCA1/2* genes suggests that the present protocol for testing 28 Finnish *BRCA1/2*-founder mutations and PTT of the largest exons is adequate for clinical use to detect a majority of harmful mutations in these two genes in the Finnish population.

Two of the *CHEK2* variants, c.470T>C and c.1100delC, have been widely studied in BrCa predisposition in Finland and elsewhere. Previous studies have shown that the c.1100delC allele confers about two-fold elevated BrCa risk in women, whereas c.470T>C is a lower risk variant [34, 35]. Both variants also associate with other cancers in the Finnish population [36-38]. In our study, two of the *CHEK2* variants, c.470T>C and c.1100delC, were identified in 10 out of 82 analyzed individuals (12.2%) suggesting that the contribution of the two *CHEK2* variants to BrCa risk is remarkable in the high-risk Finnish

BRCA1/2-founder mutation-negative individuals. However, clinical screening of the *CHEK2* variants has not yet been justified due to unclear clinical consequences related to incomplete segregation of the variants with BrCa in the high-risk BrCa families [39, 40]. Based on the findings of this study, we agree that interpretation of the *CHEK2* mutation analysis results is very difficult, since many other gene variants were also identified in individuals with either c.470T>C or c.1100delC variants and some of the variant carriers had not (yet) been diagnosed with BrCa. Thus profound segregation analysis of the c.470T>C and c.1100delC variants for *BRCA1/2*-founder mutation-negative families would be needed to further study clinical significance of these variants. Also the novel variants identified in *CHEK2* should be further analyzed.

PALB2 has been associated to BrCa predisposition in Finland by Erkkö *et al.*[12] and the c.1592delT variant was classified as a Finnish founder mutation. In this study the founder deletion was not found, which is probably explained by the limited number of analyzed high-risk HBOC individuals. We identified two novel *PALB2* missense variants, c.814G>A (Glu272Lys) and c.1000T>G (Tyr334Asp), in affected individuals (Tables 2 and 3). Protein predictions suggested non-pathogenic role of these substitutions but further studies are needed to confirm these findings. None of the four previously reported *PALB2* missense variants, c.1010T>C, c.1676A>G, c.2794G>A and c.2993G>A have been associated to BrCa risk [12, 41]. Interestingly, these variants were identified also together with other variants in analyzed individuals (Tables 2 and 3). One of the identified intronic variants, c.2586+58C>T, has been associated with an increase of breast cancer risk in a Chinese population [25] but there is no evidence of that in the Finnish population.

BRIP1 and *RAD50* genes have been shown to have rare BrCa associated variants in familial BrCa patients [14, 42]. Here, *BRIP1* mutation analysis revealed only previously reported likely neutral variants. Whereas analysis of *RAD50* identified three novel sequence alterations, including one missense variant, c.1544A>G (Asp515Gly) (Tables 2 and 3). To further study the role of these novel variants, additional analyses are needed. Germline mutations in *CDH1* have been previously found to associate with hereditary diffuse gastric cancer (HDGC) syndrome, but mutations have been also identified in familial invasive lobular BrCa patients without HDGC [15, 43]. Here, only neutral variants were identified, and all of them have been reported earlier (Supplementary Table S1 in Additional file 1). No clear results were found that any of the identified genetic variants in *BRIP1*, *RAD50*, or *CDH1* would increase the BrCa/OvCa risk in the analyzed high-risk Finnish HBOC individuals.

Conclusions

In this study, 13.4% of the analyzed, high-risk *BRCA1/2*-founder mutation-negative HBOC individuals can be explained by previously reported mutations in BrCa susceptibility genes. *CHEK2* mutations, c.470T>C and c.1100delC, make a considerable contribution (12.2%) to these high-risk individuals but further segregation analysis are needed to evaluate the clinical significance of these mutations before applying them in clinical use. Novel variant findings warrant additional studies with special interest on the novel missense variant, *BRCA2* c.72A>T (Leu24Phe), which was predicted to bear intolerated mutations and to destabilize the protein. The complex nature of HBOC addresses the need for genome-wide approaches to further study these individuals and to create new tools for genetic counseling. This study also confirmed that our current genetic testing protocol for the 28

Finnish *BRCA1/2*-founder mutations and PTT of the largest exons is sensitive enough for clinical use in the majority of Finnish HBO/HBCO individuals.

Abbreviations

BRCA1 = breast cancer 1 gene; *BRCA2*, *BRCA2* = breast cancer 2, breast cancer 2 gene; BrCa = breast cancer; *BRIP1* = BRCA1-interacting protein 1 gene; *CDH1* = cadherin-1 gene; *CHEK2* = checkpoint kinase 2 gene; CI = confidence interval; ER = Estrogen receptor; *FGFR2* = fibroblast growth factor receptor 2 gene; GWAs = genome-wide association studies; HBC = hereditary breast cancer; HBOC = high-risk hereditary breast and/or ovarian cancer; HER2 = human epidermal growth factor receptor 2; HRM = high resolution melt; miRNA = microRNA; MLPA = multiplex ligation-dependent probe amplification; OR = odds ratio; OvCa = ovarian cancer; *PALB2*, *PALB2* = Partner and localizer of *BRCA2*, Partner and localizer of *BRCA2* gene; PCR = polymerase chain reaction; PR = Progesterone receptor; PTT = protein truncation test; *RAD50* = human homolog of *S. cerevisiae* *RAD50* gene; SNP = single nucleotide polymorphism.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KMK participated patient collection, carried out the sequencing, MLPA and statistical analysis and drafted the manuscript. AB carried out and interpreted the HRM analysis of the *CHEK2* gene and helped to draft the methods section of the manuscript. MV performed and interpreted protein prediction analysis *in silico* and helped to draft the manuscript. JS and S-L S participated in the study design and coordination, and helped to

draft the manuscript. S-L S also participated in patient collection and was responsible for genetic counseling of patients. All authors read and approved the final manuscript.

Acknowledgements

We thank all the patients for their participation into this study. We also thank personnel of the Tampere University Hospital Genetics Outpatient Clinic and JS's research group for all the help. Special thanks are given to Ms. Linda Enroth for skillful technical assistance. Also Ms. Ekaterina Slitikova and Ms. Aune Aho are greatly acknowledged for their contribution. Funding: This work was supported by the Competitive Research Funding of the Tampere University Hospital (9K119, ML16), Biocentre Finland and Sigrid Juselius Foundation. The Finnish Cultural Foundation and the Finnish Breast Cancer Group have financially supported author KMK.

References

1. Claus EB, Schildkraut JM, Thompson WD, Risch NJ: **The genetic attributable risk of breast and ovarian cancer.** Cancer 1996, **77**(11):2318-2324.
2. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W: **A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1.** Science 1994, **266**(5182):66-71.
3. Tavtigian SV, Simard J, Rommens J, Couch F, Shattuck-Eidens D, Neuhausen S, Merajver S, Thorlacius S, Offit K, Stoppa-Lyonnet D, Belanger C, Bell R, Berry S, Bogden R, Chen Q, Davis T, Dumont M, Frye C, Hattier T, Jammulapati S, Janecki T, Jiang P, Kehrer R, Leblanc JF, Mitchell JT, McArthur-Morrison J, Nguyen K, Peng Y, Samson C,

Schroeder M, Snyder SC *et al*: **The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds.** Nat Genet 1996, **12**:333-337.

4. Vehmanen P, Friedman LS, Eerola H, McClure M, Ward B, Sarantaus L, Kainu T, Syrjakoski K, Pyrhonen S, Kallioniemi OP, Muhonen T, Luce M, Frank TS, Nevanlinna H: **Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: evidence for additional susceptibility genes.** Hum Mol Genet 1997, **6**:2309-2315.

5. Syrjakoski K, Vahteristo P, Eerola H, Tamminen A, Kivinummi K, Sarantaus L, Holli K, Blomqvist C, Kallioniemi OP, Kainu T, Nevanlinna H: **Population-based study of BRCA1 and BRCA2 mutations in 1035 unselected Finnish breast cancer patients.** J Natl Cancer Inst 2000, **92**:1529-1531.

6. Li FP, Fraumeni JF, Jr: **Rhabdomyosarcoma in children: epidemiologic study and identification of a familial cancer syndrome.** J Natl Cancer Inst 1969, **43**:1365-1373.

7. Starink TM, van der Veen JP, Arwert F, de Waal LP, de Lange GG, Gille JJ, Eriksson AW: **The Cowden syndrome: a clinical and genetic study in 21 patients.** Clin Genet 1986, **29**:222-233.

8. Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, Cruz-Correa M, Offerhaus JA: **Very high risk of cancer in familial Peutz-Jeghers syndrome.** Gastroenterology 2000, **119**:1447-1453.

9. Keller G, Vogelsang H, Becker I, Hutter J, Ott K, Candidus S, Grundei T, Becker KF, Mueller J, Siewert JR, Hofler H: **Diffuse type gastric and lobular breast carcinoma in a familial gastric cancer patient with an E-cadherin germline mutation.** Am J Pathol 1999, **155**:337-342.

10. Welcsh PL, Owens KN, King MC: **Insights into the functions of BRCA1 and BRCA2.** Trends Genet 2000, **16**:69-74.
11. Vahteristo P, Bartkova J, Eerola H, Syrjakoski K, Ojala S, Kilpivaara O, Tamminen A, Kononen J, Aittomaki K, Heikkila P, Holli K, Blomqvist C, Bartek J, Kallioniemi OP, Nevanlinna H: **A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer.** Am J Hum Genet 2002, **71**:432-438.
12. Erkkö H, Xia B, Nikkila J, Schleutker J, Syrjakoski K, Mannermaa A, Kallioniemi A, Pylkas K, Karppinen S, Rapakko K, Miron A, Sheng Q, Li G, Mattila H, Bell DW, Haber DA, Grip M, Reiman M, Jukkola-Vuorinen A, Mustonen A, Kere J, Aaltonen LA, Kosma V, Kataja V, Soini Y, Drapkin RI, Livingston DM, Winqvist R: **A recurrent mutation in PALB2 in Finnish cancer families.** Nature 2007, **446**:316-319.
13. Vahteristo P, Yliannala K, Tamminen A, Eerola H, Blomqvist C, Nevanlinna H: **BACH1 Ser919Pro variant and breast cancer risk.** BMC Cancer 2006, **6**:19.
14. Heikkinen K, Rapakko K, Karppinen SM, Erkkö H, Knuutila S, Lundan T, Mannermaa A, Borresen-Dale AL, Borg A, Barkardottir RB, Petrini J, Winqvist R: **RAD50 and NBS1 are breast cancer susceptibility genes associated with genomic instability.** Carcinogenesis 2006, **27**:1593-1599.
15. Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE: **E-cadherin germline mutations in familial gastric cancer.** Nature 1998, **392**:402-405.
16. Easton DF: **How many more breast cancer predisposition genes are there?** Breast Cancer Res 1999, **1**:14-17.

17. Antoniou AC, Easton DF: **Models of genetic susceptibility to breast cancer.** Oncogene 2006, **25**:5898-5905.
18. Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R, SEARCH collaborators, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J *et al*: **Genome-wide association study identifies novel breast cancer susceptibility loci.** Nature 2007, **447**:1087-1093.
19. Tommiska J, Seal S, Renwick A, Barfoot R, Baskcomb L, Jayatilake H, Bartkova J, Tallila J, Kaare M, Tamminen A, Heikkila P, Evans DG, Eccles D, Aittomaki K, Blomqvist C, Bartek J, Stratton MR, Nevanlinna H, Rahman N: **Evaluation of RAD50 in familial breast cancer predisposition.** Int J Cancer 2006, **118**:2911-2916.
20. Rozen S, Skaletsky H: **Primer3 on the WWW for general users and for biologist programmers.** Methods Mol Biol 2000, **132**:365-386.
21. **Two by two table analysis online. Including chi-square, odds ratio, risk ratio.** [<http://www.quantitativeskills.com/sisa/statistics/twooby2.htm>]
22. Thusberg J, Vihinen M: **Pathogenic or not? And if so, then how? Studying the effects of missense mutations using bioinformatics methods.** Hum Mutat 2009, **30**:703-714.
23. **miRBase** [<http://www.mirbase.org/>]
24. **BLAST: Basic Local Alignment Search Tool** [<http://blast.ncbi.nlm.nih.gov/Blast.cgi>]

25. Chen P, Liang J, Wang Z, Zhou X, Chen L, Li M, Xie D, Hu Z, Shen H, Wang H: **Association of common PALB2 polymorphisms with breast cancer risk: a case-control study.** Clin Cancer Res 2008, **14**:5931-5937.
26. Mazoyer S: **Genomic rearrangements in the BRCA1 and BRCA2 genes.** Hum Mutat 2005, **25**:415-422.
27. Pylkas K, Erkkö H, Nikkila J, Solyom S, Winqvist R: **Analysis of large deletions in BRCA1, BRCA2 and PALB2 genes in Finnish breast and ovarian cancer families.** BMC Cancer 2008, **8**:146.
28. Ostrow KL, McGuire V, Whittemore AS, DiCioccio RA: **The effects of BRCA1 missense variants V1804D and M1628T on transcriptional activity.** Cancer Genet Cytogenet 2004, **153**:177-180.
29. Phelan CM, Dapic V, Tice B, Favis R, Kwan E, Barany F, Manoukian S, Radice P, van der Luijt RB, van Nesselrooij BP, Chenevix-Trench G, kConFab, Caldes T, de la Hoya M, Lindquist S, Tavtigian SV, Goldgar D, Borg A, Narod SA, Monteiro AN: **Classification of BRCA1 missense variants of unknown clinical significance.** J Med Genet 2005, **42**:138-146.
30. Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM: **Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2.** Mol Cell 2006, **22**:719-729.
31. Deffenbaugh AM, Frank TS, Hoffman M, Cannon-Albright L, Neuhausen SL: **Characterization of common BRCA1 and BRCA2 variants.** Genet Test 2002, **6**:119-121.

32. Wagner TM, Hirtenlehner K, Shen P, Moeslinger R, Muhr D, Fleischmann E, Concin H, Doeller W, Haid A, Lang AH, Mayer P, Petru E, Ropp E, Langbauer G, Kubista E, Scheiner O, Underhill P, Mountain J, Stierer M, Zielinski C, Oefner P: **Global sequence diversity of BRCA2: analysis of 71 breast cancer families and 95 control individuals of worldwide populations.** Hum Mol Genet 1999, **8**:413-423.
33. Mazoyer S, Dunning AM, Serova O, Dearden J, Puget N, Healey CS, Gayther SA, Mangion J, Stratton MR, Lynch HT, Goldgar DE, Ponder BA, Lenoir GM: **A polymorphic stop codon in BRCA2.** Nat Genet 1996, **14**:253-254.
34. 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**:1175-1182.
35. Kilpivaara O, Vahteristo P, Falck J, Syrjakoski K, Eerola H, Easton D, Bartkova J, Lukas J, Heikkila P, Aittomaki K, Holli K, Blomqvist C, Kallioniemi OP, Bartek J, Nevanlinna H: **CHEK2 variant I157T may be associated with increased breast cancer risk.** Int J Cancer 2004, **111**:543-547.
36. Seppala EH, Ikonen T, Mononen N, Autio V, Rokman A, Matikainen MP, Tammela TL, Schleutker J: **CHEK2 variants associate with hereditary prostate cancer.** Br J Cancer 2003, **89**:1966-1970.
37. Kilpivaara O, Laiho P, Aaltonen LA, Nevanlinna H: **CHEK2 1100delC and colorectal cancer.** J Med Genet 2003, **40**:e110.

38. Kilpivaara O, Alhopuro P, Vahteristo P, Aaltonen LA, Nevanlinna H: **CHEK2 I157T associates with familial and sporadic colorectal cancer.** J Med Genet 2006, **43**:e34.
39. Nevanlinna H, Bartek J: **The CHEK2 gene and inherited breast cancer susceptibility.** Oncogene 2006, **25**:5912-5919.
40. Ripperger T, Gadzicki D, Meindl A, Schlegelberger B: **Breast cancer susceptibility: current knowledge and implications for genetic counselling.** Eur J Hum Genet 2009, **17**:722-731.
41. Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Breast Cancer Susceptibility Collaboration (UK), Easton DF, Stratton MR: **PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene.** Nat Genet 2007, **39**:165-167.
42. Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DC, Sgroi DC, Lane WS, Haber DA, Livingston DM: **BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function.** Cell 2001, **105**:149-160.
43. Masciari S, Larsson N, Senz J, Boyd N, Kaurah P, Kandel MJ, Harris LN, Pinheiro HC, Troussard A, Miron P, Tung N, Oliveira C, Collins L, Schnitt S, Garber JE, Huntsman D: **Germline E-cadherin mutations in familial lobular breast cancer.** J Med Genet 2007, **44**:726-731.
44. UCSC Genome Browser Home [<http://genome.ucsc.edu/>]

45. **ExPASy - UniProt Knowledgebase: Swiss-Prot and TrEMBL**

[\[http://au.expasy.org/sprot/\]](http://au.expasy.org/sprot/)

46. **SNP Home** [\[http://www.ncbi.nlm.nih.gov/snp\]](http://www.ncbi.nlm.nih.gov/snp)

47. **Breast Cancer Information Core** [\[http://research.nhgri.nih.gov/bic/\]](http://research.nhgri.nih.gov/bic/)

Figure legends

Figure 1. Family 249 pedigree. Family pedigree of the index individual with the identified *BRCA1* c.5095C>T variant (same variant was also identified in the daughter of the index individual and in the daughter of the index individual's paternal uncle). Individuals with breast or ovarian cancer with age at diagnosis are marked with black circles (Br: breast cancer, Ov: ovarian cancer). Other cancers are marked in grey and accompanied by age at diagnosis, if known (Co: Colon, Kid: kidney, Mel: melanoma, To: tongue, Ute: Uterus). Index individual is marked with an arrow. Deceased individuals are indicated with a slash. Current ages of healthy females are marked if known.

Figure 2. Family 129 pedigree. Family pedigree of the index individual with the identified *CHEK2* c.470T>C and *PALB2* c.1676A>G variants. Individuals with breast cancer with age at diagnosis are marked with black circles (Bil. Br: bilateral breast cancer). Other cancers are marked in grey and accompanied by age at diagnosis, if known (Co: Colon, Sto: stomach). Index individual is marked with an arrow. Deceased individuals are indicated with a slash.

Figure 3. Family 110 pedigree. Family pedigree of the index individual with the identified *CHEK2* c.470T>C, c.792+39C>T, c.1100delC, and *RAD50* c. 2398-32A>G variants. Individuals with breast cancer with age at diagnosis are marked with black circles (Br: breast cancer). Other cancers are marked in grey and accompanied by age at diagnosis, if known (Ca: cancer with unknown primary site, Pr: prostate). Index individual is marked with an arrow. Deceased individuals are indicated with a slash. Current ages of healthy females are marked if known.

Figure 4. Family 264 pedigree. Family pedigree of the index individual with the identified *CHEK2* c.1100delC variant. Individuals with breast cancer with age at diagnosis are marked with black circles (Bil. Br: bilateral breast cancer, Br: breast cancer). Index individual is marked with an arrow. Deceased individuals are indicated with a slash. Current ages of healthy females are marked if known.

Figure 5. Family 265 pedigree. Family pedigree of the index individual with the identified *CHEK2* c.1100delC and *PALB2* c.1676A>G variants. Individuals with breast cancer with age at diagnosis are marked with black circles (Br: breast cancer). Index individual is marked with an arrow. Current ages of healthy females are marked if known.

Figure 6. Family 262 pedigree. Family pedigree of the index individual with the identified *CHEK2* c.470T>C and *PALB2* c.1000T>G variants. Individuals with breast cancer with age at diagnosis are marked with black circles (Bil. Br: bilateral breast cancer, Br: breast cancer). Other cancers are marked in grey and accompanied by age at diagnosis, if known (Panc: pancreas, Si: sigma). Index individual is marked with an arrow. Deceased individuals are indicated with a slash. Current ages of healthy females are marked if known.

Figure 7. Family 131 pedigree. Family pedigree of the index individual with the identified *PALB2* c.1676A>G and *BRIP1* c.584T>C variants. Individuals with breast cancer with age at diagnosis are marked with black circles (Bil. Br: bilateral breast cancer, Br: breast cancer). Index individual is marked with an arrow. Deceased individuals are indicated with a slash. Current ages of healthy females are marked if known.

Figure 8. Family 005 pedigree. Family pedigree of the index individual with the identified *BRCA2* c.8182G>A and c.10234A>G variants. Individuals with breast cancer with age at diagnosis are marked with black circles (Bil. Br: bilateral breast cancer, Br: breast cancer). Other cancers are marked in grey and accompanied by age at diagnosis, if known (Sto: stomach). Index individual is marked with an arrow. Deceased individuals are indicated with a slash.

Tables

Table 1. Characteristics of the studied individuals

	BrCa	Bil. BrCa	OvCa	BrCa and OvCa	Non-affected
No of index individuals (n=82)	57	8	1	5	11
Age at diagnosis					
(BrCa/OvCa)	n=57	n=16 ^a	n=1	n=10 ^b	-
<30	13	0	0	1	-
<40 years	11	2	0	2	-
<50 years	15	7	0	2	-
≥50 years	18	7	1	5	-
Type of BrCa					
	n=57	n=16 ^a	-	n=5	-
Ductal	41	8	-	5	-
Intraductal	2	2	-	0	-
Lobular	9	3	-	0	-
Papillary	1	0	-	0	-
Medullary	1	0	-	0	-
Unknown	3	3	-	0	-
Ductal carcinomas,					
grade known	n=38	n=6	-	n=5	-
Grade 1	7	3	-	0	-
Grade 2	14	3	-	3	-
Grade 3	17	0	-	2	-
Estrogen receptors (ERs),					
status known	n=51	n=11	-	n=4	-
ER+	35	10	-	2	-

ER-	16	1	-	2	-
Progesterone receptors (PRs),					
status known	n=50	n=11	-	n=4	-
PR+	30	10	-	2	-
PR-	20	1	-	2	-
HER2, status known					
HER2+	14	1	-	1	-
HER2-	32	10	-	3	-
Type of OvCa					
	-	-	n=1	n=5	-
Serous	-	-	0	0	-
Endometrioid	-	-	0	0	-
Mucinous	-	-	0	2	-
Clear cell	-	-	0	1	-
Other	-	-	0	2	-
Unknown	-	-	1	0	-
No of affected (BrCa/OvCa)					
1st degree relatives	n=57	n=8	n=1	n=5	n=11
≥2	25	3	1	0	5
≥1	24	4	0	0	6
0	8	1	0	5	0
No of affected (BrCa/OvCa)					
2nd degree relatives	n=57	n=8	n=1	n=5	n=11
≥2	5	0	0	0	4
≥1	11	1	0	0	0
0	41	7	1	5	7

Bil. BrCa: Bilateral breast cancer, BrCa: Breast cancer, OvCa: Ovarian cancer, HER2: Human epidermal growth factor receptor 2, ^a8 Bilateral breast cancer cases, together 16 cancers, ^b5 Breast and ovarian cancer cases, together 10 cancers

Table 2. Identified non-synonymous and novel sequence alterations

Gene/	Nucleotide change ^a	Effect on protein	rs Number ^b	Carrier Frequency		P-values	OR; 95%CI	Status
				Individuals	Controls			
BRCA1								
	4837A>G	Ser1613Gly	rs1799966	0.634 (52/82) ^g	na	-	-	Reported ^{c, d}
	4883T>C	Met1628Thr	rs4986854	0.049 (4/82)	0.016 (6/367)	0.090	3.09; 0.85-11.19	Reported ^{c, d}
	5095C>T	Arg1699Trp	rs55770810	0.012 (1/82)	na	-	-	Reported ^{c, d}
BRCA2								
	68-80insT ^f	-	-	0.012 (1/82)	na	-	-	Novel
	72A>T	Leu24Phe	-	0.012 (1/82)	0 (0/380)	0.177	na	Novel
	793+34T>G	-	-	0.012 (1/82)	na	-	-	Novel
	8182G>A	Val2728Ile	rs28897749	0.012 (1/82)	0.003 (1/378)	0.325	4.65; 0.29-75.19	Reported ^{c, d}
	9976A>T	Lys3326Stop	rs11571833	0.012 (1/82)	0.029 (11/378)	0.702	0.41; 0.05-3.24	Reported ^{c, d}
	10234A>G	Ile3412Val	rs1801426	0.012 (1/82)	0.021 (8/379)	1.000	0.57; 0.07-4.64	Reported ^{c, d}
CHEK2								
	444+85T>A	-	-	0.012 (1/82)	0.005 (2/364)	0.457	2.23; 0.20-24.94	Novel
	470T>C	Ile157Thr	-	0.098 (8/81)	0.055 (21/381)	0.203	1.88; 0.80-4.41	Reported ^e
	792+39C>T	-	-	0.012 (1/82)	0.021 (8/375)	1.000	0.57; 0.07-4.60	Novel
	1100delC ^f	Fs, stop at codon 381	-	0.037 (3/82)	0.016 (6/380)	0.203	2.37; 0.58-9.67	Reported ^e
	1290T>C	His430His	-	0.951 (77/81) ^g	0.974 (372/382)	0.281	0.52; 0.16-1.69	Novel
	1314T>C	Asp438Asp	-	0.951 (77/81) ^g	0.974 (372/382)	0.281	0.52; 0.16-1.69	Novel
	1363G>A	Val455Ile	-	0.975 (79/81) ^g	0.976 (373/382)	1.000	0.95; 0.20-4.50	Novel
PALB2								
	814G>A	Glu272Lys	-	0.012 (1/82)	0 (0/372)	0.181	na	Novel
	1000T>G	Tyr334Asp	-	0.012 (1/82)	0.011 (4/380)	1.000	1.16; 0.13-10.52	Novel
	1010T>C	Leu337Ser	rs45494092	0.073 (6/82)	na	-	-	Reported ^c
	1676A>G	Gln559Arg	rs152451	0.122 (10/82)	0.173 (64/371)	0.323	0.67; 0.33-1.36	Reported ^c
	2205A>G	Pro735Pro	-	0.012 (1/82)	na	-	-	Novel
	2794G>A	Val932Met	rs45624036	0.037 (3/82)	na	-	-	Reported ^c
	2993G>A	Gly998Glu	rs45551636	0.012 (1/82)	0.038 (14/372)	0.491	0.32; 0.04-2.44	Reported ^c
BRIP1								
	584T>C	Leu195Pro	rs4988347	0.024 (2/82)	na	-	-	Reported ^e
	2755C>T	Pro919Ser	rs4986764	0.390 (32/82) ^g	na	-	-	Reported ^e

RAD50

1544A>G	Asp515Gly	-	0.012 (1/82)	0.010 (4/384)	1.000	1.17; 0.13-10.63	Novel
2398-32A>G	-	-	0.012 (1/82)	na	-	-	Novel
3475+33C>G	-	-	0.012 (1/82)	na	-	-	Novel

CI: confidence interval, Fs: frameshift, na: not analyzed, OR: odds ratio. ^aThe reference nucleotide sequences were obtained from the UCSC Genome Browser [44] and the accession numbers were following: *BRCA1*: [UCSC Genome Browser:NM_007295.2], *BRCA2*: [UCSC Genome Browser:NM_000059.3], *CHEK2*: [UCSC Genome Browser:NM_007194.3], *PALB2*: [UCSC Genome Browser:NM_024675.3], *BRIP1*: [UCSC Genome Browser:NM_032043.1], *RAD50*: [UCSC Genome Browser:NM_005732.3], and *CDH1*: [UCSC Genome Browser:NM_004360.3]. The accession numbers for the protein sequences obtained from the Swiss-Prot Protein knowledgebase [45] were following: *BRCA1*: [Swiss-Prot:P38398], *BRCA2*: [Swiss-Prot:P51587], *CHEK2*: [Swiss-Prot:O96017], *PALB2*: [Swiss-Prot:Q86YC2], *BRIP1*: [Swiss-Prot:Q9BX63], *RAD50*: [Swiss-Prot:Q92878], and *CDH1*: [Swiss-Prot:P12830]. ^bThe RefSNP number, obtained from the NCBI Single Nucleotide Polymorphism database (dbSNP) [46]. ^cThe NCBI dbSNP [46]. ^dThe Breast Cancer Information Core database [47]. ^eReported in the Finnish population by Vahteristo et al.[11]. ^fHeterozygous deletion or insertion. ^gDue to the high frequency of the variant observed in analyzed individuals, variant is not presented in Table 3.

Table 3. Identified variants in the studied individuals

Family id	Gene and variant	Type of cancer	BrCa/OvCa Histology,Grade	Receptor status	Other cancer cases in the family (Age at diagnosis if available)
202	<i>BRCA1</i> , 4883T>C	Br (26)	Ductal, 3	ER-, PR-, HER2-	Skin (54)
206	<i>BRCA1</i> , 4883T>C	Br (53)	Ductal, 1	ER-, PR-, HER2-	Bil. Ov (64), <u>Br (49)</u>
231	<i>BRCA1</i> , 4883T>C	-			<u>Br x2 (33, 46)</u> , Cer (60), Skin (73)
232	<i>BRCA1</i> , 4883T>C	Br (34)	Ductal, na	ER+, PR+, HER2 na	<u>Br (39)</u>
249 (Figure 1)	<i>BRCA1</i> , 5095C>T	Br (42)	Medullary, na	na	Br x5 (35, 44 , 57, 67, 71,), Co (78), Kid (67), Mel (63), Ov (45), Skin, <u>To (51)</u> , Ute (39)
115	<i>BRCA2</i> , 68-80insT	Br (59)	Lobular, 2	ER+, PR+, HER2-	Br x3 (<50), <u>Br x2 (60, 60)</u>
240	<i>BRCA2</i> , 72A>T	Br (53)	Ductal, 3	ER+, PR-, HER2+	Br x2 (<u>42</u> , 62)
207	<i>BRCA2</i> , 793+34T>G	Br (38)	na	na	Bil. Br (64)
5 (Figure 8)	<i>BRCA2</i> , 8182G>A <i>BRCA2</i> , 10234A>G	-			Bil. Br x2 (43, 48 and 54, 76), Br (43), <i>Brain (75)</i> , Lip (45), <i>Lung (81)</i> , <i>Skin (75)</i> , Sto (56)
4	<i>BRCA2</i> , 9976A>T <i>CHEK2</i> , 470T>C	-			Bil. Br x2 (53, 69 and <70), Br (<70)
212	<i>CHEK2</i> , 444+85T>A <i>PALB2</i> , 2794G>A	Bil. Br (43)	Ductal, 2 and na	ER+, PR+, HER2- and na	<u>Br (52)</u>
110 (Figure 3)	<i>CHEK2</i> , 792+39C>T <i>CHEK2</i> , 470T>C <i>CHEK2</i> , 1100delC <i>RAD50</i> , 2398-32A>G	Br (26)	Ductal, 2	ER+, PR+, HER2+	<i>Br (48)</i> , <i>Ca (84)</i> , <i>Pr (64)</i> ,
112	<i>CHEK2</i> , 470T>C <i>PALB2</i> , 1676A>G	-			Br x3 (<u>35</u> , <u>43</u> , 83), <i>Skin (76)</i> , <i>Lung (71)</i>
120	<i>CHEK2</i> , 470T>C	-			Br (64), Ov (72)
122	<i>CHEK2</i> , 470T>C	Br (25)	Ductal, 2	ER-, PR-, HER2+	<i>Brain (66)</i> , <i>Ca (83)</i> , <i>Cer (31)</i> , <i>Pr (93)</i> , <i>Re (73)</i> , <i>Skin (87)</i>
126	<i>CHEK2</i> , 470T>C	Br (48)	Ductal, na	ER, PR, HER2 na	<i>Bil. Br</i> , <i>Br x2 (51, <53)</i>
129 (Figure 2)	<i>CHEK2</i> , 470T>C <i>PALB2</i> , 1676A>G	Skin (70), Bil. Br (78), Sto (82)	Lobular, 2 and Ductal, 1	ER-, PR-, HER2- and ER+, PR+, HER2-	<u>Bil. Br (59)</u> , Co (58) , Skin (48)
262 (Figure 6)	<i>CHEK2</i> , 470T>C <i>PALB2</i> , 1000T>G	Bil. Br (45, 58)	Intraductal, na and Ductal, na	ER+, PR+, HER2+ and ER+, PR+, HER2-	Br (57), <i>Panc (83)</i> , <i>Si (79)</i>
264 (Figure 4)	<i>CHEK2</i> , 1100delC	Bil. Br (44)	Lobular, 2	ER+, PR+, HER2-	Br x2 (44, 52)

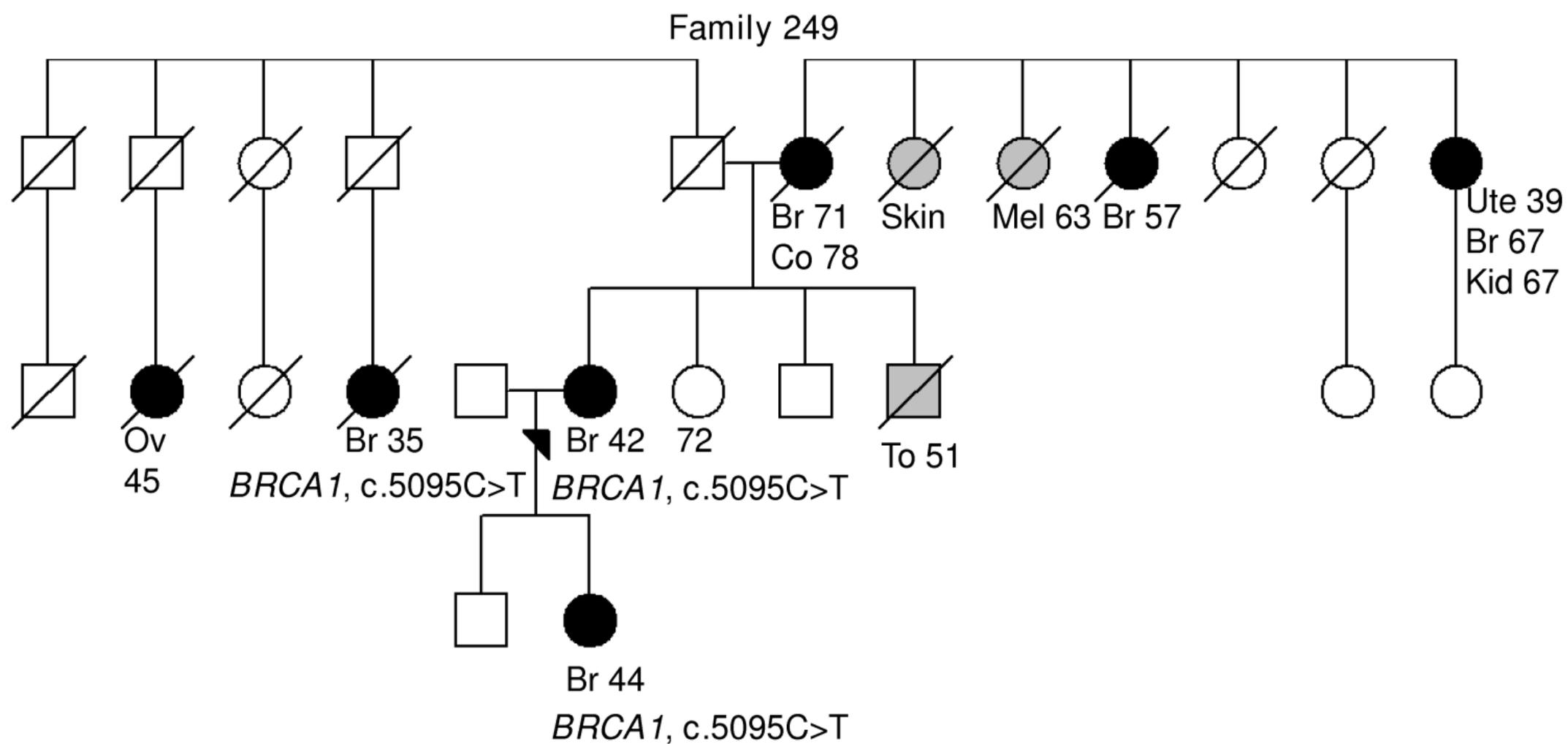
265 (Figure 5)	<i>CHEK2</i> , 1100delC <i>PALB2</i> , 1676A>G	Br (45)	Ductal, 3	ER+, PR+, HER2+	Br (38)
237	<i>PALB2</i> , 814G>A	Br (28)	Ductal, 2	ER+, PR+, HER2+	-
133	<i>PALB2</i> , 1010T>C	Br (48)	Ductal, 2	ER+, PR+, HER2-	<i>Int, Br x2 (73, 79), Skin (60)</i>
235	<i>PALB2</i> , 1010T>C	Br (52)	na	na	<u>Bil. Br (28), Br (56)</u>
239	<i>PALB2</i> , 1010T>C	Br (37)	Ductal, 2	ER+, PR+, HER2-	<i>Br (>90)</i>
250	<i>PALB2</i> , 1010T>C	Br (24)	Ductal, 3	ER+, PR+, HER2+	<u>Cer (30), Ov (83)</u>
260	<i>PALB2</i> , 1010T>C	Br (29)	Ductal, 3	ER-, PR-, HER2-	Br (58), <i>Lung (60)</i>
267	<i>PALB2</i> , 1010T>C	Br (48)	Ductal, 1	ER+, PR+, HER2 na	<u>Br x2 (51, 58)</u>
113	<i>PALB2</i> , 1676A>G ^a	Br (51), Skin (55)	Ductal, 3	ER-, PR-, HER2+	Br (35)
131 (Figure 7)	<i>PALB2</i> , 1676A>G <i>BRIP1</i> , 584T>C	Bil. Br (54)	Intraductal, na and Ductal, 2	na	<u>Bil. Br (46), Br (48)</u>
229	<i>PALB2</i> , 1676A>G	Bil. Br (68)	na	na	<u>Bil. Br (50, 70), Br x2 (45, 50)</u>
236	<i>PALB2</i> , 1676A>G	Br (29)	Intraductal, na	na	Br (52)
246	<i>PALB2</i> , 1676A>G	Thy (30), Cer (33), Br (39)	Ductal, 3	ER-, PR-, HER2+	Br x2 (<u>49, 54</u>), <i>Re (61)</i>
268	<i>PALB2</i> , 1676A>G <i>PALB2</i> , 2205A>G	Br (62)	Papillary, na	ER+, PR+, HER2-	Br x2 (36, <u>38</u>)
271	<i>PALB2</i> , 1676A>G	Thy (62), Br (65)	Lobular, 2	ER+, PR+, HER2-	Br x2 (43, 44)
102	<i>PALB2</i> , 2794G>A <i>BRIP1</i> , 584T>C	Br (29)	Lobular, na	ER-, PR-, HER2+	<i>Br (72)</i>
244	<i>PALB2</i> , 2794G>A	Br (45)	Ductal, 2	ER+, PR+, HER2-	Bil. Br (<45), Br x2 (<35, <u>46</u>), Brain (67)
270	<i>PALB2</i> , 2993G>A	Br (66)	Ductal, 3	ER+, PR+, HER2-	Br x2 (<u>48, <66</u>)
257	<i>RAD50</i> , 1544A>G	Br (39)	Lobular, 2	ER+, PR+, HER2-	Br (69)
225	<i>RAD50</i> , 3475+33C>G	Br (43)	Ductal, 1	ER+, PR+, HER2-	Br x2 (<u>52, 77</u>), <i>Kid (64)</i>

ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2, na: not available, Bil: Bilateral, Br: Breast, Ca: Cancer with unknown primary site, Cer: Cervix, Co: Colon, Int: Intestines, Kid: Kidney, Mel: Melanoma, Ov: Ovary, Panc: Pancreas, Pr: Prostate, Re: Rectum, Si: Sigma, St: Stomach, Thy: Thyroid, To: Tongue, Ute: Uterus. ^aHomozygous variant. *Cancers diagnosed in the paternal side of the family are presented in italics. Cancers diagnosed in siblings or their children of the index patients are underlined. Cancers diagnosed in the children of the index patients are presented in bold.*

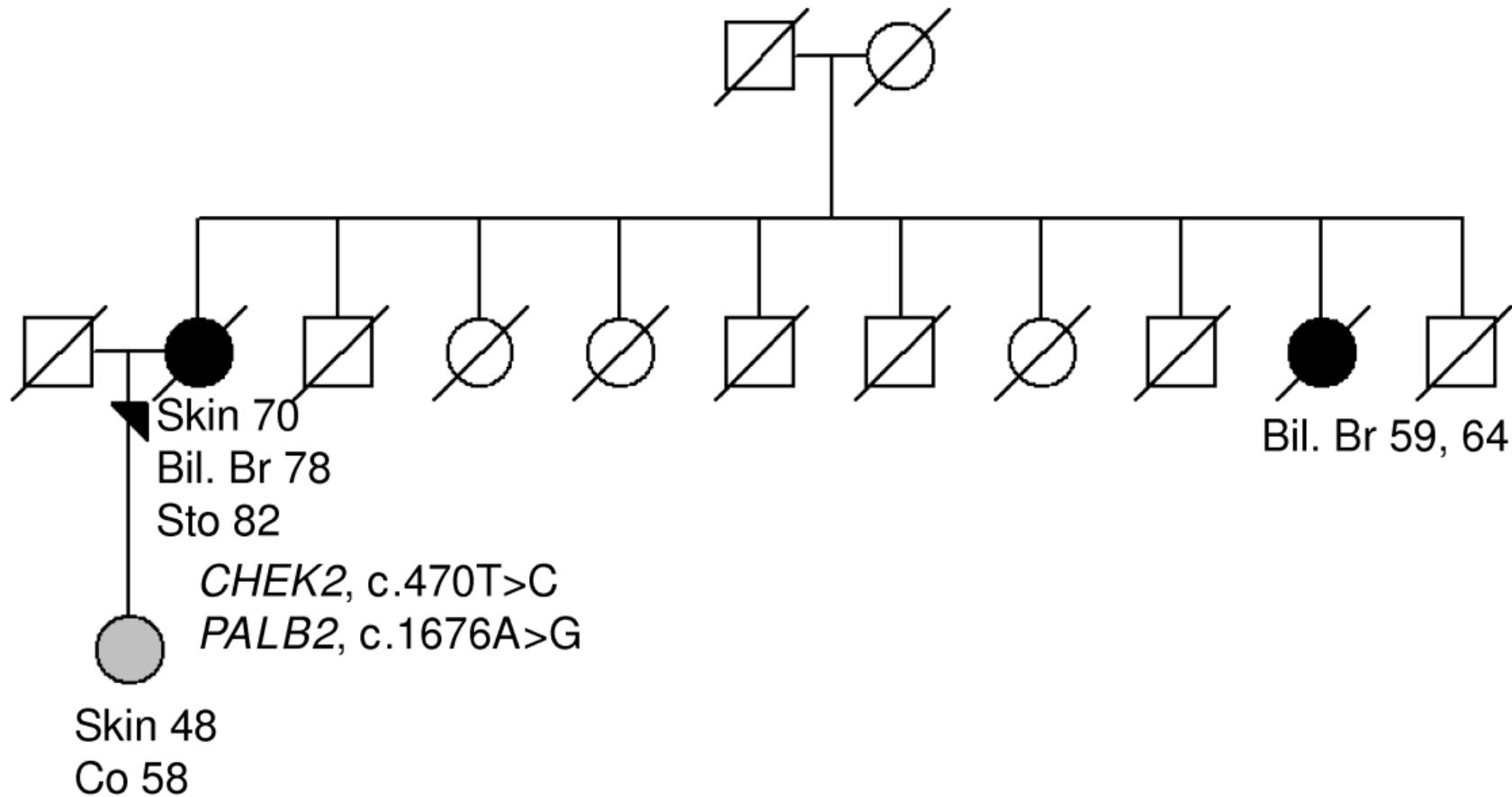
Additional files

Additional file 1: Supplementary Table S1. All of the identified 54 sequence alterations. Supplementary Table S1 include detailed information about all of the identified sequence alterations.

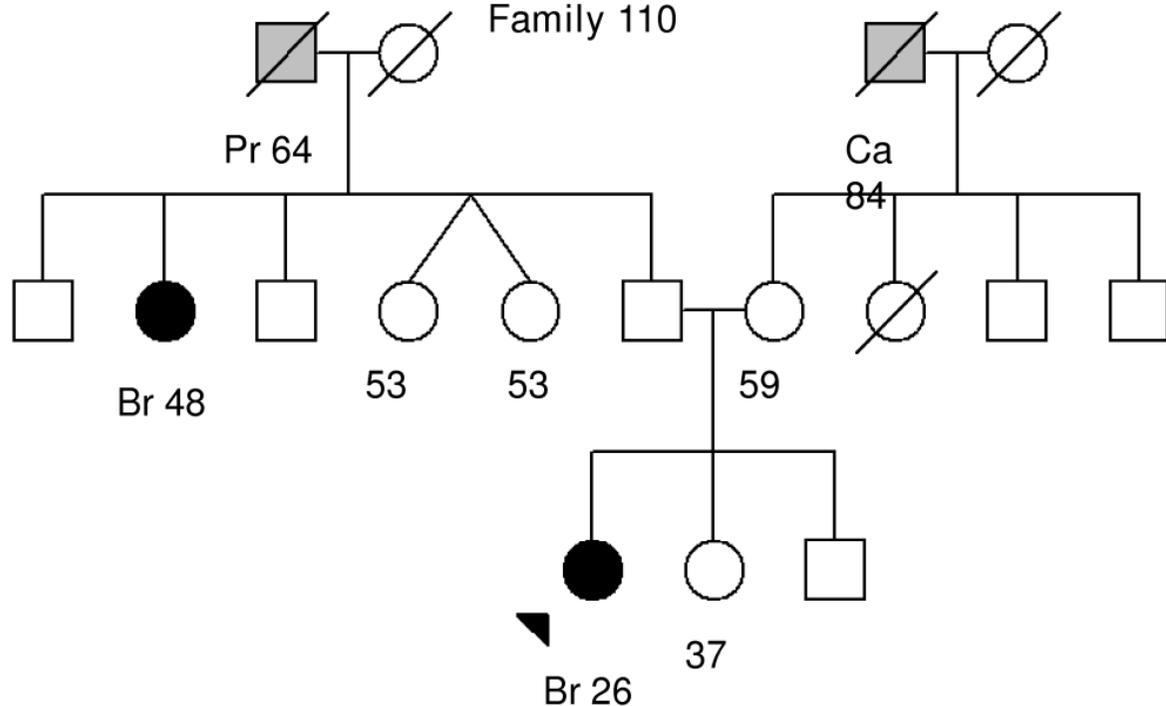
Additional file 2: Supplementary Table S2. Identified breast cancer associated variants in affected 71 individuals. Supplementary Table S2 includes re-calculated frequencies for *BRCA1* c.5095C>T, *CHEK2* c.470T>C, and *CHEK2* c.1100delC variants in affected 71 index individuals (11 unaffected index individuals excluded).



Family 129



Family 110



CHEK2, c.470T>C

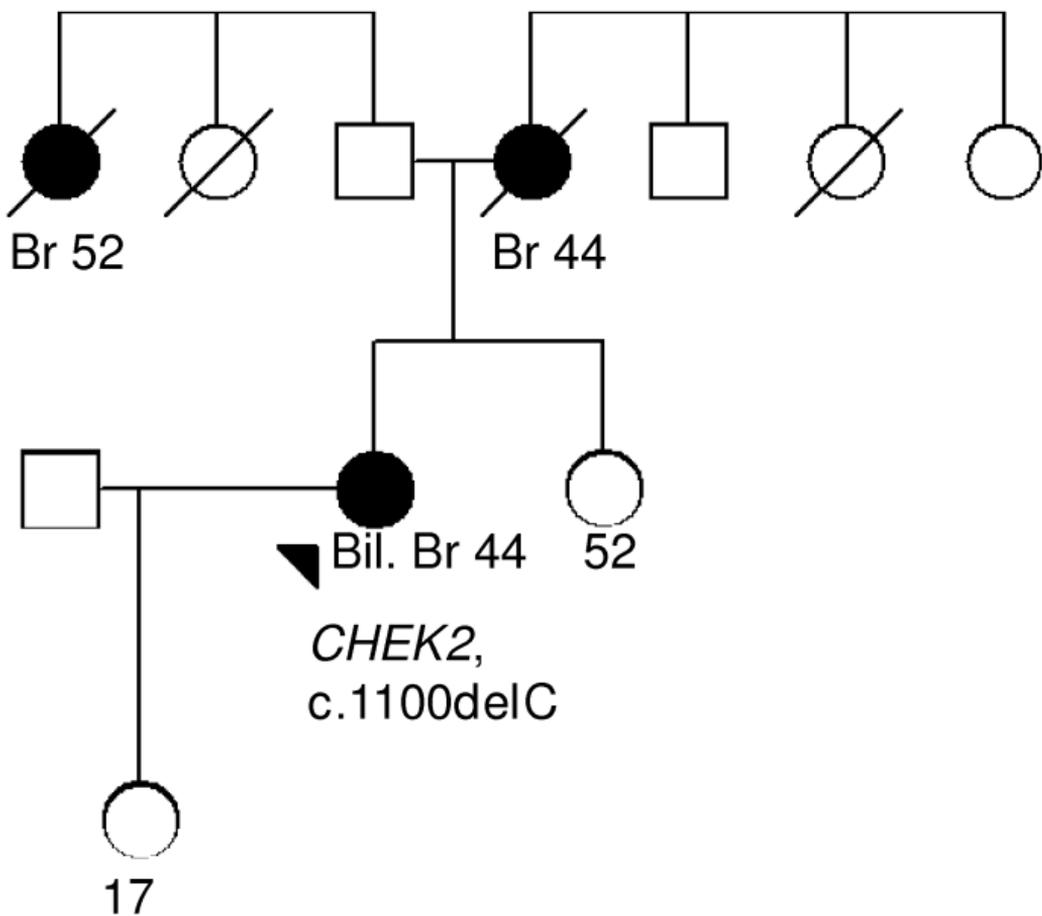
CHEK2, c.792+39C>T

CHEK2, c.1100delC

RAD50, c.2398-32A>G

Family 264

Figure 4



Family 265

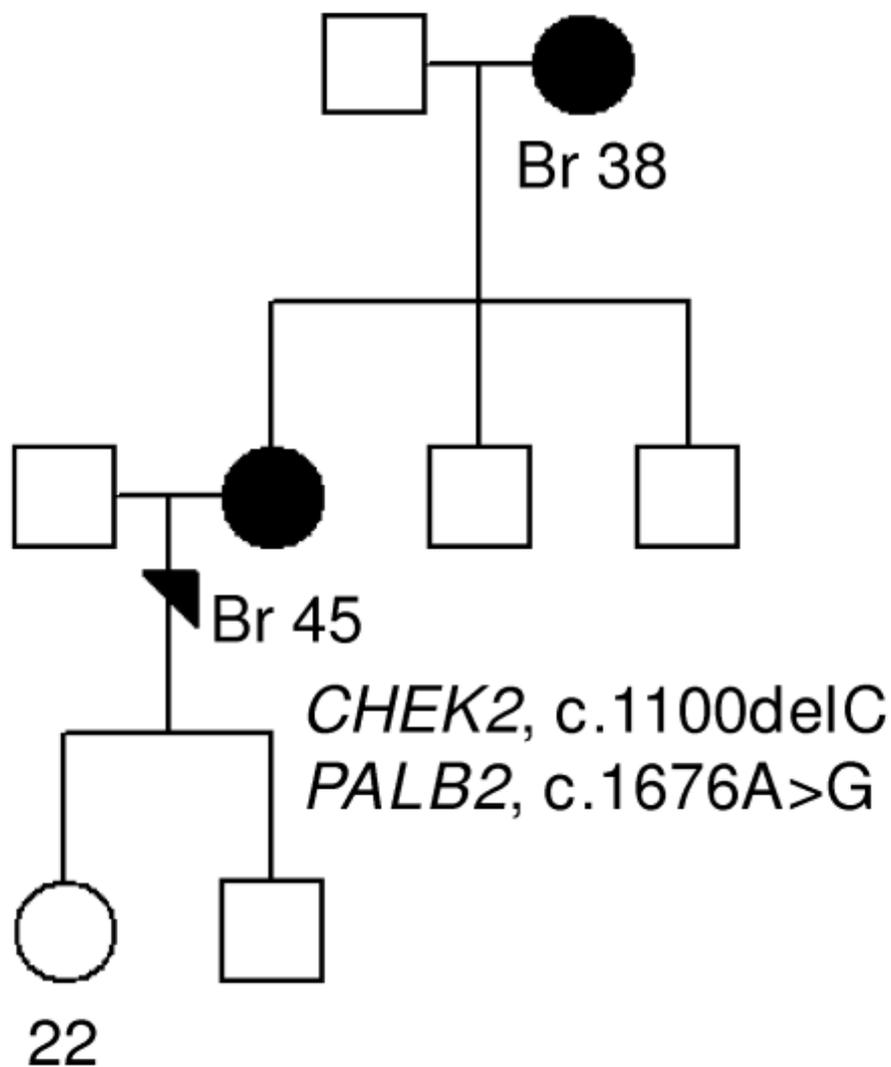


Figure 5

Family 262

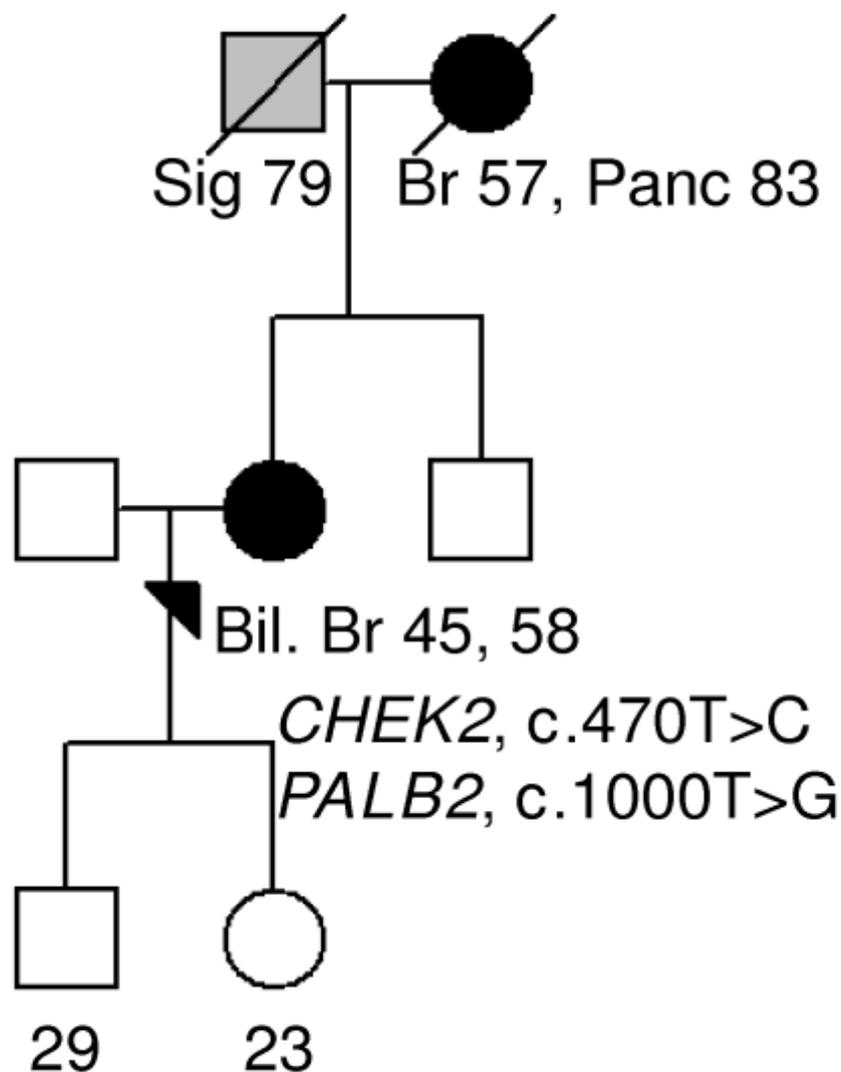


Figure 6

Family 131

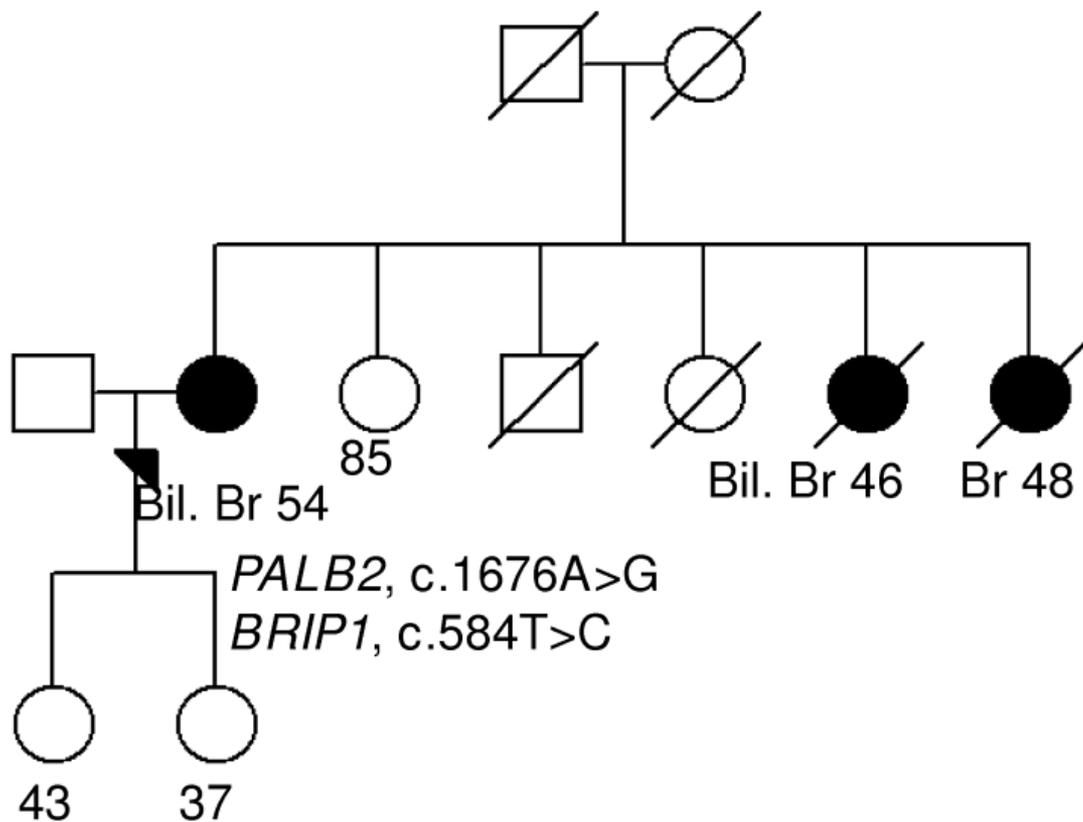
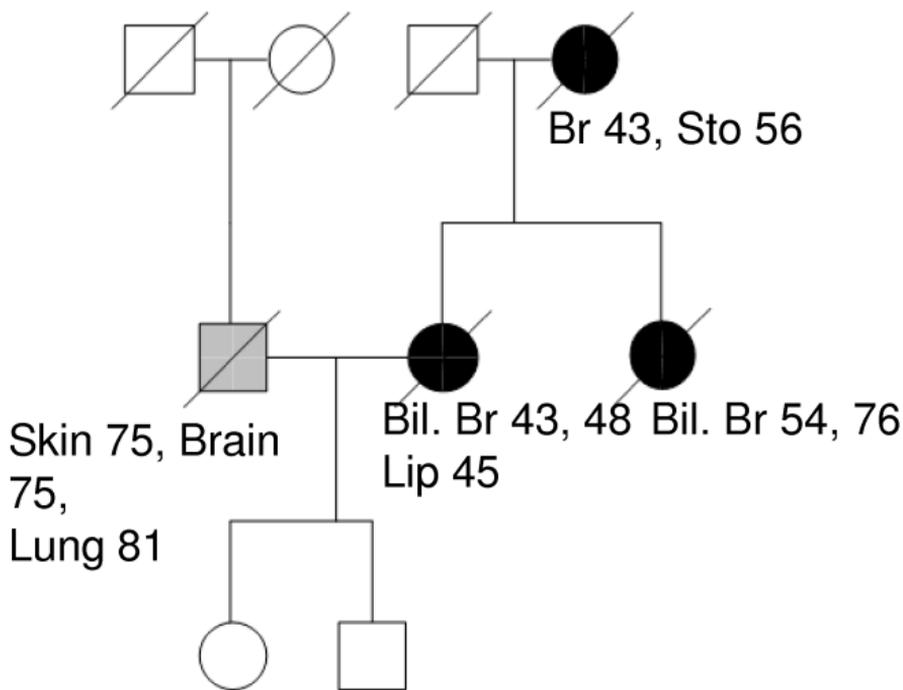


Figure 8

Family 005



▼
BRCA2, c.8182G>A
BRCA2, c.10234A>G

Additional files provided with this submission:

Additional file 1: Supplementary Table 1_270211.pdf, 33K

<http://breast-cancer-research.com/imedia/6893454835230550/supp1.pdf>

Additional file 2: Supplementary Table 2_270211.pdf, 12K

<http://breast-cancer-research.com/imedia/1962952255523055/supp2.pdf>