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## PALB2/FANCN - recombining cancer and Fanconi anemia

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### Abstract

PALB2 was originally identified as a BRCA2-interacting protein which is crucial for key BRCA2 genome caretaker functions. It subsequently became clear that *PALB2* was another Fanconi anemia (FA) gene (*FANCN*), and that monoallelic *PALB2* mutations are associated with increased risk of breast and pancreatic cancer. Mutations in *PALB2* have been identified in breast cancer families worldwide and recent studies have shown that PALB2 also interacts with BRCA1. Here we summarize the molecular functions and clinical phenotypes of this key DNA repair pathway component and discuss how its discovery has advanced our knowledge of both FA and adult cancer predisposition.

### Introduction

Genome instability is a hallmark of most cancers as well as a number of cancer susceptibility syndromes including the recessive childhood disease Fanconi anemia (FA). The clinical phenotypes of FA include diverse developmental defects, progressive aplastic anemia and heightened susceptibility to cancer (1). Although FA is genetically heterogeneous, consisting of at least 13 complementation groups (FA-A, B, C, D1, D2, E, F, G, I, J, L, M, N, and possibly RAD51C), a unifying feature of all FA subtypes is a cellular sensitivity to DNA interstrand crosslinkers (ICLs) such as mitomycin C (MMC) and diepoxybutane (DEB), indicating that a DNA repair defect is key to the development of FA (1–2). Interestingly, the gene underlying the D1 subtype of FA, *FANCD1*, was found to be *BRCA2* (3). Germline, monoallelic (heterozygous) mutations in *BRCA2* are associated with an increased risk of breast, ovarian, pancreatic and prostate cancers, whereas biallelic mutations in the gene lead to FA-D1. As the most important function of *BRCA2* thus far established is to enable homologous recombination (HR) through its control of the localization and function of recombination enzyme RAD51 (4), these discoveries suggest that the recombination function may be a cause of the associations between risks of FA and the above cancers. This notion is supported by the emerging consensus that HR is essential for the completion of ICL repair (5), a common defect of all FA cells.

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## Identification and functional studies of PALB2

PALB2 (partner and localizer of BRCA2) was identified by searching for novel components of endogenous BRCA2-containing complexes (6). The *PALB2* gene consists of 13 exons and maps to chromosome 16p12.2 (Figure 1A), which shows loss of heterozygosity in around 12% of breast cancers (7). The 1,186-aa protein has a coiled-coil motif at the N-terminus and a C-terminal domain containing a series of WD repeats (Figure 1B). The protein was found to be associated with around 50% of cellular BRCA2 and is critical for its chromatin localization and recruitment to DNA damage sites (6). Like BRCA2-deficient cells, PALB2-knockdown cells exhibited diminished HR activity, MMC sensitivity, and intra-S phase checkpoint defects. Later, it was found that PALB2-deficient FA-N cells lacked chromatin-bound BRCA2 and were completely unable to form RAD51 foci (8). The PALB2 binding site was mapped to the extreme N-terminus of BRCA2, which is both necessary and sufficient for the binding. Importantly, eight naturally occurring, breast cancer patient-derived BRCA2 unclassified variants were found to exist within or very close to the PALB2 binding region, and functional analyses of the variants showed that three of the eight variants disrupted PALB2 binding and the same three (and only the same three) variants also abrogated BRCA2 HR function (6). Together, the strong correlation between the ability of a BRCA2 variant to bind PALB2 and its ability to support HR, and the complete lack of RAD51 foci in *PALB2*-deficient cells indicate that PALB2 is crucial for BRCA2 HR function. To the extent that the HR function of BRCA2 is generally believed to be essential for its tumor suppression activity and that the three mutants are derived from breast cancer patients, the above findings further suggest that PALB2 is important for BRCA2-mediated tumor suppression. Similar to *Brca2*, homozygous *Palb2* knockout in mice causes embryonic lethality and heterozygous animals are normal (9).

Like BRCA2, BRCA1 has also been known to be important for HR, and the two breast cancer proteins co-exist in an endogenous protein complex (10). Yet how these two proteins associate with each other and whether they work together in HR remained unknown until recently. From three studies published in close succession, it was established that PALB2 physically links BRCA1 and BRCA2 to form a “BRCA complex” (11–13). Specifically, a coiled-coil motif in the N-terminus of PALB2 directly binds another coiled-coil motif in the “pre-BRCT” domain of BRCA1, which was exactly the BRCA1 domain originally found to be responsible for BRCA2 binding (10). In contrast PALB2 directly interacts with BRCA2 with its C-terminal WD repeats domain, whose crystal structure, a seven-bladed  $\beta$ -propeller, has been solved in a complex with the cognate BRCA2 N-terminal peptide (14). It was also found that PALB2 and BRCA2 focus formation was largely abolished in *BRCA1*-mutant HCC1937 cells and that acute knockdown of BRCA1 abrogated endogenous PALB2/BRCA2 foci (11,13). Furthermore, several point mutations in PALB2 specifically disrupting BRCA1 binding were generated and all resulted in a failure of the protein to support HR (12–13). Finally, multiple clinically-relevant point mutations in the coiled-coil domain that abolish PALB2 binding were identified in BRCA1 and were shown to disable its HR function (12). Taken together, these findings strongly supported the notion of a BRCA1-PALB2-BRCA2-RAD51 pathway critical for the initiation of HR and suppression of cancer and FA (Figure 1C).

However, two significant discrepancies have emerged with respect to the regulation of PALB2 function. First, it remains unclear if PALB2 recruitment to DNA damage sites is strictly dependent upon BRCA1. Two of the three studies above showed that endogenous PALB2 failed to form clear foci in the absence of BRCA1 (11,13), but the third showed that ectopically expressed PALB2 point mutant proteins largely unable to bind BRCA1 were still able to form foci with nearly normal efficiency (12), raising the question whether PALB2 may be able to form two distinct types of nuclear foci, one dependent and one independent of BRCA1. Second, the MORF-related protein MRG15, which is a component of certain chromatin remodeling complexes, has been identified as a major PALB2 binding partner (15). In the same study it

was found that down-regulation of MRG15 leads to an increase of HR and sister chromatid exchange (SCE), suggesting that MRG15 may restrict HR, be it through PALB2 or not. But a new study, which independently discovered the MRG15-PALB2 interaction, presents evidence that MRG15 may in fact facilitate HR by promoting PALB2 chromatin localization (16). Further studies will be necessary to clarify or reconcile these conflicting observations.

## PALB2 and Fanconi anemia

Immediately after *PALB2* was discovered, biallelic pathogenic mutations were identified in eight FA-N families (8,17). In some respects, FA-N cases arising in *PALB2* biallelic mutation carriers have a typical FA phenotype with growth retardation and variable congenital malformations. However, *PALB2*-related FA is associated with an unusually severe predisposition to pediatric malignancies (Table 1), with all eight described cases having developed cancer in early childhood, including five medulloblastomas, three Wilms tumors, two AMLs, one neuroblastoma and one kaposiform hemangioendothelioma (8,17). The cancer spectrum for biallelic *PALB2* mutation carriers is very similar to that of biallelic *BRCA2*/*FANCD1* mutation carriers, who also are at high risk of embryonal tumors (18). The strong similarity of cancer types and ages of onset in FA for both *PALB2* and *BRCA2* biallelic mutation carriers again supports the proposition that PALB2 is important for BRCA2 tumor suppression activity.

## PALB2 mutations and hereditary susceptibility to breast cancer

In view of the close functional relationship between PALB2 and BRCA2 and the similar phenotypes associated with biallelic mutation carriers, it was conceivable that monoallelic *PALB2* mutations may increase risk of breast cancer. Five different monoallelic *PALB2* truncating mutations were soon found in ten women from a series of 923 cases with a strong family history of breast cancer (19). These five mutations together were estimated to be associated with, on average, a moderate 2.3-fold increased risk on top of the women's underlying polygenic risk. Therefore, female monoallelic mutation carriers with a strong family history could be at high absolute risk of breast cancer (20). Moreover, as none of the 1,084 controls had a mutation, this risk estimate could be open to question. At the same time, a founder *PALB2* mutation, 1592delT, was identified in approximately 1% of all Finnish breast cancers unselected for family history (21). Using a modified segregation analyses fitted under maximum likelihood theory, the 1592delT mutation was estimated to be associated with about a six-fold increased risk of breast cancer, and the estimated age-specific cumulative risk by age of 70 for monoallelic carriers was comparable to that for *BRCA2* mutation carriers in the same country (22). Another founder *PALB2* mutation, 2323C>T, was subsequently identified and found to be present in ~0.5% of unselected French-Canadian women with early-onset breast cancer (23). *PALB2* mutations have now been identified in many countries (Table 2), with frequencies varying from 0.6% to 2.7% in familial breast cancer cases. However, penetrance estimation from multiple-case families is problematic (19), and due to the limited number of unselected cases studied to date, the average penetrance of *PALB2* mutations as a whole, let alone those of specific mutations and/or mutations in different settings (e.g. women with a family history, or with specific risk factors), is not known with certainty.

To date, information for 58 breast cancers arising in *PALB2* mutation carriers have been published (Table 2), with about two-thirds of these cases emanating from two separate studies in Finland (24–25). The cancers are frequently high grade infiltrating ductal type with 40% overall (20/50) having an ER-, PR-, HER2- (triple negative) phenotype. This phenotype does not appear to be mutation-specific because, even with exclusion of the Finnish mutation breast cancers, 8/21 (38%) of the remaining cases are triple negative. In the Finnish study, 7/12 triple negative breast cancers had a basal-like phenotype (25) and at least two other studies have

reported medullary breast cancers (23,26). It therefore appears that *PALB2*-related breast cancers might represent a separate category from *BRCA1*- and *BRCA2*-related tumors with some overrepresentation of triple negative tumors, more akin to *BRCA1*- than *BRCA2*-related tumors. It is tempting to speculate that this aspect of the phenotype could be related to the nature of the interaction and/or certain functional similarities between *PALB2* and *BRCA1* (11–12), or a direct transcriptional activation of the estrogen receptor by *PALB2* as has been shown for *BRCA1* (27). However, larger numbers of *PALB2*-related tumors will need to be studied before any firm conclusions can be drawn.

## Predisposition to other cancers

In addition to breast cancer, *BRCA2* mutation carriers are at increased risk of ovarian, pancreatic, prostate cancers and melanoma which raises the possibility that *PALB2* mutation carriers might also be at increased risk of developing these cancers. Using exomic sequencing, Jones *et al.* identified a germline *PALB2* mutation in a familial pancreatic cancer, and when they sequenced *PALB2* in 96 other highly selected pancreatic cancer families, a further three mutations were identified (28). A subsequent study of 254 less highly selected families from Canada identified one large deletion mutation (29). Four of the above five *PALB2*-related pancreatic cancer families included at least one case of breast cancer, and in two families mutations were found in women with both breast and pancreatic cancer. Recently, another study found truncating mutations in three out of 81 (3.7%) European familial pancreatic cancer families which all included breast cancers (30). Given the rarity of these mutations, *PALB2* mutation screening may be of limited help for most pancreatic cancer families, but it should be considered if there is an associated history of breast cancer. Only one prostate cancer family with a *PALB2* mutation (the 1592delT Finnish founder) segregating with disease has been reported (21) but a larger study of 178 familial and 285 unselected Finnish prostate cancer cases did not find an association with this mutation (31). A study of 95 prostate cancer families where the median age of diagnosis in the whole cohort was 49 years did not identify any pathogenic *PALB2* variants (32). *PALB2* has not been implicated as a predisposition gene in male breast cancer (24).

To date, there have only been two reports on the occurrence of *PALB2* germline mutations in ovarian cancer (26,33). In the first study, DNA samples of unselected Finnish ovarian cancer cases were screened for the presence of the *PALB2* c.1592delT founder mutation. Three out of 593 (0.5%) cancer cases were heterozygous for the founder mutation, compared to frequency of 0.2% in the controls. The second study identified a mutation in two out of 339 unselected Polish ovarian cancer cancers (0.6%) compared to a control frequency of 1 in 1310 (0.08%) (26). However, one of these cases was subsequently found to also have a *BRCA2* mutation.

## Putative genetic mechanisms of *PALB2*-related carcinogenesis

It is unclear how *PALB2* heterozygous mutations might cause cancers. Loss of heterozygosity in *PALB2* breast tumors has been demonstrated in only one case (34) out of the nine analyzed cases reported to date, so this does not seem to be a common feature of *PALB2* tumorigenesis. In addition, preliminary functional studies have not shown a dominant-negative effect of truncated *PALB2* proteins in HR (21,35), although they may be dominant negative in other as yet unknown functions. It is therefore possible that the second *PALB2* allele is somatically mutated in the tumors as was observed in a *PALB2*-related pancreas cancer (28). In a similar vein, a heterozygous somatic *PALB2* mutation was found in a genomic sequencing study of a metastatic, lobular, ER+ breast cancer (36). Another possibility is that the wild-type allele could be epigenetically silenced, although to date there are no published reports of *PALB2* promoter methylation in tumors from *PALB2* heterozygotes. In contrast, methylation in unselected tumors has been observed at a 1,512bp CpG island located in the promoter and exon

1 (37). Specifically, this study found methylation in 4/60 (7%) breast cancers, 4/53 (7.5%) ovarian tumors and 2/8 (25%) breast cancers arising in *BRCA2* mutation carriers. Finally, array CGH studies of *PALB2*-related tumors have shown differences compared with *BRCA2*-related tumors (35) with a consistent loss of chromosome 18q. However, the number of *PALB2*-related tumors was small and these observations need to be verified by larger studies.

## Summary and perspectives

It is now clear that *PALB2* physically and functionally links *BRCA1* and *BRCA2* to form a “BRCA complex” whose integrity is essential for the avoidance of cancer and FA. Though all three proteins are critical for HR, *BRCA1* and to a lesser extent *PALB2* mutations, but not *BRCA2* mutations, confer susceptibility to triple negative tumors, indicating that defective HR may increase risk of breast cancer but not necessarily triple negative disease. Identification of potential new functions shared by *BRCA1* and *PALB2* but not *BRCA2* thus hold promise to uncover the molecular genesis of triple negative breast cancer. *BRCA1* has been implicated in transcriptional regulation of many genes and in cellular redox regulation (38–39) so it would be interesting to determine if *PALB2*, a chromatin-associated protein, regulates some of the same genes as *BRCA1* and if *PALB2* also plays a role in oxidative stress response.

Similar to *BRCA2/FANCD1* and *PALB2/FANCN*, mutations in another FA susceptibility gene, *BRIP1/FANCI*, are also associated with breast cancer susceptibility (40). *BRCA2* and *PALB2* are both critical for HR, and *BRIP1* also contributes to the process although the mechanism is unclear. Mutations in yet another critical HR gene, *RAD51C*, have just been shown to be associated with both FA-like phenotypes and breast/ovarian cancers (41–42). These findings suggest that HR may be what “recombines” cancer and FA. All four proteins act “downstream” of *FANCD2/FANCI* in ICL repair, which could in part explain why mutations in their genes are associated with risk of breast cancer, unlike the other genes encoding FA proteins (43). The reason(s) for differences in the cancer risks and gene groups remain unknown (44).

A major impact of *BRCA1/BRCA2*-related research on cancer intervention has been the recent discovery that *BRCA1- or BRCA2*-deficient tumor cells are hypersensitive to PARP inhibitors (45–46), which results in persistence of unrepaired single strand breaks in DNA ultimately leading to replication fork collapse that requires HR to restore. Multiple clinical trials of several different PARP inhibitors have been conducted or are underway, and respectable anti-tumor activities have already been reported for Olaparib (AZD2281) (47). Given the similar function of *PALB2* in HR, it is not surprising that we found EUFA3141 (*FANCN*) cells are also hypersensitive to Olaparib (unpublished data). Therefore it is possible that PARP inhibitors, either as single agents or in conjunction with other drugs, could also have clinical utility in cancer patients with germline *PALB2* mutations.

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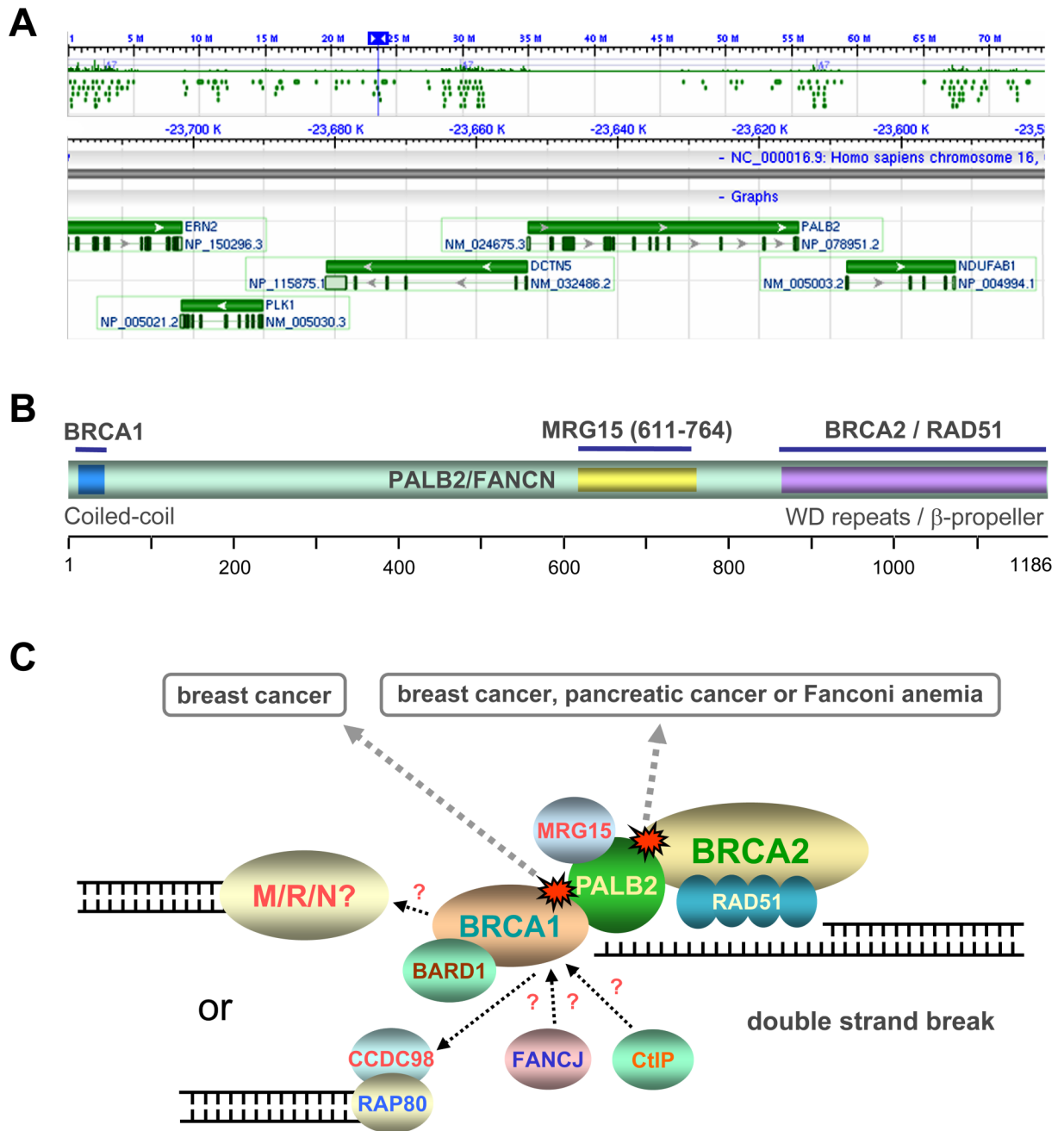
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**Figure 1. The BRCA complex of HR repair and tumor suppression**

(A) The *PALB2* gene locus in chromosome 16p12.2. The image is generated using the NCBI Sequence Viewer and slightly modified. (B) Schematic of the PALB2 protein structure showing its domains responsible for binding with BRCA1, BRCA2 and MRG15. (C) A proposed model of BRCA complex assembly at sites of DNA double strand breaks. It is currently unclear which BRCA1 BRCT domain binding partners (BRIP1/FANCF, CCDC98-RAP80 or CtIP) exist in the core BRCA1/PALB2/BRCA2 complex. Also, BRCA1 may be recruited to damage sites via two distinct mechanisms- one by interacting with the MRE11/RAD50/NBS1 (MRN) complex and the other via its binding to the CCDC98/RAP80 complex, and it remains to be seen which branch is responsible for PALB2/BRCA2/RAD51 recruitment.

**Table 1**

Malignancies associated with germline mutations in *PALB2*.

Biallelic (Fanconi Anemia)		Monoallelic
Tumor type	n (%)	
Medulloblastoma	5 (62.5)	Breast cancer
Wilms tumor	3 (37.5)	Pancreatic cancer
Acute myeloid leukemia	2 (25)	
Neuroblastoma	1 (12.5)	
Hemangioendothelioma	1 (12.5)	

n= number of malignancies in *PALB2*-related FA based on findings in eight published families (8,17)

Table 2

Distribution and frequency of published *PALB2* mutations with characteristics of 58 *PALB2*-related breast cancers in the published literature.

Country	Author	Breast Cancer Cases	DNA Mutations	Protein Change	Cases	Controls	Tumor Characteristics			LOH
							Type	Grade	Receptors	
Canada	Foulkes (23)	Familial/ Early onset	2323C>T	Q775X	2/356 (0.5%)	0/6440	IDC IDC medullary	2/3 3/3 3/3	ER+PR+HER2- ER-PR-HER2- ER-PR-HER2-	NS
Canada	Tischkowitz (35)	Familial	229delT	C77fs	1/68 (1.5%)		8 IDC 1 ILC 1 unknown	2/3 n=5 3/3 n=2	6 ER+PR+ 2 ER+PR-HER2-	no n=4
China	Cao (48)	Familial	751C>T 1050_51delA AmsTCT All	Q251X K353fs	2/360 1/360 3/360 (0.8%)	0/864 0/864	IDC IDC ILC IDC	2/3 2/3 3/3	ER+PR+HER2+ ER+PR+HER2+ ER-PR+HER2-	NS
Finland	Erkko (24)	Familial Unselected	1592delT	L531fs	3/113 (2.7%) 18/1918 (0.9%)	6/2501 (0.2%)	NS	NS	5 ER+PR+HER2- 1 ER+PR+HER2- 1 ER-PR-HER2-	no n=5
Finland	Heikkinen (25)	Familial Unselected	1592delT	L531fs	19/947 (2%) 8/1274 (0.6%)	2/1079 (0.19%)	25/33 IDC 3/33 ILC 5/33 other	1/3 n=3 2/3 n=12 3/3 n=17	14/30 ER+ 13/30 PR+ 1/22 HER2+ 12/22 ER-PR- HER2-	NS
Italy	Papi (49)	Familial	2257C>T	R753X	1/132 (0.75%)	1/300 (0.3%)	NS	NS	1 ER+PR+HER-	NS
Poland	Dansonka-Mieszkowska (26)	Familial	c.509_510 delGA	R170fs	4/648 (0.6%)	1/1310 (0.08%)	IDC IDC IDC medullary	2/3 2/3 3/3	ER-PR-HER2- ER+PR-HER2- ER-PR-HER2- ER-PR-HER2-	NS
South Africa	Sluiter (50)	Early onset	697delG	V233fs	1/48 (2.1%)		IDC	NS	ER-PR-HER2-	NS
Spain	Garcia (34)	Familial	1056_1057 delGA	K353fs	1/95 (1.05%)		IDC	3/3	ER-PR-HER2-	yes
UK	Rahman (19)	Familial	2386G>T 2982insT 3113G>A 3116delA N1039fs 3549C>G All	G796X A995fs W1038X N1039fs Y1183X	1/923 1/923 2/923 3/923 3/923 10/923 (1.1%)	0/1084 0/1084 0/1084 0/1084 0/1084				

IDC, infiltrating ductal cancer, ILC infiltrating lobular cancer, NS not stated