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PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene

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Abstract

PALB2 interacts with BRCA2, and biallelic mutations in *PALB2* (also known as *FANCN*), similar to biallelic *BRCA2* mutations, cause Fanconi anemia. We identified monoallelic truncating *PALB2* mutations in 10/923 individuals with familial breast cancer compared with 0/1,084 controls ($P = 0.0004$) and show that such mutations confer a 2.3-fold higher risk of breast cancer (95% confidence interval (c.i.) = 1.4–3.9, $P = 0.0025$). The results show that *PALB2* is a breast cancer susceptibility gene and further demonstrate the close relationship of the Fanconi anemia–DNA repair pathway and breast cancer predisposition.

PALB2 (for 'partner and localizer of BRCA2') encodes a recently discovered protein that interacts with BRCA2, is implicated in its nuclear localization and stability and is required for some functions of BRCA2 in homologous recombination and double-strand break repair1. In a paper in this issue, we show that biallelic *PALB2* mutations are responsible for a subset of Fanconi anemia cases characterized by a phenotype similar to that caused by biallelic *BRCA2* mutations2. Prompted by these observations, we investigated whether monoallelic *PALB2* mutations confer susceptibility to breast cancer by sequencing the gene

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AUTHOR CONTRIBUTIONS

The study was designed by N.R. and M.R.S. The molecular analyses were performed by S.S., P.K., A.R., S.R., K.S., R.B., T.C., H.J. and S.H. under the direction of N.R. The statistical analyses were performed by D.T., A.E. and L.M. under the direction of D.F.E. The familial collections were initiated by D.G.E. and D.E. and were collected by the Breast Cancer Susceptibility Collaboration (UK). The manuscript was written by N.R. and M.R.S.

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COMPETING INTERESTS STATEMENT

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in individuals with breast cancer from familial breast cancer pedigrees that were negative for mutations in *BRCA1* and *BRCA2* and controls (**Supplementary Methods** online).

We identified truncating *PALB2* mutations in 10/923 (1.1%) independently ascertained individuals with familial breast cancer from separate families compared with 0/1,084 (0%) controls ($P = 0.0004$) (Table 1 and Fig. 1a). Nine of the *PALB2* mutations were in the 908 families with female breast cancer only (1.0%). One occurred in the 15 families (6.7%) with cases of both female and male breast cancer ($P = 0.15$). Although this observation requires further investigation, it suggests that *PALB2* mutations may confer a higher relative risk of male breast cancer than female breast cancer, and *BRCA2* mutations are known to confer a high relative risk of male breast cancer3. One proband with a *PALB2* mutation developed melanoma at 47 years of age in addition to breast cancer at 56 years. Apart from this individual, there were no other malignancies other than breast cancer in individuals with *PALB2* mutations. Two of four first-degree affected relatives of probands with *PALB2* mutations also carried a *PALB2* mutation. This pattern of incomplete segregation in affected relatives is typical of susceptibility alleles that confer modestly increased risks and is similar to that reported in breast cancer families carrying *CHEK2, ATM* or *BRIP1* mutations4-6.

Segregation analysis incorporating the information from controls and the full pedigrees of the affected individuals estimated the relative risk of *PALB2* mutations to be 2.3 (c.i. = 1.4–3.9, $P = 0.0025$). The relative risk for women under 50 years was 3.0 (95% c.i. = 1.4–5.5), and for women over 50 years it was 1.9 (95% c.i. = 0.8–3.7, $P = 0.35$ for difference in relative risk between the age groups). The median age at diagnosis of individuals with *PALB2* mutations was 46 years (interquartile range (IQR) = 40–51) compared with a median age at diagnosis of 49 years (IQR = 42–55) in individuals with breast cancer without *PALB2* mutations ($P = 0.24$ for difference). These data suggest that the risks of breast cancer associated with *PALB2* mutations may be age dependent, but additional studies will be required to address this question. There was no difference in the extent of familial clustering of breast cancer ($P = 0.69$) or in the probability of being a bilateral case ($P = 0.23$) in families with *PALB2* mutations compared with families without mutations. Assuming a conservative sensitivity of 90% for mutation detection, we estimate the breast cancer population attributable fraction of *PALB2* mutations to be 0.23% (95% c.i.: 0.072%–0.52%) and the percentage of the familial relative risk due to *PALB2* to be 0.24% (0.02%–1.16%).

We identified 50 nontruncating variants within the *PALB2* coding sequence, including 35 nonsynonymous and 15 synonymous variants (**Supplementary Table 1** online). There was no overall evidence that *PALB2* missense variants confer susceptibility to breast cancer, with 215 (23%) affected individuals and 265 (24%) controls carrying at least one nonsynonymous missense variant. Only four missense variants had an allele frequency greater than 1%, and there was no evidence that any of these were breast cancer susceptibility alleles. This result is consistent with the data from individuals with Fanconi anemia in which all reported *PALB2* mutations result in premature protein truncation2,7.

Fanconi anemia is a genetically heterogeneous recessive condition that currently includes 13 subtypes, 12 of which have been attributed to distinct genes2,8. The known Fanconi anemia genes encode proteins that interact in an incompletely understood fashion to facilitate recognition and repair of DNA double-strand breaks. A key process in the pathway involves eight of the known Fanconi anemia proteins forming a nuclear core complex that mediates monoubiquitination and activation of FANCD2. Activated FANCD2 is translocated to DNA repair foci, where it colocalizes with BRCA2 and other proteins that effect DNA repair by homologous recombination (Fig. 1b)8.

Biallelic mutations of *BRCA2* and *PALB2* cause Fanconi anemia subtypes FA-D1 and FA-N, respectively2,7,9. The phenotypes associated with biallelic *BRCA2* and *PALB2* mutations are markedly similar to each other and differ from the other ten known Fanconi anemia genes. In particular, FA-D1 and FA-N are associated with high risks of solid childhood malignancies such as Wilms tumor and medulloblastoma, which occur very rarely in other subtypes2,8,10. Heterozygous mutations in *BRIP1*, which encodes a BRCA1-interacting protein, also confer an elevated risk of breast cancer6, and biallelic *BRIP1* mutations cause Fanconi anemia subtype FA-J11,12. However, FA-J is associated with the classical Fanconi anemia phenotype, and there have not been any reports of individuals with FA-J with a childhood solid tumor11,12.

It is plausible that heterozygosity for mutations in other Fanconi anemia genes may also be involved in breast cancer susceptibility. However, epidemiological studies of relatives of individuals with Fanconi anemia have not demonstrated this, suggesting that breast cancer susceptibility is associated with only a subset of Fanconi anemia genes. This is consistent with the negative results of mutational screens of other Fanconi anemia genes in familial breast cancer cases13. The biological features that determine whether a Fanconi anemia gene is also a breast cancer predisposition gene are unknown. However, it is notable that the three Fanconi anemia genes currently associated with breast cancer susceptibility (*BRCA2, PALB2* and *BRIP1*) are not part of the Fanconi anemia core complex and are the only known Fanconi anemia genes that act downstream of FANCD2 (Fig. 1b).

We estimate that *PALB2* mutations are associated with an approximately twofold higher risk of female breast cancer. Therefore, despite the fact that PALB2 is functionally associated with BRCA2 and that biallelic mutations in both genes cause similar phenotypes, the increase in breast cancer risk associated with *PALB2* monoallelic mutations is clearly more modest than that conferred by *BRCA2* monoallelic mutations, which result in approximately a tenfold increase in risk. These differences in risk are reminiscent of those previously reported between *BRCA1* mutations, which also confer a greater than tenfold increase in risk of breast cancer, and mutations in *BRIP1*, which confer only a twofold increase in risk6. The explanations for the apparent differences in risk associated with mutations in these genes, despite the close functional interactions between the proteins they encode, are currently unknown. Thus, our data provide further evidence of the close link between breast cancer susceptibility and the Fanconi anemia-DNA repair pathway, but they also demonstrate that the relationship is complex at both the phenotypic and molecular levels.

With the identification of *PALB2* as a new breast cancer predisposition gene, a clearer picture of the genetic architecture of breast cancer susceptibility is emerging. *BRCA1* and *BRCA2* are likely to be the only major high-penetrance breast cancer susceptibility genes (leading to more than a tenfold higher risk). Mutations in *TP53* also confer high risks of breast cancer but are much rarer14. These genes are characterized by multiple, rare, inactivating mutations that together account for approximately 15%–20% of the familial risk of the disease14. A similar mutation spectrum has now been identified in four additional genes that encode proteins that interact biologically with BRCA1, BRCA2 and/or p53. Three of these proteins, CHK2, ATM and BRIP1, interact with BRCA1, p53 or both (refs. 8, 15). PALB2 is the first that interacts with BRCA2. However, compared with risks associated with mutations in *BRCA1, BRCA2* and *TP53*, the risks associated with mutations in *CHEK2, ATM, BRIP1* and *PALB2* are much lower4-6. Moreover, inactivating mutations in each of these genes are rare, with fewer than 1% of the population being heterozygotes. As such, the contribution of each gene to the familial risk of breast cancer is small. Collectively, however, they already account for ~2.3% of the overall familial relative risk. Thus, this class of susceptibility gene may make an appreciable contribution to breast cancer predisposition.

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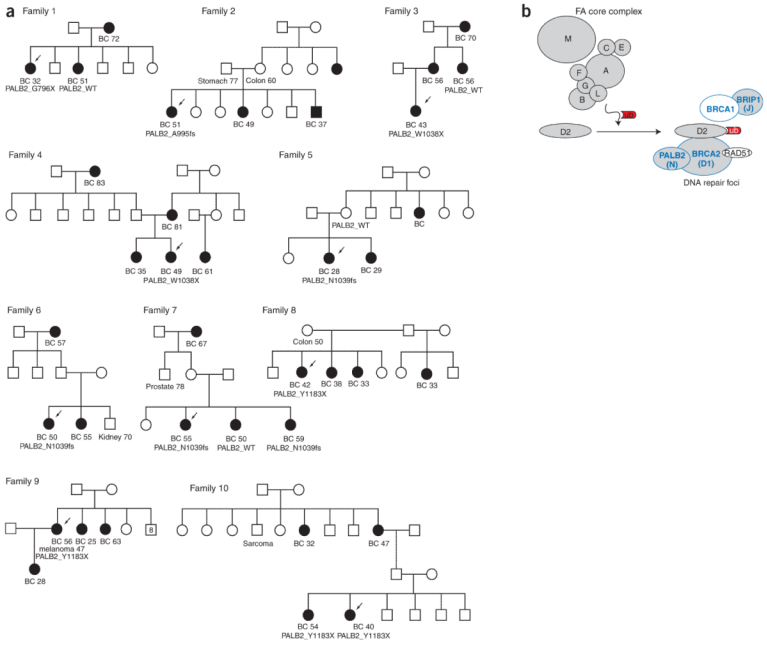

Figure 1.

PALB2 mutations in familial breast cancer. (**a**) Abridged pedigrees of ten families with breast cancer with *PALB2* mutations. The probands screened for *PALB2* mutations are indicated by arrows. Individuals with breast cancer are shown as filled circles, with the age at diagnosis given underneath. Other cancers are indicated beneath the relevant individuals, with age at diagnosis next to the cancer type. Some individuals with cancer were not genotyped either because they were deceased or because they declined to take part in the study. We obtained informed consent from all families, and the research was approved by the London Multicentre Research Ethics Committee (MREC/01/2/18). The *PALB2* mutation in each family is given under the proband and in Table 1. BC, breast cancer; PALB2_WT, *PALB2* mutation absent. (**b**) Schematic diagram of the Fanconi anemia–BRCA pathway. The Fanconi anemia core complex consists of eight Fanconi anemia proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL and FANCM) and is essential for the monoubiquitination and activation of FANCD2 (‘D2’ in the figure) after DNA damage. Activated FANCD2 is translocated to DNA repair foci, where it colocalizes with other DNA damage response proteins, including BRCA2 and RAD51, and participates in homology-directed repair. Shaded proteins are encoded by genes that cause Fanconi anemia. Proteins outlined in blue are encoded by genes that confer susceptibility to breast cancer. *BRIP1*, *BRCA2* and *PALB2* are both Fanconi anemia genes and breast cancer susceptibility genes, and they encode proteins functioning downstream of FANCD2.

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Table 1

Cancer history and *PALB2* mutations identified through analyses of individuals with familial breast cancer and controls

Family	Cancer history and age of proband	Number of relatives with breast cancer	*PALB2* mutation	PALB2 alteration
1	Breast cancer, 32 years	2	2386G→T	G796X
2	Breast cancer, 51 years	2 female, 1 male	2982insT	A995fs
3	Breast cancer, 43 years	3	3113G→A	W1038X
4	Breast cancer, 49 years	4	3113G→A	W1038X
5	Breast cancer, 28 years	2	3116delA	N1039fs
6	Breast cancer, 50 years	2	3116delA	N1039fs
7	Breast cancer, 55 years	3	3116delA	N1039fs
8	Breast cancer, 42 years	3	3549C→G	Y1183X
9	Breast cancer, 56 years Melanoma, 47 years	3	3549C→G	Y1183X
10	Breast cancer, 40 years	3	3549C→G	Y1183X

The mutations identified in families 5–10 have previously been reported as causative in individuals with Fanconi anemia subtype N (ref. 2; none of the FA-N families are part of this study). The probands with identical mutations were from separately ascertained families that are not known to be related and are from different parts of the UK. The pedigrees of families 1–10 are shown in Figure 1. We did not find any truncating mutations in sequencing the full *PALB2* coding sequence from 1,084 controls.