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A recurrent mutation in *PALB2* in Finnish cancer families

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***BRCA1*, *BRCA2* and other known susceptibility genes account for less than half of the detectable hereditary predisposition to breast cancer[1–3]. Other relevant genes therefore remain to be discovered. Recently a new BRCA2-binding protein, PALB2, was identified[4]. The BRCA2–PALB2 interaction is crucial for certain key BRCA2 DNA damage response functions as well as its tumour suppression activity[4]. Here we show, by screening for *PALB2* mutations in Finland that a frameshift mutation, c.1592delT, is present at significantly elevated frequency in familial breast cancer cases compared with ancestry-matched population controls. The truncated PALB2 protein caused by this mutation retained little BRCA2-binding capacity and was deficient in homologous recombination and crosslink repair. Further screening of c.1592delT in unselected breast cancer individuals revealed a roughly fourfold enrichment of this mutation in patients compared with controls. Most of the mutation-positive unselected cases had a familial pattern of disease development. In addition, one multigenerational prostate cancer family that segregated the c.1592delT truncation allele was observed. These results indicate that *PALB2* is a breast cancer susceptibility gene that, in a suitably mutant form, may also contribute to familial prostate cancer development.**

BRCA2, one of the two major breast cancer susceptibility proteins, primarily functions in homologous recombination (HR) and HR-based DNA double-strand break repair (DSBR)[5]. The lifetime breast cancer risk of heterozygous *BRCA2* mutation carriers is about 60–85%[1,6]. In addition, BRCA2 is also a Fanconi anaemia protein[7]. Recently, a previously unidentified BRCA2 binding factor, PALB2 (for 'partner and localizer of BRCA2'), was identified and shown to be crucial for the association of BRCA2 with chromatin and nuclear structures and for its DNA damage response functions[4]. Importantly, certain breast-cancer-associated missense variants in the PALB2-binding domain of BRCA2 abrogate BRCA2–PALB2 complex formation, and these BRCA2 variants are defective in HR-based DSBR, emphasizing the importance of proper interplay between PALB2 and BRCA2 for this essential function[4]. Given the above-noted discoveries and the dependence of BRCA2 function on PALB2, we examined whether *PALB2* is a gene predisposing susceptibility to hereditary breast cancer in its own right.

To explore this possibility, we screened for germline mutations in the exonic regions and splice junctions of the *PALB2* gene, in 113 *BRCA1*/*BRCA2* mutation-negative breast or breast–ovarian cancer families from northern Finland. As shown in Table 1, a total of six different exonic variant alleles were identified in affected index individuals. Four of these changes were also detected at similar frequencies in the control population, suggesting that they are not cancer-associated. This view was supported by the results obtained from computer simulations using PolyPhen, ESEfinder and NNSplice software. By contrast, one alteration (c.1592delT) was detected in three (2.7%) index individuals, but only in six (0.2%) of 2,501 controls ($P = 0.005$; odds ratio (OR) 11.3; 95% confidence interval (CI) 1.8–57.8), therefore suggesting a significant disease association. This alteration should result in a frame-shift at Leu 531, with the new reading frame progressing for 28 codons before termination. Another alteration, 3433G→C (G1145R), was detected in one index individual but in none of 971 controls. In addition, three sequence alterations were detected in introns (Table 1), but none of them seemed disease-related.

c.1592delT and 3433G→C were then introduced into PALB2-expressing complementary DNA vectors and tested functionally. As shown in Fig. 1a, b, c.1592delT resulted in a truncated protein (PALB2-L531Fs), which had a markedly decreased BRCA2-binding affinity without affecting endogenous BRCA2 abundance upon transient overexpression (Fig. 1b). Consistent with this observation and the functional importance of BRCA2–PALB2 complex formation, PALB2-L531Fs failed to support HR in PALB2-knockdown cells (Fig. 1c) or to restore crosslink repair in PALB2-deficient cells (Fig. 1d). Thus, c.1592delT is a genuine loss-of-function mutation. In contrast, PALB2-G1145R seemed to be fully capable of BRCA2 binding and was functional in these two assays.

Subsequently, *PALB2* c.1592delT was sought in germline DNAs of unselected (not selected for or against family history of cancer) female breast cancer cases ($n = 1{,}918$), unselected male breast cancers ($n = 141$), colorectal cancers ($n = 476$; 188 were familial and 288 belonged to the unselected group) and prostate cancer cases ($n = 639$; 164 were familial and 475 belonged to the unselected group), all from Finland. In the unselected female breast cancer group, 18 additional mutation-positive cases were identified, and a

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Table 1 | *PALB2* sequence variants observed in comprehensive mutation screening in familial breast cancer patients

Exon/intron	Nucleotide change	Effect on protein	Carrier frequency, % (*n*/*N*)		*P* (OR; 95% CI)
			Familial cases	Controls	
Ex 4	1010*T*→C	Leu337Ser	8.0 (9/113)	10.0 (31/304)	0.5 (0.8; 0.4–1.7)
Ex 4	1592delT	Leu531→Fs→Stop	2.7 (3/113)	0.2 (6/2,501)	0.005 (11.3; 1.8–57.8)
Ex 4	1676A→G	Gln559Arg	8.8 (10/113)	15.0 (150/999)	0.08 (0.6; 0.3–1.1)
Ex 8	2794G→A	Val932Met	1.8 (2/113)	5.1 (16/315)	0.1 (0.3; 0.1–1.5)
Ex 12	3300*T*→G	Thr1100Thr	3.5 (4/113)	1.6 (5/322)	0.2 (2.3; 0.6–8.8)
Ex 13	3433G→C	Gly1145Arg	0.9 (1/113)	0 (0/971)	0.1 (n.a.)
IVS1–46	G→A	–	8.8 (10/113)	6.4 (19/295)	0.4 (1.4; 0.6–3.1)
IVS4–70	*T*→G	–	1.8 (2/113)	0 (0/302)	0.07 (n.a.)
IVS4–58	A→C	–	6.1 (7/113)	2.3 (7/302)	0.07 (2.8; 0.95–8.1)

The following sequence information was used: NC_000016.8 (genomic DNA) and NM_024675.2 (mRNA and protein). Fs, frameshift; n.a., not available.

significant association between c.1592delT and female breast cancer was observed (0.9% versus 0.2% in controls ($n = 2{,}051$; 70.6% females and 29.4% males), $P = 0.003$, OR 3.94, 95% CI 1.5–12.1). In addition, the mutant allele was detected in one familial prostate cancer individual, but none of the male breast cancer or colorectal cancer cases revealed any evidence of *PALB2* involvement (Supplementary Table 1). Because all unselected cases and the familial prostate and colorectal cases were only screened for c.1592delT, possible involvement of other, potentially disease-related *PALB2* mutations in these cancers cannot be ruled out. Of a total of 22 identified unrelated cancer patients (21 breast and 1 prostate) heterozygous for *PALB2* c.1592delT, 16 were tested for possible co-segregation of known Finnish *BRCA1* and *BRCA2* mutations[8], and none was detected.

The average age of disease onset for c.1592delT mutation-positive individuals was 52.9 years (variation 39–73 years), which seems slightly younger than the average of the remaining individuals in the unselected breast cancer group (57.8 years, variation 23–95 years; $P = 0.17$) but older than those with Finnish *BRCA1* (46 years, variation 32–57 years) and *BRCA2* (48 years, variation 45–67 years) mutations[9]. However, additional studies will be required to address the influence of the *PALB2* mutation on the age of disease onset. The mutation was also observed in six controls (0.2%; 6/2,501), suggesting that the penetrance of c.1592delT is incomplete. However, most control individuals heterozygous for the mutation were relatively young (five females aged between 27 and 51 years and one male aged 28 years), compared with the above-noted average age of disease onset for affected c.1592delT carriers. The actual penetrance might therefore be higher than currently observed.

For the 18 unselected mutation-positive breast cancer patients, available records were analysed for evidence of a positive family history, and at least half of these families were found to have an apparently heritable disease history (for example, a–d and g in Fig. 2). In addition to breast cancer, all families studied showed other forms of cancer, including colorectal, stomach, endometrial and pancreatic cancers and leukaemia (Fig. 2). Segregation analysis of the truncation allele with regard to cancer incidence was attempted in three of the families with breast cancer studied (Fig. 2a–c) but was not sufficiently informative to draw meaningful conclusions because of a lack of DNA samples from suitable family members. For the remaining families, the analysis was restricted only to the affected index individual who initially displayed the c.1592delT allele. A segregation

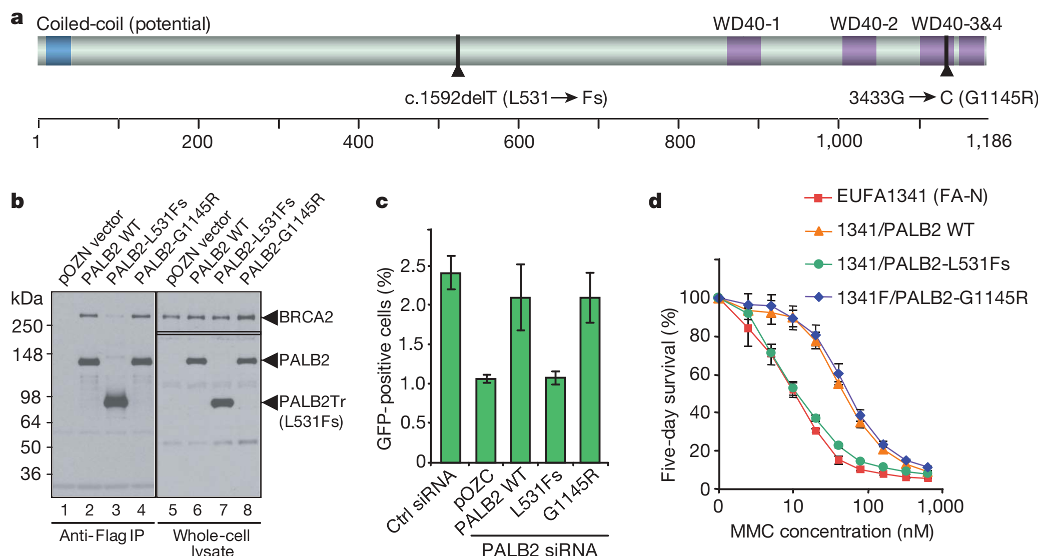

Figure 1 | PALB2 protein structure and assessment of functional consequences of the c.1592delT and 3433G→C sequence alterations. **a**, Schematic diagram of the protein showing predicted functional domains and the sites of the two main sequence changes observed. **b**, 293T cells were transfected with the indicated plasmids and PALB2 proteins double-tagged with Flag and haemagglutinin (HA) were immunoprecipitated (IP) with anti-Flag M2 agarose beads. The abundance of tagged PALB2 proteins and BRCA2 in the precipitates was analysed by western blotting (lanes 1–4). The endogenous BRCA2 abundance and relative levels of ectopic PALB2 expression are shown on the right (lanes 5–8). WT, wild type. The numbers at the left indicate the positions of molecular mass markers. **c**, DR-U2OS HR reporter cells were treated with control or PALB2 short interfering RNAs (siRNAs) and then co-transfected with pCBASce together with the pOZC vector or cDNA constructs. Cells positive for green fluorescent protein were counted 72 h later. The PALB2 cDNAs contain seven silent base changes and are resistant to the PALB2 siRNA. The results shown are means ± s.d. for three independent experiments, each performed in duplicate. **d**, EUFA1341 (FA-N) fibroblasts stably expressing indicated PALB2 proteins were treated with the indicated concentrations of MMC and their survival was assayed 120 h after treatment. The results are means ± s.d. from a representative experiment performed in triplicate.

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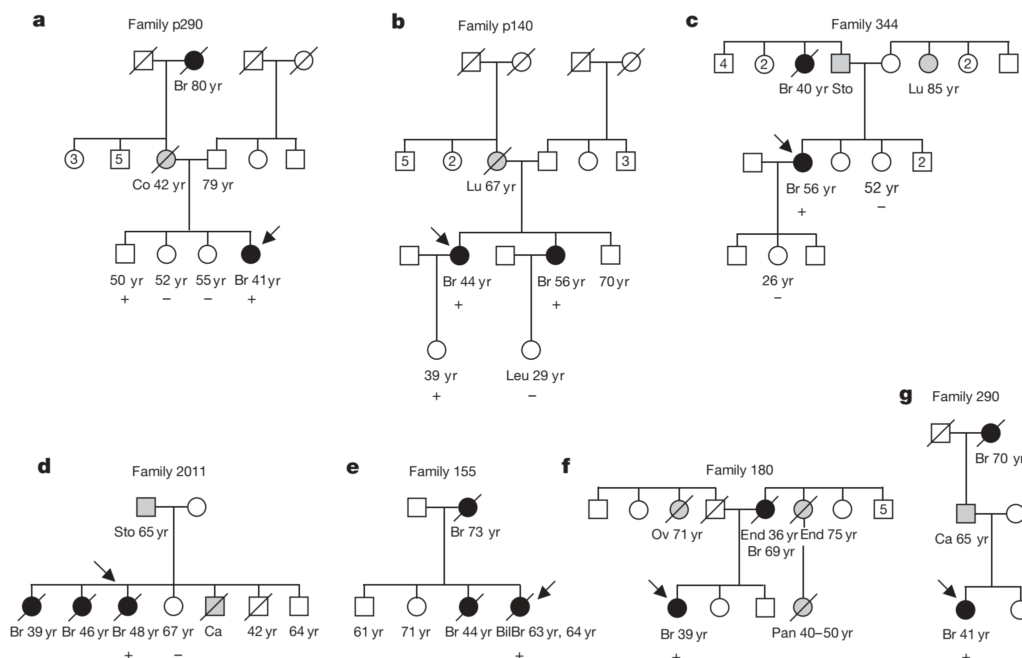

Figure 2 | Examples of pedigrees of breast cancer families exhibiting the c.1592delT allele. a–c, Families attempted for partial segregation analysis. **d–g**, Families displaying the mutant allele but in which segregation analysis was not possible because of a lack of DNA samples from suitable family members. Patients with breast cancer are marked with black circles (BilBr, bilateral breast cancer). Other cancer types are marked in grey and mentioned when known (Ca, cancer of unknown type with liver metastases (the patient died at the age of 68 years); Co, colorectal; End, endometrial; Lu, lung; Leu, leukaemia; Ov, ovarian; Pan, pancreatic; Sto, stomach). Age at cancer diagnosis is shown, when known. Individuals genotyped for c.1592delT are marked with either a plus sign (if mutation-positive) or a minus sign (if mutation-negative). Age at monitoring is shown for healthy individuals genotyped for c.1592delT. Index individuals are marked with arrows. A slashed pedigree symbol indicates a deceased individual.

study was also performed on the family of the mutation-positive patient with prostate cancer (Fig. 3). Other than the individual who died early at 52 years of age, all male carriers developed prostate cancer by the age of 76 years, indicating high penetrance of the mutation in the two generations of this family that were studied.

To test whether loss of heterozygosity (deletion of the wild-type allele) had occurred in tumours of individuals heterozygous for the mutation, genomic DNA extracted from formalin-fixed, paraffin-embedded tumour sections from six patients was analysed. Before DNA extraction, laser-capture microdissection was performed to isolate pure cancer cell populations from tumours from five patients. *PALB2* gene segments (about 200 base pairs) surrounding the c.1592delT mutation were amplified by polymerase chain reaction with multiple primer pairs, and sequenced. The existence of the c.1592delT mutation was confirmed in all tumours, whereas no reproducible evidence of loss of heterozygosity was ever observed, implying that these tumours were likely to have been driven, at least in part, by PALB2 haploinsufficiency, perhaps in combination with a dominant-negative effect of the truncated protein product.

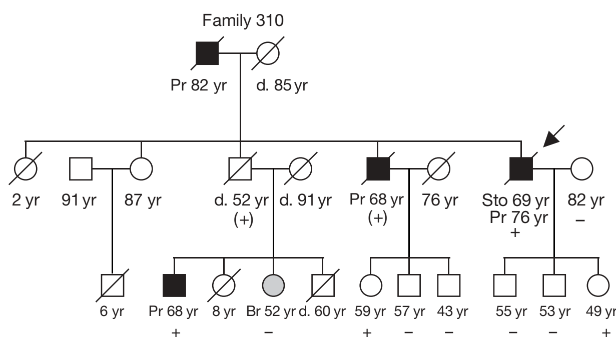

Figure 3 | Pedigree of a prostate cancer family segregating the *PALB2* c.1592delT truncation allele. Individuals with prostate cancer are marked with black squares. In addition to prostate cancer, the index individual (marked with an arrow) also had stomach cancer (Sto). The single individual with breast cancer (Br) is shown in grey. No other individuals with cancer were known to have occurred in this family. Individuals genotyped for c.1592delT are marked with either a plus sign (if mutation positive) or a minus sign (if mutation negative). Age at monitoring is shown for healthy individuals typed for c.1592delT. A slashed symbol indicates a deceased individual. A plus sign in parentheses indicates an obligate mutation carrier. It was not possible to obtain analysable DNA from the first-generation male ancestor.

Immunohistochemistry was also performed on sections from the same six tumours noted above and from one further sample. As shown in Supplementary Table 2, all except one revealed strong expression of oestrogen receptor, and five of seven showed expression of progesterone receptor. These results imply that *PALB2* tumours share the above phenotypic properties with those generated by *BRCA2* mutations[10,11]. However, because of the limited number of specimens analysed, more extensive analysis is merited.

Very recent discoveries have revealed that, like BRCA2, PALB2 is also a Fanconi anaemia gene product, and *PALB2* and *BRCA2* patients with Fanconi anaemia share similar severe phenotypes characterized by childhood cancers and early mortality[12–14]. Thus, in addition to their role in the development of Fanconi anaemia and childhood cancers, the present results indicate that suitable germline mutations in *PALB2* also confer an elevated risk of breast cancer and perhaps prostate cancer.

PALB2 c.1592delT, which subsequently proved to be a founder mutation (data not shown), seems to be associated with a roughly fourfold increased hereditary propensity for female breast cancer and to make a limited contribution to familial prostate cancer. The truncated protein product seems to be stable but is functionally defective, being unable to support intact BRCA2 DNA repair function; this observation, together with the above-noted significant statistical difference in its prevalence between patients with breast cancer and controls, indicates that this mutation is a significant component of heritable susceptibility to breast cancer in Finland. The roughly 1%

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occurrence rate of c.1592delT in unselected breast cancer patients is remarkable, especially because the 19 different pathogenic mutations identified in *BRCA1* and *BRCA2* together account for about 1.8% of Finnish breast cancer cases[8]. In Finland, about 4,000 women are diagnosed with breast cancer annually. This single mutation could therefore be responsible for about 40 new cases per year. Furthermore, because the *PALB2* gene was comprehensively screened in only 113 cancer families, the existence of other cancer-predisposing *PALB2* alterations in the Finnish population remains possible. The present results also imply that *PALB2* might be a significant new cancer susceptibility gene in other populations. In keeping with this notion, two of the mutations identified in Fanconi anaemia patients in non-Finnish populations seem to be associated with incidences of familial breast cancer[12,13].

METHODS

Sample selection. See Supplementary Information for details.

Mutation screening. The entire coding region and exon–intron boundaries of the *PALB2* gene were screened for germline mutations by conformation-sensitive gel electrophoresis[15,16] and direct sequencing.

Statistical and bioinformatic methods. Carrier frequencies were compared using Pearson's χ^2 test or Fisher's exact test. SPSS version 12.0 for Windows was used. The Mann–Whitney *U*-test was used to compare mean ages of disease onset between mutation carriers and non-carriers. PolyPhen simulation, ESEfinder 2.0 and NNSplice software were used to predict functions of the observed sequence changes. The cutoff value for statistical significance was $P = 0.01$.

Functional analysis. The retroviral PALB2 cDNA vectors, pOZN-PALB2 and pOZC-PALB2, have been described previously[4]. The mutations, c.1592delT and 3433G→C, were introduced into these vectors by site-directed mutagenesis with the QuikChange method (Stratagene). The HR/DSBR assay was performed as described[4]. The generation of EUA1341(FA-N) fibroblasts stably expressing various PALB2 species and subsequent mitomycin-C (MMC) sensitivity assays were as described[12].

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1. Wooster, R. & Weber, B. L. Breast and ovarian cancer. *N. Engl. J. Med.* **348,** 2339–2347 (2003).
2. Seal, S. *et al.* Truncating mutations in the Fanconi anemia J gene *BRIP1* are low-penetrance breast cancer susceptibility alleles. *Nature Genet.* **38,** 1239–1241 (2006).
3. Renwick, A. *et al.* ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nature Genet.* **38,** 873–875 (2006).
4. Xia, B. *et al.* Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Mol. Cell* **22,** 719–729 (2006).
5. Shivji, M. K. & Venkitaraman, A. R. DNA recombination, chromosomal stability and carcinogenesis: insights into the role of BRCA2. *DNA Repair (Amst.)* **3,** 835–843 (2004).
6. King, M. C., Marks, J. H. & Mandell, J. B. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science* **302,** 643–646 (2003).
7. Howlett, N. G. *et al.* Biallelic inactivation of BRCA2 in Fanconi anemia. *Science* **297,** 606–609 (2002).
8. Syrjäkoski, K. *et al.* Population-based study of BRCA1 and BRCA2 mutations in 1035 unselected Finnish breast cancer patients. *J. Natl Cancer Inst.* **92,** 1529–1531 (2000).
9. Sarantaus, L. *et al.* Multiple founder effects and geographical clustering of BRCA1 and BRCA2 families in Finland. *Eur. J. Hum. Genet.* **8,** 757–763 (2000).
10. Borg, Å. Molecular and pathological characterization of inherited breast cancer. *Semin. Cancer Biol.* **11,** 375–385 (2001).
11. Hedenfalk, I. *et al.* Molecular classification of familial non-BRCA1/BRCA2 breast cancer. *Proc. Natl Acad. Sci. USA* **100,** 2532–2537 (2003).
12. Xia, B. *et al.* Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. *Nature Genet.* advance online publication, doi:10.1038/ng1942 (31 December 2006).
13. Reid, S. *et al.* Biallelic mutations in *PALB2* cause Fanconi anemia subtype FA-N and predispose to childhood cancer. *Nature Genet.* advance online publication, doi:10.1038/ng1947 (31 December 2006).
14. Alter, B. P., Rosenberg, P. S. & Brody, L. C. Clinical and molecular features associated with biallelic mutations in FANCD1/BRCA2. *J. Med. Genet.* **44,** 1–9 (2007).
15. Ganguly, A., Rock, M. J. & Prockop, D. J. Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. *Proc. Natl Acad. Sci. USA* **90,** 10325–10329 (1993).
16. Körkkö, J., Annunen, S., Pihlajamaa, T., Prockop, D. J. & Ala-Kokko, L. Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. *Proc. Natl Acad. Sci. USA* **95,** 1681–1685 (1998).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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