Author/s:
Teo, ZL;Provenzano, E;Dite, GS;Park, DJ;Apicella, C;Sawyer, SD;James, PA;Mitchell, G;Trainer, AH;Lindeman, GJ;Shackleton, K;Cicciarelli, L;Buys, SS;Andrulis, IL;Mulligan, AM;Glendon, G;John, EM;Terry, MB;Daly, M;Odefrey, FA;Nguyen-Dumont, T;Giles, GG;Dowty, JG;Winship, I;Goldgar, DE;Hopper, JL;Southey, MC

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Tumour morphology predicts *PALB2* germline mutation status

Z L Teo[1], E Provenzano[2], G S Dite[3], D J Park[1], C Apicella[3], S D Sawyer[4], P A James[4,5,6], G Mitchell[4,5], A H Trainer[4,5,6,7], G J Lindeman[6,7,8], K Shackleton[6,8], L Cicciarelli[4], kConFab[9], S S Buys[10], I L Andrulis[11], A M Mulligan[12,13], G Glendon[11], E M John[14,15], M B Terry[16,17], M Daly[18], F A Odefrey[1], T Nguyen-Dumont[1], G G Giles[3,19], J G Dowty[3], I Winship[7,20], D E Goldgar[10], J L Hopper[3] and M C Southey*[,1]

[1]*Genetic Epidemiology Laboratory, The University of Melbourne, Melbourne, Victoria 3010, Australia;* [2]*Department of Pathology, The University of Melbourne, Melbourne, Victoria 3010, Australia;* [3]*The Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, The University of Melbourne, Melbourne, Victoria 3010, Australia;* [4]*Familial Cancer Centre, Peter MacCallum Cancer Centre, East Melbourne, Victoria, 3002, Australia;* [5]*Sir Peter MacCallum, Department of Oncology, The University of Melbourne, Melbourne, Victoria, 3010, Australia;* [6]*Familial Cancer Centre, The Royal Melbourne Hospital, Parkville, Victoria 3050, Australia;* [7]*The Department of Medicine, The University of Melbourne, Melbourne, Victoria 3010, Australia;* [8]*The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia;* [9]*The Peter MacCallum Cancer Centre, St Andrews Place, East Melbourne, Victoria 3002, Australia;* [10]*Huntsman Cancer Institute, The University of Utah School of Medicine, Salt Lake City, UT 84112, USA;* [11]*Department of Molecular Genetics and Laboratory Medicine and Pathobiology, University of Toronto, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5;* [12]*Laboratory Medicine Program, University Health Network, Toronto, Ontario, Canada M5G 2C4;* [13]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Ontario, Canada M5G 1X5;* [14]*Cancer Prevention Institute of California, Fremont, CA 94538, USA;* [15]*Department of Health Research and Policy and Stanford Cancer Center Institute, Stanford, CA 94305, USA;* [16]*Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032, USA;* [17]*The Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY 10032, USA;* [18]*Fox Chase Cancer Center, Philadelphia, PA 19111, USA;* [19]*Cancer Epidemiology Centre, Cancer Council, Carlton, Victoria 3053, Australia and* [20]*The Royal Melbourne Hospital, Parkville, Victoria 3050, Australia*

Background: Population-based studies of breast cancer have estimated that at least some *PALB2* mutations are associated with high breast cancer risk. For women carrying *PALB2* mutations, knowing their carrier status could be useful in directing them towards effective cancer risk management and therapeutic strategies. We sought to determine whether morphological features of breast tumours can predict *PALB2* germline mutation status.

Methods: Systematic pathology review was conducted on breast tumours from 28 female carriers of *PALB2* mutations (non-carriers of other known high-risk mutations, recruited through various resources with varying ascertainment) and on breast tumours from a population-based sample of 828 Australian women diagnosed before the age of 60 years (which included 40 *BRCA1* and 18 *BRCA2* mutation carriers). Tumour morphological features of the 28 *PALB2* mutation carriers were compared with those of 770 women without high-risk mutations.

Results: Tumours arising in *PALB2* mutation carriers were associated with minimal sclerosis (odds ratio (OR) = 19.7; 95% confidence interval (CI) = 6.0–64.6; $P = 5 \times 10^{-7}$). Minimal sclerosis was also a feature that distinguished *PALB2* mutation carriers from *BRCA1* ($P = 0.05$) and *BRCA2* ($P = 0.04$) mutation carriers.

Conclusion: This study identified minimal sclerosis to be a predictor of germline *PALB2* mutation status. Morphological review can therefore facilitate the identification of women most likely to carry mutations in *PALB2*.

*Correspondence: Professor MC Southey; E-mail: msouthey@unimelb.edu.au

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PALB2, a partner and localiser of BRCA2, is crucial for proficient homologous recombination repair of DNA double-strand breaks through its regulation of BRCA2 and its interaction with BRCA1 (Xia *et al*, 2006; Sy *et al*, 2009; Zhang *et al*, 2009). Bi-allelic inactivating mutations in *PALB2* underlie Fanconi anaemia subtype N and have been shown to be associated with high risk of childhood cancers (Reid *et al*, 2007; Xia *et al*, 2007). Heterozygous germline loss-of-function mutations in *PALB2* have been associated with increased risk of breast cancer (Rahman *et al*, 2007).

The first study that reported an association between *PALB2* mutations and breast cancer risk involved familial breast cancer cases and unaffected controls from the United Kingdom. Using only some information obtained from just 10 families, and under strong modelling assumptions, the average relative risk associated with 5 protein-truncating *PALB2* mutations was estimated indirectly to be 2.3-fold (95% confidence interval (CI) = 1.4–3.9) (Rahman *et al*, 2007). Subsequent population-based studies estimated the risk associated with *PALB2* mutations to be higher (Erkko *et al*, 2008; Southey *et al*, 2010). For example, *PALB2* c.1592delT was identified in 18 out of 1918 (0.9%) Finnish breast cancer cases unselected for family history compared with 6 out of 2501 (0.2%) unaffected controls (odds ratio (OR) = 3.94; 95% CI = 1.5–12.1). Using the family histories of the case carriers, *PALB2* c.1592delT was estimated to be associated with a 40% (95% CI = 17–77%) risk of breast cancer to the age of 70 years (Erkko *et al*, 2008). Similarly, *PALB2* c.3113G > A was identified in 5 out of 1403 (0.4%) unselected Australian breast cancer cases and 0 out of 764 (0%) unaffected controls (Southey *et al*, 2010). Using the family histories of the five carrier cases, the estimated cumulative risk for *PALB2* c.3113G > A was 91% (95% CI = 44–100%) to the age of 70 years. Therefore, population-based studies of breast cancer that have directly used the family history data have estimated that at least some *PALB2* mutations are associated with a breast cancer risk (penetrance) comparable to that of the average pathogenic mutation in *BRCA2*: 45% (95% CI = 31–56%) (Antoniou *et al*, 2003).

Mutations in *PALB2* are rare (varying from 0.1% to 1.5% depending upon the population) (Foulkes *et al*, 2007; Rahman *et al*, 2007; Tischkowitz *et al*, 2007; Dansonka-Mieszkowska *et al*, 2010; Papi *et al*, 2010; Southey *et al*, 2010; Bogdanova *et al*, 2011; Casadei *et al*, 2011; Ding *et al*, 2011; Hellebrand *et al*, 2011; Teo *et al*, 2013a, b) but for women carrying them, and their relatives who might also be mutation carriers, knowing their mutation status has the potential to be clinically important as carriers are at high risk of breast cancer. Identified mutation carriers could be informed of optimal, risk appropriate clinical screening and treatment. Potential therapies could include those that target homologous DNA repair dysfunction (Buisson *et al*, 2010). As *PALB2* mutations have also been associated with increased risk of developing a second breast cancer (Tischkowitz *et al*, 2012), risk reducing surgery and treatment might also be considered by *PALB2* mutation carriers. The integration of *PALB2* mutation testing into clinical practice is still in progress and strategies that effectively identify potential *PALB2* mutation carriers could help facilitate this important process.

Characterisation of the morphology of breast cancers arising in *PALB2* mutation carriers and non-carriers offers the possibility of identifying tumour morphological features predictive of an underlying germline *PALB2* mutation, as they have been shown for underlying *BRCA1* mutations (Lakhani *et al*, 1998; Southey *et al*, 2011; Hopper *et al*, 2012). This could be conducted at the time of diagnosis and therefore, be used to facilitate personalised treatment strategies, as well as enabling identification of those relatives who have also inherited a similar high breast cancer risk.

Breast cancer tumour morphology can be suggestive of underlying familial, if not heritable, risk. We recently reported that, in a population-based sample of 375 women with early-onset breast cancer cases with no known high-risk mutation in a breast cancer susceptibility gene, minimal sclerosis, presence of circumscribed growth, extensive intraductal carcinoma and lobular growth patterns were independent predictors of increased breast cancer risk for their first-degree female relatives (2.0-fold to 3.3-fold increased risk for relatives, $P < 0.02$ for all listed features). Relatives of the 128 (34%) index cases with none of these 4 features were at population risk (standardised incidence ratio = 1.03, 95% CI = 0.57–1.85), while relatives of the 37 (10%) index cases with two or more features were at high risk (standardised incidence ratio = 5.18, 95% CI = 3.22–8.33) (Dite *et al*, 2012).

Breast cancer morphological features can also be used to identify women most likely to carry germline mutations in breast cancer susceptibility genes. It has been known for some time that some morphological features are more common in cancers arising in *BRCA1* mutation carriers (Lakhani *et al*, 1998). These features have been identified by studying carriers across a wide range of ages at diagnosis and ascertained either because of their strong family cancer history or through population-based sampling. Lack of oestrogen receptor (ER) and progesterone receptor (PR) expression has also been reported to improve prediction of *BRCA1* mutation status based on family history (Lakhani *et al*, 2002; James *et al*, 2006; Mavaddat *et al*, 2010). Using a population-based sample of 452 young women with breast cancer, we found that just two breast tumour morphological features (trabecular growth pattern and high mitotic index) were sufficient to identify 28 of the 29 *BRCA1* mutation carriers in the study (Southey *et al*, 2011). Moreover, prediction of mutation status using these two features was more sensitive and specific than using family history alone, and when combined, the area under the receiver operator curve was in excess of 0.9.

A detailed analysis of the morphological features of *PALB2* mutation-associated breast cancers has not been previously conducted. Some information about the general morphology of breast tumours arising in *PALB2* mutation carriers is available from work studying breast tumours carrying the Finnish founder mutation *PALB2* c.1592delT. Mutation carriers with a family history of breast cancer were more likely to have 'triple negative' tumours (negative for ER, PR, and human epidermal growth factor receptor 2 (HER2) expression) when compared with familial non-*PALB2* mutation-associated breast cancers (54.5% and 12.2%, respectively; $P < 0.0001$). The *PALB2* c.1592delT-associated tumours were reported to be more often of higher grade and to have greater expression of Ki67, which is a cellular marker for proliferation than tumours arising in non-carriers of the mutation. Carrying this *PALB2* mutation was also reported to be associated with reduced survival; comparing affected *PALB2* mutation carriers, negative for HER2 expression, with a family history of breast cancer with affected non-carriers of *BRCA1*, *BRCA2*, or *PALB2* mutations, the hazard ratio was estimated to be 4.57 (95% CI = 1.96–10.64; $P = 0.0004$) (Heikkinen *et al*, 2009).

In this study, we conducted a standardised pathology review of 28 invasive breast cancers arising in women who carry a germline loss-of-function *PALB2* mutation. The morphological characteristics of these 28 tumours were compared with those of a population-based sample of 770 unselected breast tumours that had undergone the same standard pathology review.

MATERIALS AND METHODS

Subjects. The women in this study were participants in three breast cancer research resources: the Breast Cancer Family Registry (BCFR) (John *et al*, 2004), in particular the Australian BCFR; the Victorian Familial Breast Cancer Cohort (VFBCC) (Sawyer *et al*,

2012); and the Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab) (Mann *et al*, 2006). All participants provided written informed consent to participate in these research programs that were approved by the relevant ethics committees, including the Cancer Council Victoria and the New South Wales Cancer Council, and all participating sites/centres of the BCFR, kConFab, and the VFBCC. This study was approved by the Human Research Ethics Committee of The University of Melbourne.

***PALB2* mutation carriers.** A total of 28 women with invasive breast cancers who had been found to carry a *PALB2* germline mutation were included in this study. This included 24 women who carried *PALB2* c.3113G>A (5 from the Australian BCFR, 2 from the Ontario BCFR, 1 from the Utah BCFR, 5 from the VFBCC, and 11 from kConFab). The remaining four women were from kConFab; one was a carrier of *PALB2* c.196C>T, another carried *PALB2* c.1947_1948insA, and two were carriers of *PALB2* c.2982_2983insT.

The *PALB2* mutation carriers in the Australian BCFR, kConFab, and the VFBCC have been reported previously (Southey *et al*, 2010; Teo *et al*, 2013a, 2013b). The *PALB2* c.3113G>A carriers in the Ontario BCFR and Utah BCFR were identified via Taqman assay as described in Southey *et al* (2010) and Teo *et al* (2013a) by screening 1831 and 68 probands from these BCFRs, respectively. Probands from the California ($n=2052$), New York ($n=849$), and Philadelphia ($n=403$) BCFRs had also been genotyped for *PALB2* c.3113G>A using Taqman assay but no carriers were identified. The 28 *PALB2* mutation carriers were from 21 participating families as described in Table 1.

The diagnostic haematoxylin and eosin pathology slides, blocks, or digital images of the haematoxylin and eosin sections for each of the 28 *PALB2* mutation carriers were retrieved from the diagnostic centres. A pathology review was conducted by an expert breast pathologist (EP) using a standardised pathology review tool (described below). Data on ER, PR, and HER2 status of the *PALB2* mutation-associated tumours were collected, if available, from diagnostic laboratories and pathology reports. The HER2 status was considered to be positive if immunohistochemical test results were ranked 3+ (higher than normal amount of HER2 protein was present) or if tested as positive via fluorescence *in situ* hybridisation. An immunohistochemical test result of 1+ (normal amount of HER2 protein was present) was classified as negative for HER2 expression while an immunohistochemical test result of 2+ (moderate amount of HER2 protein was present) without a confirmatory fluorescence *in situ* hybridisation test was classified as equivocal.

Non-*PALB2* mutation carriers: population-based sample. The Australian BCFR used population-based sampling to recruit 1485 population-based probands between 1993 and 1999. The DNA derived from the Australian BCFR probands diagnosed before the age of 40 years ($n=692$) was screened for genetic mutations in the coding and flanking intronic regions of *PALB2* using high-resolution melt analysis (Southey *et al*, 2010). The Australian BCFR probands diagnosed at ages 40 or older ($n=793$) were genotyped for *PALB2* c.3113G>A using Taqman assay (Southey *et al*, 2010). First, primary invasive breast tumours from 836 (56%) of these probands were retrieved from diagnostic centres and systematically reviewed by pathologists as described below and elsewhere (John *et al*, 2004; Southey *et al*, 2011; Dite *et al*, 2012). Among the breast tumours that were reviewed, 40 (5%) were from *BRCA1* mutation carriers, 18 (2%) were from *BRCA2* mutation carriers, 1 (0.1%) was from a carrier of *ATM* c.7271T>G and 4 (0.5%) were from *TP53* mutation carriers (Southey *et al*, 1999; Andrulis *et al*, 2002; Chenevix-Trench *et al*, 2002; Apicella *et al*, 2003; Dite *et al*, 2003; Bernstein *et al*, 2006; Smith *et al*, 2007; Neuhausen *et al*, 2009; Mouchawar *et al*, 2010; Dite *et al*, 2012). Three breast tumours were from *PALB2* mutation carriers (Southey *et al*, 2010) and were included in the *PALB2* mutation carrier group (Table 1). The remaining 770 (93%) tumours were from women not found to carry a mutation in *BRCA1*, *BRCA2*, *ATM*, *PALB2*, or *TP53* after extensive screening (Southey *et al*, 1999; Dite *et al*, 2003; Mouchawar *et al*, 2010).

Pathology review. The haematoxylin and eosin-stained breast tumour tissue was reviewed and scored for morphology features by one or more trained pathologists using a standardised tool as previously applied (Armes *et al*, 1998; Southey *et al*, 2011; Dite *et al*, 2012) and validated (Longacre *et al*, 2006). Briefly, tumour grade was scored using the modified system of Bloom-Richardson by assessing mitotic rate, nuclear pleomorphism, and tubular differentiation (Elston *et al*, 1999). Tumours were typed into primary growth pattern (representing 75% or more of the tumour or ~60% of the tumour if a secondary pattern was present) and secondary pattern (representing ~40% of the tumour) using the World Health Organization breast carcinoma classification with minor modifications (Page *et al*, 1987). The carcinomas were categorised into 17 histological types: infiltrating ductal not otherwise specified, tubular, cribriform, micropapillary, mucinous (colloid), secretory, medullary (classical), medullary (atypical), adenoid cystic, metaplastic, lobular (classical), lobular (trabecular), lobular (alveolar), lobular (solid), tubulo (lobular), pleomorphic lobular, or other. Tumours were classified as having a primary histological type with no secondary type if >70% of the tumour presented with features characteristic of the histological

Table 1. Basic demographics of 28 *PALB2* mutation carriers with tumour material available for pathology review

Mutation	Probands	Relatives	Ages of diagnosis	Resource	Reference
PALB2 c.3113G>A	3	2	28, 35, 37, 42, 47	Australian BCFR	Southey *et al* (2010)
	2	0	45, 57	Ontario BCFR	—
	1	0	48	Utah BCFR	—
	7	4	32, 40, 41, 47, 47, 48, 49, 49, 54, 61, 63	kConFab	Southey *et al* (2010)
	5[a]	0	33, 38, 42, 44, 45	VFBCC	Teo *et al* (2013b)
PALB2 c.196C>T	1	0	43	kConFab	Teo *et al* (2013a)
PALB2 c.1947_1948insA	1	0	42	kConFab	Teo *et al* (2013a)
PALB2 c.2982_2983insT	1	1	47, 54	kConFab	Teo *et al* (2013a)

Abbreviations: BCFR = Breast Cancer Family Registry; kConFab = Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer; VFBCC = Victorian Familial Breast Cancer Cohort.
[a]One proband in the VFBCC was a relative of a proband participating in the Australian BCFR, and both were carriers of *PALB2* c.3113G>A.

type. Tumours were also classified as having both a primary (60%) and a secondary histological type (40%) if the tumour presented with features characteristic of two histological types. Sclerosis of the tumour was defined as fibrosis composed of fibroblasts and/or collagen that is devoid of tumour cells (Van den Eynden *et al*, 2008; Dite *et al*, 2012). The presence of extensive sclerosis is similar to a fibrotic focus as defined by Van den Enden *et al* (2008), which has been shown to be easily assessable and reproducible morphological feature in breast cancer (Van den Eynden *et al*, 2007). A tumour was defined to have minimal sclerosis if $\leqslant$20% of the tumour volume contained sclerosis and defined to contain extensive sclerosis if $>$20% of the tumour volume consisted of sclerosis. Information of the remaining tumour features from the pathology reviews was extracted as 'present' or 'absent' for statistical analysis as presented in Table 2.

The ER and PR status were obtained from immunohistochemical testing of tumour tissues or from histopathology reports held by cancer registries (Armes *et al*, 1999) or diagnostic laboratories (McCredie *et al*, 2003). The ER and PR status were available for $\sim$90% of the retrieved tumours of non-carriers of *PALB2* mutations (746 and 745 tumours, respectively).

Statistical analyses. Missing data for tumour morphology features (average 4 (0.5%) missing per feature) were estimated using multiple imputation, made possible by the correlations between different morphological features (see Southey *et al*, 2011 and Dite *et al*, 2012). Multiple linear logistic regression was used to estimate the OR and 95% CI for associations between each of the morphological features and carrier status (*PALB2* mutation carriers *vs* non-carriers of high-risk mutations, *PALB2* mutation carriers *vs* *BRCA1* mutation carriers and *PALB2* mutation carriers *vs* *BRCA2* mutation carriers), after adjusting for the number of affected first-degree relatives, number of affected second-degree relatives and age at diagnosis. These adjustments were necessary given that sampling of some carriers was from cases selected specifically because they had a family history and/or an early age at diagnosis. For the multivariate models, the best-fitting model was identified by stepwise selection, starting with the most significant variable and testing the addition of each of the remaining variables. All analyses were performed with Stata Version 11 (StataCorp, 2009). Following convention, all statistical tests were two-sided and *P*-values <0.05 were considered as nominally statistically significant. The positive and negative predictive values of a morphological feature for *unselected cases* were calculated based on the prevalence of *PALB2* c.3113G$>$A affected carriers in a population-based study (0.36%) (Southey *et al*, 2010) and the prevalence of the morphological feature in the Australian BCFR breast cancer cases recruited by population-based sampling.

Table 2. Classification criteria of standardised pathology review tool to assess tumour features in invasive breast cancer

	Criteria for classification	
	Present	**Absent**
Nuclear grade	Malignant	Bland/intermediate
Minimal tubule formation	Tubule formation observed in $<$10% of tumour	Tubule formation observed in $\geqslant$10% of tumour
Number of mitotic cells $\geqslant$20	$\geqslant$20 Mitotic cells identified per 10 high powered fields	$<$20 Mitotic cells identified per 10 high powered fields
Syncytial growth pattern	$\geqslant$75% of the tumour was observed to consist of broad sheets of tumour cells with indistinct cell borders	Absent
Pushing margins	$>$50% of tumour border observed to be well defined by a continuous pushing front of tumour cells	Absent
Circumscribed growth pattern	$>$50% of tumour border observed to be well defined	Absent
Lymphocytic infiltration site	Diffuse within tumour	Absent or observed to be at the border of the tumour
Lymphocytic infiltration level	Intense	Absent/minimal/moderate
Minimal sclerosis	Minimal: $\leqslant$20% of tumour is observed to contain sclerosis	Extensive: $>$20% of tumour consists of sclerosis which is defined as fibrosis composed of fibroblasts and/or collagen that is devoid of tumour cells
Necrosis	Present	Absent/uncertain
Apoptosis	Intense	Absent/minimal/moderate
Lymphovascular invasion	Cancerous cells observed in blood and/or lymphatic vessels	Uncertain or absence of cancerous cells in blood and lymphatic vessels
Acinar growth pattern	Present	Absent
Lobular growth pattern	Present	Absent
Trabecular growth pattern	Present	Absent
Tubular growth pattern	Present	Absent
Atypical lobular hyperplasia	Present	Absent
Atypical ductal hyperplasia	Present	Absent
Lobular carcinoma *in situ*	Present	Absent
Ductal carcinoma *in situ*	Present	Absent

RESULTS

Tumour morphological features associated with *PALB2* mutation status. Table 3 shows that having minimal sclerosis was associated with *PALB2* mutation status (OR = 19.7; 95% CI = 6.0–64.6; $P = 5 \times 10^{-7}$). This association of minimal sclerosis remains strongly significant even after correcting for multiple comparisons (Bonferroni correction). There was marginal evidence for an association between *PALB2* mutation status and having minimal tubule formation (OR = 5.6; 95% CI = 1.3–24.2; $P = 0.02$), having lobular carcinoma *in situ* (OR = 5.7; 95% CI = 1.1–29.4; $P = 0.04$), having circumscribed growth (OR = 2.9; 95% CI = 1.0–8.5; $P = 0.05$), and being ER positive (OR = 3.9; 95% CI = 0.95–16.3; $P = 0.06$). There was no evidence that any of the other tumour morphological features was associated with *PALB2* mutation status. Figure 1 shows examples of tumours with and without sclerosis, circumscribed growth, and tubule formation. After adjusting for having minimal sclerosis, no other feature was significantly associated with *PALB2* mutation status.

With respect to the immunohistochemistry of tumours arising in *PALB2* mutation carriers, information on ER and PR expression was available for 19 *PALB2* mutation carriers; 11 (58%) were ER+/PR+, 6 (32%) were ER+/PR−, and only 2 (11%) were ER−/PR−. This distribution was different to that for non-carriers from the Australian BCFR ($P = 0.002$). Of the non-carriers from the Australian BCFR with information available on ER and PR expression, 387 (56%) were ER+/PR+, 56 (8%) were ER+/PR−, 78 (11%) were ER−/PR+, and 167 (24%) were ER−/PR−. Expression status of HER2 was available for five *PALB2* mutation-associated tumours (data not shown), and only one of these tumours had the triple negative (ER−/PR−/HER2−) phenotype. The Australian BCFR does not currently have data on HER2 expression.

For *unselected cases*, the positive and negative predictive values of minimal sclerosis as a predictive feature of the carrier status of *PALB2* c.3113G>A were 2.5% and 99.9%, respectively.

Comparison with breast tumours arising in carriers of high-risk mutations in other breast cancer susceptibility genes. Table 4 presents the individual associations of minimal sclerosis with *PALB2*, *BRCA1*, and *BRCA2* mutation-associated tumours when compared with tumours of non-carriers of high-risk mutations.

When compared with tumours arising in *PALB2* mutation carriers, those arising in *BRCA1* mutation carriers were more likely to have a high mitotic count (>50; $P = 0.004$), extensive sclerosis (OR = 0.21; 95% CI = 0.05–0.99, $P = 0.05$), and necrosis ($P = 0.01$), be ER negative ($P = 0.001$) and PR negative ($P = 0.03$), and less likely to have a lobular growth pattern ($P = 0.02$). When compared with tumours arising in *PALB2* mutation carriers, those arising in *BRCA2* mutation carriers were more likely to have extensive sclerosis (OR = 0.06, 95% CI = 0.004–0.88, $P = 0.04$).

DISCUSSION

This report brings together several lines of evidence that support the relevance of genetic information about *PALB2* to breast cancer clinical genetics services. Is it now time for this information to be made available to women who are seeking advice and explanation for their person and family history of breast cancer?

The appropriate translation of new genetic information requires clear evidence and cost-benefit analysis. In the specific example of *PALB2,* there are several characterised genetic epidemiological features of the mutation spectrum that need to be considered and managed in the process of translation.

Table 3. Morphological features of *PALB2* mutation-associated tumours compared with those of non-carriers of high-risk genetic mutations

	PALB2		Non-carrier		PALB2 *vs* non-carrier		
	N	**%**	***N***	**%**	**OR**	**95% CI**	***P*-value**
Malignant nuclear grade							
Present	20	71	603	78	0.58	0.21–1.61	0.3
Absent	8	29	165	21			
Missing	0	0	2	0.3			
Minimal tubule formation							
Present	25	89	525	68	5.56	1.28–24.18	0.02
Absent	3	11	243	32			
Missing	0	0	2	0.3			
Number of mitotic cells ⩾20							
Present	10	36	237	31	2.34	0.85–6.39	0.1
Absent	16	57	530	69			
Missing	2	7.1	4	0.5			
Syncytial growth pattern							
Present	1	4	42	6	0.62	0.06–5.99	0.7
Absent	27	96	723	94			
Missing	0	0	5	0.7			
Pushing margins							
Present	2	7	17	2	2.81	0.41–19.27	0.3
Absent	26	93	744	97			
Missing	0	0	9	1.2			
Circumscribed growth pattern							
Present	8	29	100	13	2.92	1.00–8.51	0.05
Absent	20	71	661	86			
Missing	0	0	9	1.2			
Lymphocytic infiltration site							
Present	8	29	258	34	0.75	0.27–2.11	0.6
Absent	20	71	502	65			
Missing	0	0	10	1.3			
Lymphocytic infiltration level							
Present	5	18	117	15	0.6	0.14–2.56	0.5
Absent	23	82	639	83			
Missing	0	0	14	1.8			
Minimal sclerosis							
Present	14	50	30	4	19.68	6.00–64.59	5×10^{-7}
Absent	14	50	734	95			
Missing	0	0	6	0.8			
Necrosis							
Present	7	25	224	29	1.12	0.38–3.34	0.8
Absent	21	75	541	70			
Missing	0	0	5	0.7			
Apoptosis							
Present	17	61	563	73	0.89	0.33–2.45	0.8
Absent	11	39	206	27			
Missing	0	0	1	0.1			
Lymphovascular invasion							
Present	6	21	237	31	1.18	0.37–3.79	0.8
Absent	21	75	531	69			
Missing	1	3.6	2	0.3			

Table 3. (Continued)

	PALB2		Non-carrier		PALB2 *vs* non-carrier		
	N	**%**	***N***	**%**	**OR**	**95% CI**	***P*-value**
Atypical lobular hyperplasia							
Present	0	0	9	1	N/A		
Absent	28	100	758	98			
Missing	0	0	3	0.4			
Atypical ductal hyperplasia							
Present	2	7	25	3	1.43	0.24–8.63	0.7
Absent	26	93	741	96			
Missing	0	0	4	0.5			
Lobular carcinoma *in situ*							
Present	2	7	30	4	5.65	1.09–29.38	0.04
Absent	26	93	734	95			
Missing	0	0	6	0.8			
Ductal carcinoma *in situ*							
Present	17	61	206	27	0.5	0.19–1.36	0.2
Absent	11	39	562	73			
Missing	0	0	2	0.3			
Acinar growth pattern							
Present	22	79	671	87	0.53	0.17–1.70	0.3
Absent	6	21	99	13			
Missing	0	0	0	0			
Lobular growth pattern							
Present	9	32	283	37	0.86	0.31–2.39	0.8
Absent	19	68	487	63			
Missing	0	0	0	0			
Trabecular growth pattern							
Present	8	29	121	16	1.8	0.60–5.35	0.3
Absent	20	71	649	84			
Missing	0	0	0	0			
Tubular growth pattern							
Present	4	14	125	16	0.65	0.17–2.44	0.5
Absent	24	86	645	84			
Missing	0	0	0	0			
Lobular/pleomorphic lobular							
Present	5	17.9	132	17	1.05	0.39–2.81	0.9
Absent	23	82.1	638	83			
Oestrogen receptor							
Present	17	61	444	58	3.93	0.95–16.25	0.06
Absent	2	7	246	32			
Missing	9	32.1	80	10.4			
Progesterone receptor							
Present	11	39	465	60	0.91	0.28–2.90	0.9
Absent	8	29	224	29			
Missing	9	32.1	81	10.52			

Abbreviations: CI = confidence interval; OR = odds ratio. N/A: unable to be analysed due to zero observations of atypical lobular hyperplasia.

First, is information about *PALB2* mutation status clinically relevant? Several reports now provide evidence that the risk of breast cancer associated with at least some *PALB2* mutations is of the same magnitude as that associated with 'high-risk' mutations in other cancer susceptibility genes such as *BRCA2* and *MSH2* (Antoniou *et al*, 2003; Erkko *et al*, 2008; Southey *et al*, 2010; Win *et al*, 2012). Risks of this magnitude support the relevance of this information to clinical genetic services, but what use is this information to women who might be carriers of mutations in *PALB2* and at high risk of cancer? For affected women, and especially those identified as carriers of *PALB2* mutations at the time of diagnosis, there is the potential for treatment that target homologous DNA repair dysfunction (Buisson *et al*, 2010). There is also the importance of advising on and managing the high risk of breast cancer that could involve risk reducing surgery (for both affected and unaffected carriers) and the potential for gene-specific medical risk reduction.

Second, mutations in *PALB2* are very rare and thus, without additional information, application of traditional genetic counselling and testing regimes would be costly and identify very few carriers. We estimated that the positive predictive value of minimal sclerosis for unselected cases would be about 2.5%, but this estimate has a wide CI due to the lack of precise knowledge about the prevalence of *PALB2* mutations in such cases. It should be noted, however, that given the high penetrance of *PALB2* mutations, they will be more common in cases with a family history of breast cancer, as are *BRCA1* and *BRCA2* mutations. Therefore, it would be expected that the positive predictive value of minimal sclerosis will be substantially greater for cases with a family history. In the Australian and other settings, there is also the potential to consider testing for specific mutations in *PALB2* that are found more commonly in these populations (Rahman *et al*, 2007; Erkko *et al*, 2008; Southey *et al*, 2010; Teo *et al*, 2013a, b). At present, this might represent some cost saving at the level of genetic testing at the laboratory bench. The increasing introduction of massively parallel sequencing into the diagnostic testing laboratory continues to reduce the cost of testing and expand the genetic distance that can be covered in single instrument runs. This advancement in technology could result in making the detection of *PALB2* mutations a natural part of clinical genetic testing, even in contexts other than breast cancer.

Third, this study provides important information that could help translation of genetic information about *PALB2* into clinical use. Similar to the way that pathology has been used to facilitate the identification of women who carry germline mutations in *BRCA1* and the identification of carriers of mismatch repair genes (Southey *et al*, 2005, 2011; Hopper *et al*, 2012), the new information presented here could be used to facilitate the identification of carriers of *PALB2* mutations at the time of diagnosis, even irrespective of family history. It is also of note that the key feature associated with carrying a *PALB2* mutation (minimal sclerosis in the breast tumour) is also a feature that distinguishes *PALB2* mutation carriers from *BRCA1* ($P = 0.05$) and *BRCA2* ($P = 0.04$) mutation carriers. Moreover, we have previously shown that, even without knowledge of germline *PALB2* mutation status, minimal sclerosis is associated with about a five-fold increased risk for relatives of women with early-onset breast cancer (Dite *et al*, 2012). The presence of central sclerosis is more frequently identified in basal-like breast cancers, and has been associated with a worse prognosis (Fulford *et al*, 2006; Marginean *et al*, 2010). Therefore, inclusion of this feature in standard pathology review, particularly for early-onset cases, could help identify families carrying high-risk genetic mutations through means other than conventional approaches based on family cancer history.

Despite the key interactions of PALB2 with both BRCA1 and BRCA2 in the same complex during homologous recombination repair, our results, overall, do not provide evidence of similarities in tumour morphological features between tumours arising in *PALB2*, *BRCA2*, or *BRCA1* mutation carriers. However, it is interesting that we observed five lobular or pleomorphic lobular

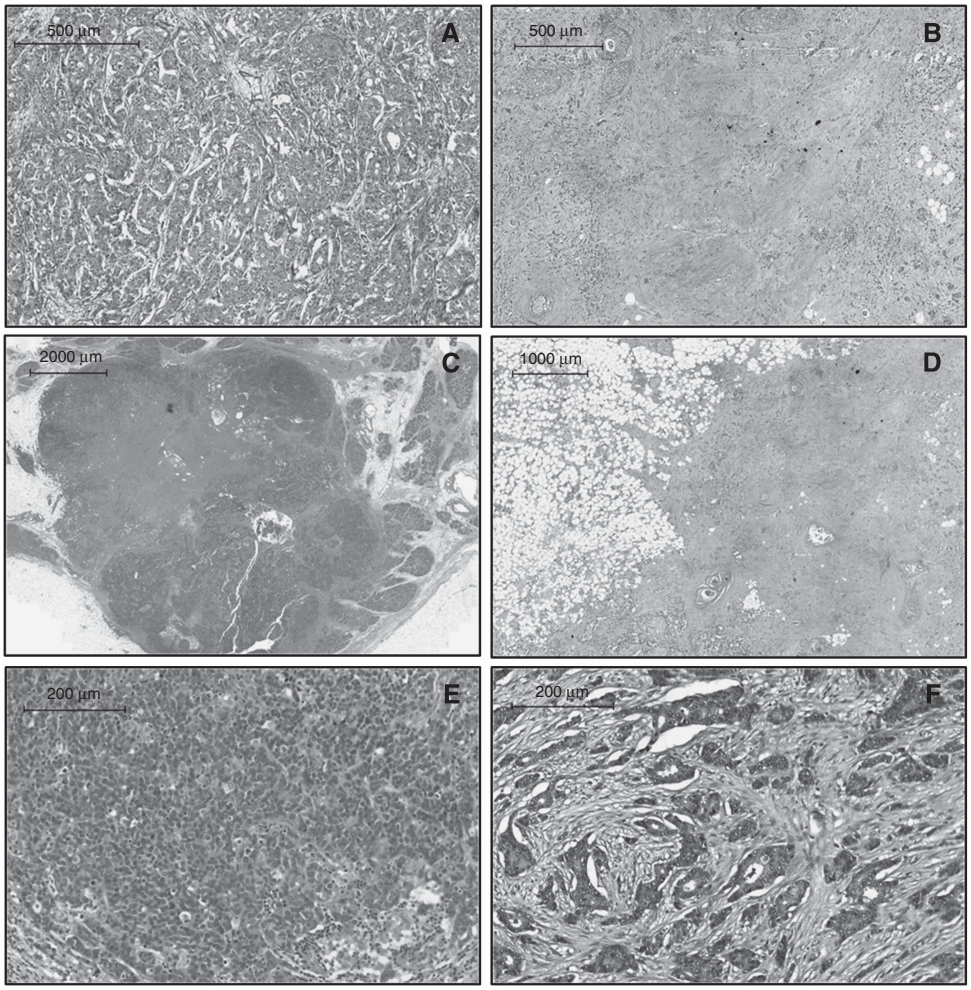

Figure 1. Morphological characteristics of *PALB2* tumours. (**A**) Minimal sclerosis (× 5 magnification), (**B**) extensive sclerosis (× 5 magnification), (**C**) circumscribed growth (× 1 magnification), (**D**) absence of circumscribed growth (× 2 magnification), (**E**) minimal tubule formation (× 10 magnification), and (**F**) intermediate tubule formation (× 10 magnification).

Table 4. Minimal sclerosis in BRCA1, BRCA2, and PALB2 mutation-associated tumours and in non-carriers of high-risk mutations

	Minimal sclerosis			
	Present *N* (%)	**Absent *N* (%)**	**Odds ratio (95% CI)**	***P*-value**
Non-carriers	30 (3.9)	734 (95.3)		
PALB2 mutation carriers	14 (50)	14 (50)	*PALB2* vs non-carriers	
			19.7 (6–64.6)	5×10^{-7}
BRCA1 mutation carriers	9 (22.5)	31 (77.5)	*BRCA1* vs non-carriers	
			3.15 (1.3–7.7)	0.01
BRCA2 mutation carriers	2 (11.1)	16 (88.9)	*BRCA2* vs non-carriers	
			1.29 (0.27–6.17)	0.8

Abbreviation: CI = confidence interval.

carcinomas (observed as primary or as secondary histological type) in women with *PALB2* mutations that were diagnosed before the age of 50 years (ranging from 37 years to 47 years) and to note that in a population-based study of early-onset breast cancer (diagnosis under the age of 40 years), tumours arising in *BRCA2* mutation carriers were more frequently pleomorphic lobular carcinomas compared with those arising in non-carriers of *BRCA1* or *BRCA2* mutations (Armes *et al*, 1998). There has also been consistent evidence that the proportion of ER-negative breast tumours increases with age at diagnosis for *BRCA2* mutation carriers ($P = 1.2 \times 10^{-5}$ and $P = 0.02$ reported by Mavaddat *et al*, 2010 and Eerola *et al*, 2005, respectively).

It is important to note that the majority of tumours (24 out of 28) that have undergone pathology review in this study have been derived from carriers of the *PALB2* c.3113G>A mutation. Therefore, it is unclear whether the predictive value of having minimal sclerosis is specific to *PALB2* c.3113G>A or whether it could be extended to all *PALB2* mutations.

Due to the rarity of *PALB2* loss-of-function mutations, an international effort to combine data for a large number of carriers of *PALB2* loss-of-function mutations is required to validate tumour morphological features associated with *PALB2* mutation status observed in this study. A larger study would also allow for the data to be stratified by age at diagnosis to examine the potential for age-dependent associations with tumour morphology (as is evident for

BRCA1 mutation carriers; Hopper *et al*, 2012) and for some *PALB2* mutations to be associated with triple negative breast cancer (Heikkinen *et al*, 2009; Tischkowitz & Xia, 2010). Note, however, that our study has found no evidence that the tumours of *PALB2* mutation carriers are more likely to be triple negative, and instead found that if anything they might be less likely.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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