The incidence of PALB2 c.3113G>A in women with a strong family history of breast and ovarian cancer attending Familial Cancer Centres in Australia.

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Abstract: The familial aggregation of breast cancer has been well-described with approximately 25% of breast cancers attributable to inherited mutations in currently known breast cancer susceptibility genes. *PALB2* c.3113G>A (p.Trp1038*) is a protein-truncating mutation which has been associated with high estimated risk of breast cancer in Australian women (91%; 95% CI = 44-100) to age 70 years. This study screened for *PALB2* c.3113G>A in germline DNA representing 871 unrelated individuals from “high-risk” breast and/or ovarian cancer families evaluated in the setting of a Familial Cancer Centre in Australia. The *PALB2* c.3113G>A mutation was identified in eight of 871 probands (0.92%) from these families. Median age of diagnosis was 42 years. Five of these eight women had contra-lateral breast cancers. Available data suggests that the *PALB2* c.3113G>A is a rare mutation with estimated breast cancer risks similar in magnitude to that associated with *BRCA2* mutations. Although the proportion of high-risk women carrying this *PALB2* mutation is low, research efforts should continue in order to effect its translation into clinical genetic testing practice.

Keywords:

PALB2

Hereditary breast cancer

Familial cancer centre

BRCA1 and BRCA2 mutation negative

Letter to the Editor,

Breast cancer is a common disorder with a strong hereditary component resulting in at least a two-fold increase in risk of the disease for women with an affected first-degree relative [1-8]. Approximately 20% of women in the population are reported to have one or more first-degree relatives affected with breast cancer [9]. The familial aggregation of breast cancer is thought to be largely due to heritable factors [10] yet mutations in *BRCA1* and *BRCA2* account for only around 16% of the genetic contribution to familial breast cancer [11,12]. Recent studies have investigated the possibility that rare germline mutations in additional genes explain the remaining familial risk. This has led to the description of a number of new breast cancer susceptibility genes including *ATM* [13], *CHEK2* [14], *BRIP1* [15] and *PALB2* [16].

Normal *PALB2* function is essential for homologous recombination (HR) repair of DNA double strand breaks where the protein acts as a bridge, forming an integral link between *BRCA1* and *BRCA2* [17,18]. Mono-allelic mutations in *PALB2* have been estimated to confer an increased risk of breast cancer of two- to six-fold [16] but the statistical approach applied had some limitations [19].

Modified segregation analysis involving breast cancer cases, unselected for family history and their first- and second-degree relatives estimated the penetrance of the *PALB2* c.3113G>A mutation to age 70 years to be 91%, albeit with wide confidence intervals (95% confidence interval = 44-100) [20]. The confidence interval of the estimate encompasses and is, therefore, comparable to the average estimated risk for breast cancer for *BRCA2* mutation carriers to the age of 71[21]. However, the segregation pattern in some families cannot exclude the possibility of additional genetic breast cancer risk modifiers being present in these families.

The *PALB2* c.3113G>A (p.Trp1038*) mutation appears to be a recurrent mutation associated with a high estimated risk of breast cancer. Hence, we aimed to establish the contribution of *PALB2* c.3113G>A to breast cancer in the group of women assessed as 'high risk' on the basis of their personal and family history of breast and/or ovarian cancer following clinical assessment at a Familial Cancer Centre (FCC). To determine the prevalence of *PALB2* c.3113G>A in this clinic-based population, targeted screening for this mutation was performed in unrelated

probands from the Victorian Familial Breast Cancer Cohort (VFBCC) of “high risk” breast and/or ovarian cancer families [22]. The cohort includes individuals assessed by one of the three Familial Cancer Centers in Victoria, Australia (The Peter MacCallum Cancer Centre, The Royal Melbourne Hospital, and the Victorian Clinical Genetic Service) between 1997 and 2010. For all families, the cancer history was assessed through questionnaire and person consultation and was verified through local cancer registries or individual medical records. Initially, classification of a proband as ‘high risk’ was based on national guidelines requiring a family history involving a minimum of two, first- or second-degree relatives with breast cancer with at least one additional risk factor (additional affected close relatives, diagnosis before 40 years, multiple primary breast or ovarian cancers in one individual, male breast cancer, or Ashkenazi Jewish ancestry). From 2003, individuals were classified as ‘high risk’ where the proband was estimated to have a $\geq 10\%$ probability of a mutation in *BRCA1* or *BRCA2* by an established mutation prediction tool [23,24].

Diagnostic testing was conducted on all DNA samples provided by probands. Prior to 2003, diagnostic testing was a combination of PTT, dHPLC and Sanger sequencing of all exons. After this date, diagnostic testing consisted of Sanger sequencing of all coding and flanking intronic regions of *BRCA1* and *BRCA2* and SALSA[®] Multiplex Ligation-dependent Probe Amplification (MLPA)[®] analysis (MRC-Holland, Amsterdam, Netherlands). A proportion of probands underwent retrospective MLPA analysis; consequently, 83% of the cohort completed screening for large deletions or rearrangements within the *BRCA1* and *BRCA2* genes. Within the cohort, 7% of probands only underwent diagnostic testing for Ashkenazi Jewish founder mutations via Sanger sequencing.

Ethics approval was obtained from human research ethic committees at all sites and at the University of Melbourne prior to study commencement.

A total of 972 individuals were recruited by the VFBCC. Of these, 95 were excluded as i) their DNA samples were found to be unsuitable for screening (n=2), ii) they were identified as carriers of *BRCA1*, *BRCA2*, or *TP53* mutations (n=22), iii) they were not affected with breast cancer or that their cancer status could not be determined (n=71) iv) or they failed genotyping (n=6). (Figure 1). The remaining 871 individuals included 848 women and 23 men who were affected with breast and/or ovarian cancer. DNA extracted from peripheral blood

of the participants was whole genome amplified (WGA) using the Repli-G Phi-mediated amplification system (Qiagen, Hilden, Germany) and analysed for *PALB2* c.3113G>A using the Custom Taqman® SNP Genotyping Assay (Life Technologies, Carlsbad, California, USA). The Taqman® probe-based assays were performed as per manufacturer's instructions on the LightCycler480 (Roche Applied Science, Penzberg, Upper Bavaria, Germany). Non-WGA DNA samples were used to confirm positive results of the Taqman® probe-based assay by Sanger sequencing. DNA samples from six individuals (0.6%) failed the Taqman® probe-based assays.

Of the 871 unrelated participants who were successfully genotyped for *PALB2* c.3113G>A, eight women (0.92%) were identified as carriers. The median age of onset of the eight mutation carriers was 42 (33-49) years compared to a median age of onset of 45 (20-84) years in non-carriers ($p = 0.07$). (Table 1).

Information on the segregation of the *PALB2* c.3113G>A mutation in the families of the carriers was limited, as blood samples of the relatives were typically not available. However, one female sibling of an affected *PALB2* c.3113G>A carrier (Figure 2h), diagnosed with a grade 2 infiltrating ductal carcinoma (ER+/PR+/HER2+) at the age of 36 years was also a participant in the VFBCC; genotyping confirmed that she also carried the *PALB2* c.3113G>A mutation.

Figure 2 shows the pedigrees of the carriers of *PALB2* c.3113G>A. Five of the eight carriers had two primary diagnoses of breast cancer. The median age of onset for the second diagnoses of these five unrelated participants was 51 (range, 38-58). Five of the nine *PALB2* c.3113G>A carriers were affected by contra-lateral breast cancer (63%) compared to 123/740 (17%) ($p = 0.009$, Fischer's exact test) of the non-carrier women with breast cancer. This finding supports the association with contra-lateral breast cancer that has been reported previously [25].

There were a total of 14 breast cancer diagnoses among the nine *PALB2* mutation carriers who had personal diagnoses of breast cancer. Pathology reports were available for 11 of the 14 cancers; ten cases were infiltrating ductal carcinomas and one an infiltrating lobular carcinoma (Table 2). Information on histological grade and receptor status was either unavailable or incomplete on two of the pathology reports. For the remaining nine tumours, seven of the cancers were

either histological grade 2 or 3 using the modified Bloom-Richardson system [26], and two of the nine were triple negative, i.e. negative for estrogen and progesterone receptors (ER and PR), and human epidermal growth factor receptor 2 (HER2) expression (Table 2).

Our study demonstrates that the *PALB2* c.3113G>A mutation contributes to the risk of breast cancer in approximately 1% of the high-risk breast and/or ovarian cancer families presenting to FCCs in Victoria, Australia. The mutation was found at a similar level in two other studies that involved breast and/or ovarian cancer multiple-case families attending clinical genetics services in Australia and/or New Zealand [20,27]. Table 3 compares the carrier frequency of *PALB2* c.3113G>A observed in this study with the four previous studies.

The prevalence of *PALB2* c.3113G>A observed in Victorian FCCs, together with its high estimated breast cancer risk [20] that is similar, on average, to that conferred by *BRCA2* mutations [21], supports the progress of *PALB2* c.3113G>A into a clinical translation phase. Nonetheless, the translation of *PALB2* genetic testing into routine clinical practice will be complex as the value of a negative pre-symptomatic test for family members is not always clear. This is a challenging and dynamic phase that requires further studies to exclude the presence of other susceptibility alleles, health economic analyses and educational activities to ensure smooth and appropriate use of this new genetic information.

The authors declare that they have no conflict of interest.

Abbreviations:

CI: Confidence interval

FCC: Familial Cancer Centre

VFBCC: Victorian Familial Breast Cancer Cohort

WGA: Whole genome amplified

ER: Estrogen receptor

PR: Progesterone receptor

HER2: Human epidermal growth factor-2

MLPA: Multiplex Ligation-dependent Probe Amplification

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Fig. 1 Flowchart of the probands included in the study

Fig. 2 Pedigrees of carriers of *PALB2* c.3113G>A. Symbols with filled lower left quadrant: cancer other than breast cancer. Symbols that are completely filled: breast cancer. Arrow: Proband. UK: Unknown cancer or unknown age of diagnosis. +: Genotyped and identified as a carrier of *PALB2* c.3113G>A. -: Genotyped and identified as a non-carrier of *PALB2* c.3113G>A. ^a: previously reported by Southey et al [20]

Table 1: Demographics of unrelated participants screened for *PALB2* c.3113G>A

	Non-carriers of <i>PALB2</i> c.3113G>A	Carriers of <i>PALB2</i> c.3113G>A
Total number of participants	863	8
Deceased	39	2
Age at last contact ^a	55	51
Follow up from diagnosis ^a	9	6
Unilateral Breast Cancer	618	3
Age of first diagnosis (median, range)	45 (20-84)	42 (33-49)
Bilateral breast cancer	120	5
Age of first diagnosis (median, range)	45 (23-71)	46 (32-49)
Age of second onset (median, range)	52 (27-85)	51 (38-58)
Breast and/or ovarian cancers	29	0
Age of first diagnosis (median, range)	53 (28-75)	
Ovarian Cancer	57	0
Age of first diagnosis (median, range)	55 (27-80)	
Male Breast Cancers	23	0
Age of first diagnosis (median, range)	59 (31-77)	
Other cancer Diagnosis	16	0
Family History, 1 st -3 rd degree relatives (n, mean)		
Female Breast Cancer	1545 (1.8)	27 (3.4)
Male Breast Cancer	25 (0.03)	0
Ovarian cancer	165 (0.2)	2 (0.3)
Isolated cases ^b	6	0
Total no. 1st-3rd degree Relatives	22754	312

^aMedian, years. ^bNo 1st-3rd degree relatives affected by breast or ovarian cancer

Table 2: Histopathological features of tumours from carriers of *PALB2* c.3113G>A

	Cancer	Age of diagnosis	Histopathology subtype	DCIS (presence)	Grade	Hormone Receptor Status		
						ER	PR	HER2
1	Breast	33	Ductal -Invasive	Yes	III	neg	neg	neg
2	Breast	42	Ductal -Invasive	Yes	-	pos	pos	-
3	Breast	44	Ductal -Invasive	Yes	III	pos	pos	pos
4	Breast	48	Ductal -Invasive	-	I	pos	neg	neg

	Breast	51	-	-	-	-	-	-
5	Breast	49	Ductal -Invasive	Yes	II	pos	neg	neg
	Breast	58	-	-	-	-	-	-
6	Breast	46	Lobular -Invasive	Yes	-	-	-	-
	Breast	52	Ductal -Invasive	Yes	III	neg	pos	neg
7	Breast	38	Ductal -Invasive	Yes	III	neg	neg	neg
	Breast	38	Ductal -Invasive	-	-	-	-	-
8 ^a	Breast	32	-	-	-	-	-	-
	Breast	46	Ductal -Invasive	Yes	III	pos	neg	neg
9 ^a	Breast	36	Ductal -Invasive	Yes	II	pos	pos	pos

DCIS: Ductal carcinoma *in situ*. ER: Estrogen receptor. PR: Progesterone receptor. HER2: Human epidermal growth receptor -2. -Unable to locate cancer pathology report. ^aRelated carriers, mutation carrier 8 is the proband.

Reference	Ascertainment	Geographic region	<i>PALB2</i> c.3113G>A carrier frequency in cases
Rahman <i>et al.</i> , 2007	Women with breast cancer and a family history of breast cancer. All breast cancer cases were previously screened for mutations and large deletions/duplications in <i>BRCA1</i> and <i>BRCA2</i> and were found to be negative.	United Kingdom	2/923 (0.23%)
Southey <i>et al.</i> , 2010	This study reported the carrier frequencies of <i>PALB2</i> c.3113G>A in two study groups. A population-based study that recruited women with a histologically confirmed first primary invasive cancer of the breast regardless of family history and women with breast cancer and a family history of breast cancer recruited through Australian and New Zealand cancer clinics by the Kathleen Cuninghame Foundation Consortium for Research in Familial Breast Cancer [30].	Population-based study cohort: Metropolitan areas of Victoria and New South Wales, Australia Multiple-case study cohort: Australia and New Zealand	Population-based : 5/1,403 (0.4%) Multiple-case: 8/778 (1.0%)
Wong <i>et al.</i> , 2011	Women with breast or ovarian cancer (68 breast cancer and two ovarian cancer cases). These cases had an early age at diagnosis (under the age of 50 years or pre-menopausal), a strong family history of breast and ovarian cancers in first- or second-degree relatives and were previously screened for mutations in <i>BRCA1</i> and <i>BRCA2</i> and were found to be non-carriers.	New South Wales, Australia	1/70 (1.4%) ^a
Casadei <i>et al.</i> , 2011	Study cohort consisted of breast cancer cases with at least two first- or second-degree relative with breast cancer. The breast cancer cases were screened for and not found to be carriers of <i>BRCA1</i> or <i>BRCA2</i> mutations. 172 breast cancer cases of the study cohort (all females) were of the Ashkenazi Jewish ancestry and 972 (959 females and 13 males) were of other unselected ancestries.	USA	Ashkenazi Jewish ancestry: 0/172 Unselected ancestries: 5/972 (0.5%)
This study	Study cohort consisted of breast cancer cases with at least two first- or second-degree	Victoria, Australia	8/878 (0.91%)

	<p>relatives with breast cancer with at least one additional risk factor: 1) additional affected close relatives; 2) diagnosis before 40 years; 3) multiple primary breast or ovarian cancers in one individual; 4) male breast cancer; or 5) Ashkenazi Jewish ancestry.</p>		
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^a Mutation carrier is affected with breast cancer.

a)

b)^a

c)

d)^a

e)

f)