A comprehensive pathway of PALB2 gene that provides negative biofeedback paying regards to women breast cancer

Dr. Partha Majumder and Debolina Chatterjee

Abstract
PALB2 has taken its place with genuine/legitimate breast cancer susceptibility genes. It is now well established that women who carry loss-of-function in the PALB2 gene are in the position of similarly elevated breast cancer risks to those who carry mutations in BRCA2. Information about PALB2 is now being used in breast cancer clinical genetics practice and is routinely included in breast cancer predisposition gene panel tests. However, prospective data related to the clinical outcomes of PALB2 mutation carriers is lacking and very little information (beyond mutation penetrance) is available to guide current clinical management for carriers (affected and unaffected by cancer). In addition, clinical classification of the vast array of non-loss-of-function genetic variants identified in PALB2 is in its infancy. These are key areas of current research efforts and are important foundations on which to move information about PALB2 into the accuracy of public health arena.

Keywords: PALB2, Breast cancer, Cancer susceptibility, familial cancer, PALB2 mutation carrier

Introduction
For the last two decades, women have been offered genetic testing of BRCA1 and BRCA2 in various clinical contexts. The vast majority of these women are seeking an explanation for a personal or family history of breast and/or ovarian cancer, and an accurate means of risk assessment, to facilitate risk management across the family. Indeed clinical criteria used to determine eligibility for BRCA1 and BRCA2 testing in many settings have been founded on the number of affected relatives and their age at diagnosis and then developed over time with increased evidence and local practice issues. Of those women who undergo testing, up to 20% are found to carry a clinically actionable mutation in BRCA1 or BRCA2. Until very recently additional genetic testing was not possible unless other clinical indicators were present (such as Li-Fraumeni syndrome that might indicate genetic testing of TP53). Women and their families who received uninformative genetic test results for BRCA1 and BRCA2 were clinically managed solely on the basis of their personal and family history. This limited the use of invasive strategies such as risk reduction surgery. Continued research and a recent revolution in genetic technology that can be applied to this research has identified a number of additional breast cancer predisposition genes and reported a large number of additional candidate breast cancer predisposition genes that are yet to be validated. This same technology has also transitioned into molecular diagnostic laboratories and has enabled a shift from high cost single gene genetic tests to lower cost multi-gene panel tests. The uptake of gene panel tests has been rapid and included a volume of successful direct-to-the-public marketing. In some areas of clinical genetics, panel testing is now the standard of care [1]. With some important caveats and considerations discussed in this review, current data suggests that gene panel testing offers breast cancer clinical genetics practice increased opportunity to identify “actionable” genetic variants in a greater proportion of women [2, 3, 4].

However, few of the large number of genes included in many gene panel tests are bona fide breast cancer predisposition genes and the vast majority of genetic variation identified by these gene panel tests cannot be interpreted in terms of breast or ovarian cancer risk. This is currently a controversial area of breast cancer research and clinical genetics practice, and is discussed in detail elsewhere [8].
Fig 1: Pathways of PALB2 gene in Human (Homologous Recombination)

Fig 2: Pathway of Fanconi Anemia in Human
PALB2 has now firmly taken its place with the small number of genuine breast cancer susceptibility genes. It is now well established that women who carry mutations in the PALB2 gene are at similar breast cancer risks as those who carry mutations in BRCA2 \([6,7]\) making many rethink the appropriateness of the initial “moderate or intermediate risk gene” label \[8\].

PALB2 now plays a legitimate role in breast cancer clinical genetics practice and takes a valid place on breast cancer predisposition gene panel tests. Internationally, tens of thousands of women, including those who have gone direct to the test provider, have had genetic tests for PALB2 mutations in the context of breast cancer susceptibility. Today, many nations have (or are preparing) best practice guidelines that include recommendations for PALB2 genetic testing and risk management \([5,9]\).

Currently, prospective data related to the clinical outcomes of PALB2 mutation carriers is lacking and very little information (beyond mutation penetrance) is available to guide clinical management for carriers (affected and unaffected by breast cancer). Over the last two decades, evidence has slowly been accumulated to support recommendations around risk management and targeted treatment regimes for BRCA1 and BRCA2 mutation carriers. Very little of this evidence currently exists for PALB2 mutation carriers.

Accumulating this evidence is challenging due to the very low frequency of women with PALB2 mutations, even in affected women with a strong family history of breast and ovarian cancer. However, with new technology and international coordination there is promise that further evidence could be gathered for PALB2 mutation carriers that will improve their clinical care within a few years.

In addition, risk estimates for PALB2 mutations have been based on collections of loss-of-function mutations. Clinical classification of the vast array of non-loss-of-function genetic variants identified in PALB2 is in its infancy. Informed by prior research in this area involving unclassified genetic variants in BRCA1 and BRCA2, international initiatives are moving quickly to identify the best approaches to assess PALB2 genetic variants on a variant-by-variant basis, to enable personalized use in clinical genetics practice.

PALB2 has made it over the first hurdle and is now included in the breast cancer genetics arena but to extend current utility and have an impact on improving the clinical outcomes for carriers of PALB2 mutations and incorporate use of this genetic information into precision public health initiatives, additional data is still urgently required.

**PALB2: A bona fide breast cancer susceptibility gene**

Mutations in PALB2 make a small contribution to heritable breast cancer susceptibility in most populations. Germline PALB2 mutations and carrier frequencies have been reviewed elsewhere \([10]\). Briefly, protein truncating mutations in PALB2 are distributed throughout the coding region \([6, 10]\) yet four PALB2 mutations are of note in terms of multiple observations. As few studies have been conducted within unselected breast cancer cases, estimation of the age-specific cumulative risk (penetrance) of breast cancer associated with PALB2 mutations has been limited.

To consider penetrance of a larger number of PALB2 genetic variants and a larger number of families, the PALB2 interest group \([19]\) embarked on a collaborative effort that collected data from 362 members of 154 families who had deleterious truncating, splice, or deletion mutations in PALB2 \([6]\). The estimated average cumulative risk of breast cancer risk ranged from 33% (95% CI, 25–44%) for a female carrier without affected relatives to 58% (95% CI, 50–66%) for a female carrier with two first-degree relatives who had breast cancer diagnosed by 50 years of age. Supported by other similar observations \([20]\), some recommend that both family history and PALB2 genotype should be considered together for clinical breast cancer risk management.

Thus, all published estimates of penetrance of PALB2 mutations are comparable to the breast cancer risk associated with BRCA2 mutations: 45% (95% CI, 31–56%) \([7]\). PALB2 is now regarded as a bona fide breast cancer predisposition gene and is justifiably included on current breast cancer gene testing panels with the above evidence.

**PALB2 mutations and risk of other cancer types**

As PALB2 functions together with BRCA1 and BRCA2, in the same DNA-damage response pathway, it has been thought plausible that PALB2 mutations, similar to BRCA1 and BRCA2 mutations, could predispose to other cancer types. The rarity of mutations in PALB2 and the rarity of some of the other cancers likely to be involved (pancreatic, male breast cancer, ovarian cancer, prostate cancer) make the estimation of the risk (if any) extremely challenging. Data in this area continues to come from small (yet important) studies and case reports that accumulatively may assist this interpretation \([21]\) and many others. By pooling international resources, the PALB2 Interest Group estimated that the relative risk of ovarian cancer and male breast cancer for PALB2 mutation carriers was 2.31 (95% CI, 0.77–6.97; \(P = 0.18\)) and 8.30 (95% CI, 0.77–88.56; \(P = 0.08\)) respectively \([6]\).

There is still very little data and no evidence supporting an association between PALB2 mutations and prostate cancer risk \([11, 36, 37, 38, 39]\) although several pedigrees have been presented and a possible trend toward aggressive disease in carriers has been noted \([19]\). iCOGS measured PALB2 (c.1592delT, p.Leu531Cysfs and c.3113G > A, p.Trp1038*) in 22,301 prostate cancer cases and 22,320 controls and found no evidence for association with prostate cancer risk OR 2.06, 95% CI 0.59–7.11, \(p = 0.24\) and OR 0.49, 95% CI 0.18–1.36, \(p = 0.16\) respectively. The PALB2 interest group continues work to further refine breast and other cancer risks for PALB2 mutation carriers \([19]\).
It has taken decades of research to provide the evidence base for BRCA1 and BRCA2 mutation carriers to make informed decision about the use of chemo-preventive agents, the use of the bilateral salpingo-oophorectomy, the use of mammography, magnetic resonance imaging (MRI) and other screening modalities, risk reducing mastectomy and targeted treatment regimes. Accumulating this evidence was challenging due in part to the very low frequency of women with BRCA1 and BRCA2 mutations, the historically laborious and expensive process of testing for mutations in these genes and the need to follow these women prospectively. However, in today’s context where PALB2 is being included in gene panel tests that are being conducted rapidly in large numbers at reduced costs and research can be conducted in a coordinated fashion internationally involving well established research resources (including resources founded to assess these questions for BRCA1 and BRCA2 mutation carriers) and in community-academic-industry partnerships—there is promise that evidence can be found for PALB2 mutation carriers that will impact clinical practice in the short term. Further studies are required to test if women who carry PALB2 mutations are at increased risk of death from breast cancer compared to non-carriers. More information is needed to understand the options for prevention and risk reduction. Intuitively, given the similar biological role of the protein, it is likely that some of the recommendations for BRCA1 and BRCA2 mutation carriers, including therapeutic regimes, may be relevant for PALB2 mutation carriers— but much work is needed to resolve these questions. To this end, a new academic-industry partnership named PROMPT—Prospective Registry of Multiplex Testing [42, 43], and many other large research initiatives are underway to address these important questions for carriers. PROMPT has scope beyond addressing these questions for PALB2 alone and will support the rapid translation of similar information for several new breast cancer predisposition genes including ATM, CDH1, CHEK2, RAD51C, RAD51D, STK11, TP53 in addition to BRCA1 and BRCA2. PROMPT is an online research registry for people who have undergone gene panel testing and been found to have a genetic variation in one of the above genes. PROMPT is one of several initiatives that create a new paradigm for research study participation that directly involves the most relevant community. PROMPT is designed to involve those who want to share their genetic results, learn more from sharing these results and engage at a level of their choosing/comfort as a collaborator alongside physicians and researchers to learn more about how mutations in these genes (such as PALB2) may affect their health and cancer risks.
In contrast to several other breast cancer predisposition genes, there is no evidence that missense variants in \textit{PALB2} (as a combined group) are associated with increased risk for breast cancer \cite{44, 45}. We and others have reported that the breast cancer risk fraction contributed by missense variants in \textit{BRCA1}, \textit{BRCA2}, \textit{ATM}, and \textit{CHEK2} is as high, if not higher, than protein-truncating variants in these genes \cite{46, 47, 48}. However, interpretation of the rare genetic variation observed in \textit{PALB2} on a variant-by-variant basis, especially the rare missense variants, remains challenging \cite{44}. That is, on a variant-by-variant basis it is difficult to provide any information that can be used to guide clinical management of carriers of rare missense mutations.

In some practices, the previous approach of breast cancer clinical genetics to generalize risk within groups of similar mutations (e.g., protein truncating mutations in \textit{BRCA1}) has not been automatically applied in the context of \textit{PALB2} due to i) a perception that the \textit{PALB2} risk estimates currently include data from a disproportionate number of the founder \textit{PALB2} mutations (and thus may not represent the average risk associated with all loss-of-function mutations) and ii) the more recent characterization of variants in \textit{BRCA1} (e.g., R1699Q \cite{49, 50}) and \textit{BRCA2} (e.g., K3326* \cite{51, 52}) with more moderate or low risk of breast cancer that is also a plausible scenario for variants in other genes, including \textit{PALB2}.

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\textbf{Fig 5:} Complete information of mutation in \textit{BRCA1}

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\textbf{Fig 6:} Complete information of \textit{BRCA2} Mutant in human
There is therefore a need to extend international efforts that are currently trying to classify rare variants identified in BRCA1 and BRCA2 for clinical use to include rare variants identified in PALB2 (and other genes) to assist the clinical management of the individuals who carry them. Several activities are well underway.

Fig 7: Complete informations of ATM Serine/Threonine Kinase in human

The most extensive and internationally set groups working in this area include The PALB2 Interest Group [6, 19] and The Evidence-based Network for the Interpretation of Germline Mutation Alleles (ENIGMA) [53, 54, 55] whose members are providing a range of data to accumulate new evidence on a variant-by-variant basis to be assessed in multifactorial risk models. These groups are also providing expert opinion to global databases and classification initiatives and working to communicate new information to clinical genetics practices urgently in need of individualized information.

Fig 8: Complete informations of CHEK2 Variant in human
The assessment and clinical classification of rare missense variants in \textit{PALB2} are likely to require incorporation of many pieces of evidence to enable clinical utility. Some of this evidence is likely to come from so-called functional assays. Fortunately, several functional domains of PALB2 are recognized including a coiled-coil structure, an ETGE-type KEAP1 binding motif, a chromatin-association motif (ChAM) at the N-terminus and a WD repeat motif in the C-terminus (reviewed elsewhere \cite{10}). These domains, coupled with PALB2’s role in DNA repair and Fanconi anemia, are facilitating work that is pitched at assessing the functional differences between wildtype PALB2 and PALB2 carrying rare missense mutations in key functional domains. Park \textit{et al}., characterized effects of missense mutations of the PALB2 WD40 domain and demonstrated that PALB2 L939W (c.2816 T \textgreater G) and PALB2 L1143P (c.3428 T \textgreater A) display a decreased capacity for DNA double-strand break-induced homologous recombination and an increased cellular sensitivity to ionizing radiation \cite{50}. This data offers much potentially useful information for rare variant classification.

Calibrated assays for functional assessment of variants in \textit{BRCA1} and \textit{BRCA2} have been developed and reported \cite{57, 58}. Recently, a publically available resource for functional analysis of missense variants \textit{in BRCA1 (BRCA1 Circos)} has been made available to facilitate meta-analysis of functional data and improve classification of variants in that gene \cite{59}. It is anticipated that groups such as the Functional Working Group of ENIGMA \cite{60} will be able to develop similar resources for PALB2 once assays are further developed and data is available.

\textbf{Conclusions}

This gene encodes a protein that functions in genome maintenance (double strand break repair). This protein binds to and colocalizes with the breast cancer 2 early onset protein (\textit{BRCA2}) in nuclear foci and likely permits the stable intranuclear localization and accumulation of \textit{BRCA2}. PALB2 binds the single strand DNA and directly interacts with the recombinase RAD51 to stimulate strand invasion, a vital step of homologous recombination. \textit{PALB2} can function synergistically with a \textit{BRCA2} chimera (termed piccolo, or \textit{piBRCA2}) to further promote strand invasion. Variants in the \textit{PALB2} gene are associated with an increased risk of developing breast cancer of magnitude similar to that associated with \textit{BRCA2} mutations and \textit{PALB2}-deficient cells are sensitive to PARP inhibitors.

\textbf{Author’s Contribution}

Author, Debolina Chatterjee has extended in depth research and exclusive study regarding the present spectrum of \textit{PALB2} gene that has been manifested to write her this review in favour of mankind and well being of health science and cultivation.

In this paper, the author achieved extreme guidance favoring the in depth cultivation with a positive output from Dr. Partha Majumder, Human Physiologist and Systems Biologist, Former Principal Scientist of Helixinfosystems and Former Head & Coordinator of Sikkim Manipal University (CC:1637), Kolkata, India. Dr. Partha Majumder contributed a pioneer role to the design of the study, data analysis, and revision of the manuscript.

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\textbf{References}


