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# Association of Common PALB2 Polymorphisms with Ovarian Cancer: A Case-Control Study

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**Abstract:** ***Background: The partner and localizer of breast cancer 2 (PALB2) has an essential role in BRCA2 mediated DNA double-strand break repair by serving as a bridging molecule and acting as the physical and functional link between BRCA1& 2 proteins. Truncating mutations in the PALB2 gene are rare but are thought to be associated with increased risk of developing breast and /or ovarian cancer in different populations. The present study was designed to investigate the variants of PALB2 and their association with OC.***
***Material &Methods: A total of 150 histopathologically confirmed ovarian cancer patients and 250 healthy age matched controls were collected. Three SNPs c.2794 G/A( rs45624036), c.1010 T/C(rs45494092), and c.1676A/G(rs152451) of PALB2 gene were selected and genotyped by ARMS-PCR followed by agarose gel electrophoresis. Appropriate statistical tests were applied to test for the significance of the results.***
***Results: A significant association of G/A (rs45624036) in inheritance models was observed & at the allelic level, the A allele conferred four-fold increased risk compared to G allele. Regarding T/C (rs45494092) polymorphism all the models revealed an association with OC and C allele showing eight-fold increased risk. With respect to A/G (rs152451) polymorphism, the protective role was observed in tested inheritance models in OC patients.***
***The Haplo analysis for the combination of all the three variants revealed increased risk with A-T-A and G-C-G haplotypes.(OR=4.50 ;95%CI 1.85-10.94;p=0.001,OR=26.36 ;95%CI 2.33 -297.91;p= 0.0085), whereas other haplotypes conferred a protective role in OC.***
***Conclusions: The present study suggests an essential role of PALB2 in the etiology of ovarian cancer.***

***Keywords: Ovarian Cancer, PALB2, BRCA1, BRCA2, Haplo analysis***.

## INTRODUCTION

Ovarian cancer (OC) is an asymptomatic gynecologic malignancy, especially in postmenopausal women with high incidence rate. Developed countries like the US showed a number of women die annually from OC than other gynecologic malignancies, approximately22,000 new cases diagnosed yearly and 15,000 of these women will die of the disease [1,2,3].Symptoms usually do not become apparent until the tumor invades and ascites develop. 70% of the patients are not diagnosed with the disease until cancer has metastasized beyond the ovaries, the fact that ovaries are deep within the pelvic cavity and difficult to palpate is an obstacle for early diagnosis. OC is a multifactorial disease resulting due to the interaction of genetic and environmental risk factors.

Previous studies revealed that family history, reproductive status, nulli- parity and hormonal disturbances play an important role in the development of OC. Patients with advanced stage have 20-30% survival rate only, hence there is a great need for better understanding of the etiology of ovarian cancer with the development of the efficient serum based tumor markers and effective screening methods for the early detection of the disease [4].

There are three types of OC based on the type of cell origin.90% are Epithelial Ovarian Cancer(EOC), 6%Stromal cell, 4%Germ cell. Stages are differentiated by the extent of metastasis.

Stage I confined to ovaries, Stage II involves other pelvic structures and the disease spread beyond the pelvis into the upper abdominal cavity in stage III. Stage IV defined as disease outside of the peritoneal cavity and often includes parenchymal lesions in the liver or malignant pleural effusions. Histopathologically OC is specifically classified as type 1 and type 2 ovarian tumors where type 1tumors, include endometrioid, mucinous, and low-grade serous, which are at a slow pace in development, whereas type 2 tumors progress to high-grade serous carcinomas at a rapid pace [5] characterized by an etiopathogenic variant. High-grade tumors are associated with genomic instability, aggressive clinical appearance, and high mortality [6]. *BRCA1* and *BRCA2* genes are two inherited breast and/or ovarian cancer susceptibility genes, mutations in these genes render the proteins unable to perform their intended functions. Patients who carry these germline mutations have improved survival compared with ovarian cancer patients without these mutations, so these mutation carriers reflect a better response to chemotherapy and prognosis [7, 8, and 9]. The lifetime risk of ovarian carcinoma is estimated to be 60% in BRCA1 mutation carriers and 30% in BRCA2 mutation carriers. However, these estimates were based on families with multiple cases of breast and/or ovarian cancer [10, 11, and 12].

PALB2 (Partner and Localizer of BRCA2) encodes for a protein which indirectly affects the expression of BRCA2 leads to genetic instability and disturbs the defense system and results in uncontrolled cell proliferation and tumorigenesis. The *PALB2* gene, also known as *FANCN*, forms a bond and co-localizes with BRCA2 in DNA repair. This gene is located on 16p12.2 spanning approximately 38 kb, containing 13 exons and encodes for a protein involved in BRCA2-related pathways [13]. It also interacts with *BRCA1*, effectively bridging these two well-known high-risk breast cancer susceptibility genes by serving as a linker between BRCA1 and BRCA2, and aiding to regulate their function in DNA damage response and homologous recombination [14,15,16]. The role of PALB2 in the etiopathogenesis of OC needs to be elucidated in view of its key contribution in ovarian cancer [17,18, 19].

## 2. MATERIALS & METHODS

### Sample Collection

A case-control study was conducted with a total of 400 individuals, which include 150 clinically and histopathologically confirmed ovarian cancer patients and 250 age-matched control samples of healthy women over a period of 2012-2016 from Yashoda Hospitals, Secunderabad, and Telangana. A total of 4ml peripheral blood was collected in EDTA vacutainers for DNA isolation was carried out by using the phenol-chloroform method [20] and stored at -20 $^0$C for further use. The concentration of the DNA was measured spectrophotometrically.

Details of demographic variables such as age, menopause status, consanguinity, gravida, dietary habits and family history etc., were obtained with the help of a structured Proforma. Informed written consent was obtained from all the subjects and the study was approved by the Ethical committee of Institute of Genetics, Osmania University, and Hyderabad.

### Inclusion & Exclusion Criteria

Primarily diagnosed ovarian cancer patients were included and patients with other chronic diseases were excluded from the study.
OF-Outer forward; OR-Outer reverse; IF-Inner forward; IR-Inner reverse primers

**Table 1: Oligonucleotide Sequences for the Detection of PALB2 Gene Polymorphisms**

| PALB2 | Primer Sequence | Amplicon Size |
|---|---|---|
| **G/Ars45624036** | OF: GAGTTTTCTGAGCCTTCAAATGATG | |
| | OR: CCTGCACTTAAAACCAGCTGACAG | 294bp |
| | IF: CCA GTG CCT GAT GTG TAT AAT CTC G (G wild) | 294,135bp |
| | IR: CCAAATTTCCCAAAGCTACACACAT (A mutant) | 294,208 bp |
| **T/Crs45494092** | OF:GGAGGCACAAGGCAAAAAAATG | |
| | OR:CAGAAGGCCTTCAGGCACTGTG | 343bp |
| | IF: CTAAATGAACTCACCTACAATAACTT [WILD] | 343,224bp |
| | IR: GTTTTGGTTTTCATTTGCTGGTG [MUTANT] | 343,166bp |
| **A/Grs152451** | OF:GATAATGACTTGTCTAGGAAGGCAG | |
| | OR: GACGTAAGCCACCACACTTGG | 322bp |
| | IF:TATCAGCACGAAAAATTATTTATTCG [WILD] | 322,157bp |
| | IR: ACACATCTTGATTTACCTTTCACTT [MUTANT] | 322,215bp |

**Molecular Analysis**

Detection of single nucleotide polymorphisms of PALB2 gene was carried out using the oligonucleotide primers listed in Table 1. In each PCR reaction, 25ng of genomic DNA was added to 20µl of reaction mixture consisting of 10 × PCR reaction buffer (10mMTris-HCl, pH 8.3, 50mMKCl, 10 µg/ml gelatin), 3.25mM MgCl2, 0.2mMdNTPs, and 3 U Taq DNA polymerase (Roche, Penzberg, Germany). The concentrations of primers used were 2.0 µM for OF and OR, 0.4 µM for IF, 0.12 µM for IR. Each PCR reaction consisted of an initial 4 min at 94°C, followed by 35 cycles of 94ºC for 30 sec, 57ºC for 30 sec, and 72ºC for 45 sec, and a final extension step of 10 min at 72°C.The amplified products were electrophoresed on 2% agarose gel stained with ethidium bromide. Genotypes were determined by allele-specific polymerase chain reaction (ARMS-PCR), followed by agarose gel electrophoresis. Statistical analysis was done to test the significance of results obtained.

**Statistical Analysis**

Genotypic distribution and allele frequencies were analyzed by chi-square test to assess the Hardy-Weinberg equilibrium for patient and control groups. All the p-values were determined by two-tailed Fisher's exact test at p≤0.05.Statistical analysis of the differences between groups was determined by chi-square test using SNP stat (Sole et al., 2006) [21]. Co-efficient (D') of pair-wise linkage disequilibrium (LD) between the SNPs was calculated using the software Haplo-view version 4.2 (Barrett et al., 2005) [22].

**Results:**

The demographic characteristics of patients and control groups were represented in Table2, revealed a significant association with respect to age (p<0.0001), menopause status (p <0.0001). However, there was no variation with respect to consanguinity and diet.

**Table 2: Demographic Variables Distributed in Controls and Ovarian Cancer Groups**

| **Variables** | **Controls N=250 %** | **Patients N=150 %** | **Odd's ratio** | **95% CI** | **p-value** |
|---|---|---|---|---|---|
| **Age(yrs)** | | | | | |
| ≤40 | 143 (57%) | 44 (29%) | 3.22 | 2.09-4.95 | <**0.0001**** |
| >40 | 107 (43%) | 106(71%) | | | |
| **Menopause status** | | | | | |
| Pre | 138 (55%) | 38 (24%) | 3.63 | 2.32-5.66 | < **0.0001**** |
| Post | 112 (45%) | 112(76%) | | | |
| **Consanguinity** | | | | | |
| + | 57(23%) | 31(21%) | 1.13 | 0.69-1.85 | 0.708 |
| - | 193(77%) | 119 (79%) | | | |
| **Diet Intake** | | | | | |
| Veg | 61 (24%) | 43 (28%) | 0.803 | 0.508-1.268 | 0.409 |
| Non-Veg | 189 (76%) | 107(72%) | | | |
| ***p≤0.05, **P ≤0.01** | | | | | |

**Table 3: Genotypic and Allele Frequencies of G/A (rs45624036), C/T (rs45494092), G/A (rs152451) Polymorphisms of PALB2 in Ovarian Cancer Patients and Control Subjects**

| **c.2794 G/A (rs45624036)** | | | | |
|---|---|---|---|---|
| **Co-dominant** | **Controls n=250(%)** | **Cases n=150(%)** | **OR (C.I.)** | **p-value** |
| GG | 241(96.4%) | 128(85.3%) | 1(Ref) | |
| GA | 09(3.6%) | 20(13.4%) | 4.46(1.97-10.1) | **0.0002**** |
| AA | 00 | 02(1.3%) | ----- | |
| **Dominant Model** | | | | |
| GG | 241 | 128 | | |
| GA+AA | 09 | 22 | 4.60(2.05-10.2) | **0.0001**** |
| **Recessive Model** | | | | |
| GG+GA | 250 | 148 | | |
| AA | 00 | 2 | -------- | |
| **Over Dominant Model** | | | | |
| GG+AA | 250 | 130 | | |
| GA | 09 | 20 | 4.274(1.892-9.652) | **0.00039**** |
| **Allele Frequency** | | | | |
| G | 491(0.98) | 276(0.92) | | |
| A | 09(0.02) | 24(0.08) | 4.74(2.17-10.35) | **0.000044**** |
| **c.1010 T/C (rs45494092)** | | | | |
| **Co-dominant** | **Controls** | **Cases** | **OR (C.I.)** | **p-value** |
| TT | 246(98.4%) | 133(88.6%) | 1(Ref) | |
| TC | 04(1.6%) | 14(9.4%) | 6.47(2.08-20.06) | **0.0006**** |
| CC | 00 | 03(2%) | ---------- | |
| **Dominant Model** | | | | |
| TT | 246 | 133 | | |
| TC + CC | 04 | 17 | 7.86(2.5-23.8) | **<0.0001**** |
| **Recessive Model** | | | | |
| TT+TC | 250 | 147 | | |
| CC | 00 | 3 | ---------- | |
| **Over Dominant Model** | | | | |
| TT+CC | 246 | 136 | | |
| TC | 4 | 14 | 6.331(2.04-19.6) | **0.0007**** |
| **Allele Frequency** | | | | |

| | | | | |
|---|---|---|---|---|
| T | 496(0.99) | 280(0.93) | | |
| C | 04(0.01) | 20(0.07) | 8.85(2.9-20.1) | **<0.0001**** |
| **c.1676A/G (rs152451)** | | | | |
| **Co-dominant** | **Controls** | **Cases** | **OR (C.I.)** | **p-value** |
| AA | 223(89.2%) | 123(82%) | 1(Ref) | |
| AG | 25(10%) | 22(14.6%) | 1.59(0.863-2.947) | 0.1800 |
| GG | 02(0.8%) | 05(3.4%) | 4.53(0.866-23.71) | 0.1193 |
| **Dominant Model** | | | | |
| AA | 223 | 123 | | |
| AG+GG | 27 | 27 | 1.82(1.01-3.22) | 0.05 |
| **Recessive Model** | | | | |
| AA+AG | 248 | 145 | | |
| GG | 2 | 5 | 0.23(0.04-1.22) | 0.13 |
| **Over Dominant Model** | | | | |
| AA+GG | 225 | 128 | | |
| AG | 25 | 22 | 0.64(0.35-1.19) | 0.2143 |
| **Allele Frequency** | | | | |
| A | 496(0.99) | 280(0.93) | | |
| G | 04(0.01) | 20(0.07) | 8.85(2.9-26.1) | **<0.0001**** |

p value<0.05- "*" p value <0.001-"**"

The distribution of genotype and allele frequencies of *PALB2* polymorphisms is presented in Table 3. A significant association of G/A (**rs45624036**) polymorphism with OC was observed in co-dominant (OR=4.46; 95%CI=1.97-10.1; p=**0.0002)**, dominant (OR=4.60; 95%CI 2.05-10.2; p=**0.0001**) and over dominant (OR=4.27; 95%CI 1.89-9.65; p=**0.0003**) tested inheritance models. On the other hand, at the allelic level, the A allele was found to be strongly associated and conferred four-fold increased risk compared to G allele in Ovarian cancer group. Regarding T/C (**rs45494092**) polymorphism the results demonstrated that co-dominant(OR=6.47;95%CI=2.08-20.0; p=**0.0006),** dominant(OR=7.86;95%CI 2.5-23.8; p<**0.0001**) and over dominant(OR=6.33;95%CI 2.04-19.6;p=**0.0007**) models revealed an association with OC with the C allele showing eight-fold increased risk to the cancer. With respect to A/G (**rs152451**) polymorphism, the protective role of tested inheritance models in ovarian cancer patients is highlighted, where G allele revealed eight fold risk to OC.

**Haplotype Analysis of PALB2 Gene**

The Haplo- analysis for the combination of three polymorphisms of PALB2, G/A (**rs45624036**), T/C (**rs45494092**), A/G (**rs152451**) was performed, Eight possible haplotype frequencies were noticed in case and control subjects which were represented in Table 4. Haplotypes A-T-A and G-C-G revealed an increased risk (OR=**4.50; 95%CI 1.85 - 10.94;p=0.001,**OR=**26.36 ;95%CI 2.33 - 297.91;p= 0.0085)**.

**Table 4: PALB2 Haplotype Distributions in Patients with OC and Controls**

| S. No. | Haplotype | Controls | Cases | OR(95%CI) | p-value |
|---|---|---|---|---|---|
| 1 | G-T-A | 0.9208 | 0.8078 | 1.00 | ---------- |
| 2 | G-T-G | 0.0552 | 0.0488 | 0.94 (0.49 - 1.83) | 0.86 |
| 3 | A-T-A | 0.015 | 0.0613 | **4.50 (1.85 - 10.94)** | **0.001*** |
| 4 | G-C-G | 0.0017 | 0.0425 | **26.36 (2.33 -297.91)** | **0.0085**** |
| 5 | G-C-A | 0.0043 | 0.0209 | 3.78 (0.78 - 18.34) | 0.1 |
| 6 | A-T-G | 0.0011 | 0.0154 | --------- | |
| 7 | A-C-A | 0.0019 | 0.0033 | ---------- | |
| 8 | A-C-G | NA | 0 | ---------- | |

* *P-value* <0.05, ** *p*-value <0.001

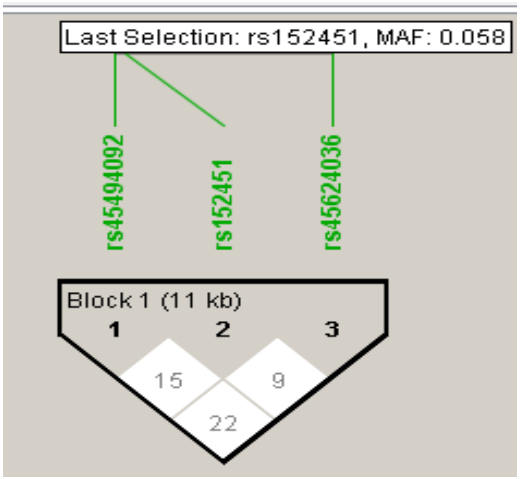
**Linkage Disequilibrium Analysis (LD)**

Linkage Disequilibrium Analysis (LD) was estimated in controls and cases for the 3 SNPs of PALB2 gene. The D' values were represented in Table 5 and the LD graph in controls and cases were given in Fig.1,2 respectively. Moderate LD was observed between rs45624036 and rs45494092 of cases (D'=0.58) while no such linkage disequilibrium was observed in control subjects.

**Table 5: Linkage Disequilibrium in Controls & Cases of OC Groups**

| **Subjects** | **L1** | **L2** | **D'** | **LOD** | **$r^2$** | **CI (low)** | **CI (high)** |
|---|---|---|---|---|---|---|---|
| Controls | rs45494092 | Rs152451 | 0.153 | 0.12 | 0.003 | 0.02 | 0.63 |
| | rs45494092 | rs45624036 | 0.223 | 0.53 | 0.022 | 0.04 | 0.63 |
| | rs152451 | rs45624036 | 0.092 | 0.0 | 0.0 | 0.03 | 0.96 |
| Cases | rs45494092 | rs152451 | 0.587 | 5.89 | 0.206 | 0.38 | 0.76 |
| | rs45494092 | rs45624036 | 0.077 | 0.0 | 0.0 | 0.02 | 0.83 |
| | rs152451 | rs45624036 | 0.121 | 0.44 | 0.011 | 0.01 | 0.31 |

'*' D' value = 1.0



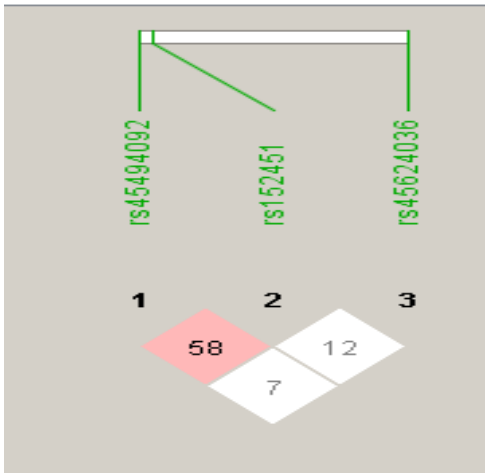




**Fig.1: Controls** **Fig.2: Cases**

**Fig.1,2 Linkage Disequilibrium Pattern Between Single Nucleotide Polymorphisms of PALB2 are G/A (rs45624036), T/C (rs45494092), A/G (rs152451) in controls and cases respectively**

## DISCUSSION

Mutations in BRCA1 and BRCA2 are associated with susceptibility to breast and ovarian cancer with a low frequency related to familial cases, and consequently, a rigorous search for additional target gene/s is warranted.PALB2 also known as FANCN (Fanconi Anemia) was identified as a BRCA1- and BRCA2-interacting protein, which is known to act as the physical and functional link between the breast cancer 1(BRCA1) and breast cancer 2 (BRCA2) proteins as a BRCA1-PALB2-BRCA2 complex.
The spectrum of malignancies associated with PALB2 mutations still remain unclear, therefore, it was postulated that *PALB2* genetic variants may also be significantly associated with the development and progression of different cancers [23]. Pathogenic mutations of PALB2 are rare and vary in frequency in different ethnic groups [24]. Since genetic variation/diversity is vastly associated with Indian population, the association of DNA repair proteins confer genomic stability through repair mechanism and are essential to prevent human cancer initiation and development [25].

Recent studies have demonstrated that mutations of *PALB2* had a significant impact on susceptibility to breast cancer [26].The functional interaction between the DNA helicase *PALB2* and *BRCA2* genes predisposes specific genotype association in a similar way in OC cases [27].
Variants of *PALB2*, include c.2794 G/A(rs45624036), c.1010 T/C(rs45494092) and c.1676 A/G(rs152451) respectively [28,29].The role of these SNPs was evaluated in the present study with respect to age, menopausal status to understand the role of PALB2 in the etiology of OC. The rs152451 revealed an impact on PALB2 functioning, as the variant is found inside the PALB2 motif, which interacts with chromatin domains, however, the studies are still at infancy to establish the specific function [30].

Several studies have shown that mutations of *PALB2* may play an extremely important role in the development and progression of breast and /or ovarian cancer. In various ethnic and diverse population like Australia [31] Dutch [32], North America, Polish, Russia, South Africa and Spanish studies, a strong association of PALB2 with a familial OC was revealed. However, contradictory to the previous studies the exact role of *PALB2* mutations in cancer risk is still a debate. Cao et al. (2009) analyzed in Chilean population, the complete coding sequence and exon-intron boundaries of PALB2 variants in BRCA1/2-negative Breast Cancer patients and could not find any specific association [33]. Gunnarson et.al (2008) conducted a study in Iceland population and found no mutations in that ethnic group [34].

The highlight of the study was a selective disadvantage of the heterozygotes conferring a risk to OC and a significant association of specific alleles to OC. The haplotypes A-T-A and G-C-G were found to confer a statistically significant increased risk to OC. Among all the three SNPs G/A (rs45624036), T/C (rs45494092) are in linkage disequilibrium ( D'=0 .58). The differences in the distribution of genotypes of the reported SNPs could be due to ethnicity, the source of controls and diversity of the population. The significant association pinpoints to DNA damage response for the activation, selective disadvantage of heterozygotes to OC and chromatin disintegration as commonly seen in cancers.

## CONCLUSIONS

The mutations in PALB2 are rare, but along with BRCA1 and BRCA2 may play a vital role in familial cancer cases. There were low-penetrance effects of PALB2 variants on ovarian cancer risk in our population. Our data shows that PALB2 pathogenic germline mutations (rs152451 and rs45551636) are associated with increased risk of OC. To the best of our knowledge, this is the first study to evaluate the common variants in PALB2 and their association with ovarian cancer risk in our ethnicity.

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