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Analysis of the Gene Coding for the BRCA2-Interacting Protein PALB2 in Hereditary Prostate Cancer

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BACKGROUND. The genetic basis of susceptibility to prostate cancer (PRCA) remains elusive. Mutations in *BRCA2* have been associated with increased prostate cancer risk and account for around 2% of young onset (<56 years) prostate cancer cases. *PALB2* is a recently identified breast cancer susceptibility gene whose protein is closely associated with BRCA2 and is essential for BRCA2 anchorage to nuclear structures. This functional relationship made *PALB2* a candidate PRCA susceptibility gene.
METHODS. We sequenced PALB2 in probands from 95 PRCA families, 77 of which had two or more cases of early onset PRCA (age at diagnosis <55 years), and the remaining 18 had one case of early onset PRCA and five or more total cases of PRCA.
RESULTS. Two previously unreported variants, K18R and V925L were identified, neither of which is in a known *PALB2* functional domain and both of which are unlikely to be pathogenic. No truncating mutations were identified.
CONCLUSIONS. These results indicate that deleterious *PALB2* mutations are unlikely to play a significant role in hereditary prostate cancer. *Prostate 68: 675–678, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: **hereditary prostate cancer; PALB2; BRCA2**

INTRODUCTION

Prostate cancer is a leading cause of morbidity and mortality in men and it is diagnosed in almost one-fifth of US men during their lifetime. Epidemiological studies suggest that up to 5% of all cases may be due to autosomal dominant genes [1–4], and twin studies suggest that approximately 42% of PRCA cases diagnosed under the age of 70 years are likely to be due to heritable factors [5]. Men with an affected father or brother are twice as likely to develop PRCA as men with no affected relatives [6], and the relative risk of developing PRCA rises considerably as the number of cases in a family cluster increases and the average age at diagnosis in the cluster decreases [7]. A meta-analysis of PRCA risk among men with a positive family history found a 1.8–2.1-fold increased risk if a second degree relative is affected compared to a 2.9-fold increased risk if the father or a brother is affected [8]. The results from the Breast Cancer Linkage Consortium (BCLC) showed a RR of 4.65 (95% confidence interval, CI 3.48–6.22) of

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PRCA in male *BRCA2* mutation carriers, data supported by a case–control study of 251 unselected Ashkenazi Jewish PRCA cases and 1,472 controls which estimated the odds ratio for PRCA in *BRCA2* mutation carriers to be 4.78; 95% CI 1.87–12.25) [9]. Edwards et al. [10] screened the complete *BRCA2* coding sequence in 263 British men under the age of 55 years and identified six truncating mutations (2.3%) compared to a *BRCA2* mutation prevalence in the UK of a round 0.13%. From these data, they estimated that there was a 23-fold relative risk of developing prostate cancer by age 56 years in *BRCA2* mutation carriers. Moreover, a recent Icelandic study has shown that PRCA arising in *BRCA2* mutation carriers has a significantly worse prognosis compared to sporadic PRCA [11]. However, other groups have shown limited [12] or no association [13,14] of *BRCA2* mutations with familial PRCA.

PALB2/FANCN is a recently identified protein which interacts closely with BRCA2 [15]. This interaction is essential for BRCA2 anchorage to nuclear structures and for its function in double strand break repair by homologous recombination [15]. Recent studies have shown that, as with *BRCA2*, mutations in *PALB2* are a cause of hereditary breast cancer [16–18]. To date, there are no published studies on *PALB2* in other cancer types. In view of the close functional relationship between PALB2 and BRCA2 and their joint role in breast cancer predisposition, it is conceivable that *PALB2* mutations may also predispose to PRCA and that *PALB2* mutations account for a proportion of hereditary PRCA. We therefore screened *PALB2* in a cohort of highly selected PRCA families.

METHODS

Study Subjects

Subjects were selected from the University of Michigan Prostate Cancer Genetics Project (PCGP), a large, ongoing, family-based study of hereditary PRCA. All participants gave written informed consent, and all consents and protocols were approved by the Institutional Review Board at the University of Michigan Medical School. To be eligible for the PCGP, families must have either two or more living members affected with prostate cancer or at least one living member with early onset PRCA (diagnosed at <55 years of age). For each participant a blood sample was taken for DNA extraction, the medical charts were reviewed and extended family history information was obtained. For the current study, 95 families with either multiple cases of early onset PRCA (defined as diagnosis prior to age 55 years) or a single case of early onset PRCA and multiple (defined as five or more) cases of PRCA diagnosed at any age were identified. Of those 95 families, 77 had two or more cases of early onset PRCA, and the remaining 18 had one case of early onset PRCA and five or more total cases of PRCA. The individual with the youngest age of diagnosis for whom DNA was available was selected from each family for *PALB2* sequencing. Characteristics of the families and individuals tested are given in Tables I and II, respectively. *PALB2* mutation analysis was performed by DNA sequencing as previously described [18]. Primers were designed to include an average of 75 base pairs either side of each exon, and at the 5′ and 3′ ends of *PALB2* the analyzed sequence included 50 bases upstream of exon 1 (150 bases upstream of the translation start site) and 50 bases downstream of exon 13 (350 bases downstream of the stop codon).

RESULTS AND DISCUSSION

Probands from a total of 95 hereditary prostate cancer families were screened for *PALB2* mutations and no truncating mutations were identified. A number of sequence variants were detected, the majority of which have been previously characterized [17] (Table III) and therefore it seems unlikely that these contribute to PRCA risk. Two previously unreported variants, however, were identified, namely K18R and V925L. Neither variant was located in a known functional domain of PALB2, and both were classified as ''tolerated'' using SIFT [19]. The K18R variant was found in the proband as well as his unaffected brother. Moreover, this family is of African American descent which most likely explains why the variant has not been seen in the control group screened by Rahman et al. [17], 97% percent of which were of white ethnicity. The V925L variant was detected in both the proband and his affected brother. And as with K18R, no further samples were available from other family members. However as this amino acid change is relatively conservative (Grantham score = 32), it seems unlikely that it is pathogenic.

TABLE I. Characteristics of Families (n = 95)

Trait	Median [range]
Race[a]	
Caucasian	84 (88%)
African-American	10 (11%)
Asian	1 (1%)
Minimum age of diagnosis	48.8 [37–54]
Average age of diagnosis[b]	56.0 [52–60.3]
Number of early onset PRCA cases	2.0 [1–5]
Total number of PRCA cases	2.4 [2–13]

[a]Number of families and (percentage of total families) are given.
[b]Median and [interquartile range] are given.

TABLE II. Characteristics of Individuals Resequenced (n = 95)

Trait	No. (%)
Age at diagnosis[a]	49.0 [47–52]
Pre-diagnosis PSA[a]	7.7 [4.0–7.5]
Surgery (% yes)	82 (86%)
Gleason	
≤6	50 (54%)
7	36 (39%)
≥7	6 (7%)

Column subtotals do not sum to 95 due to missing data.
[a]Median and [interquartile range] are given.

Our study was designed to determine whether or not *PALB2* is a moderate to high penetrance gene in PRCA by screening families that were highly enriched for hereditary characteristics including early age at cancer diagnosis and/or multiple affected family members. Note that in this report, men who were tested for *PALB2* mutations had a median age of prostate cancer diagnosis of 49 years, which is approximately 20 years earlier than the median age of diagnosis for sporadic PRCA. We specifically selected families from the PCGP resource with early-onset PRCA based on the findings of Edwards et al. [10]. These investigators screened 265 men with PRCA diagnosed at or before age 55 years for *BRCA2* mutations and identified 6 men (2.3%) with protein truncating mutations. BRCA2 and PALB2, together with BRCA1-interacting protein (BRIP1 or FANCJ), comprise Group III Fanconi Anemia (FA) proteins [20]. These proteins appear to act downstream from the ID complex which is formed from Group II FA proteins FANCD2 and FANCI. Monoallelic mutations in all three Group III FA genes are associated with breast cancer susceptibility [21–23]. In our study we screened sufficient familial cases to have an 85% probability of detecting at least one deleterious mutation if the expected *PALB2* carrier rate is 2%. This frequency is not unreasonable given that 1) our families were highly selected and enriched specifically for early-onset PRCA, and 2) the *BRCA2* mutation frequency from Edwards et al. [10] in sporadic young onset prostate cancer was 2.3%.

In their recent study Erkko et al. [16] screened 475 unselected and 164 familial (defined as having at least two affected members) PRCA cases, all from Finland, for the *PALB2* 1592delT founder mutation. The mean age at diagnosis in the study group was 69.5 years. They identified the mutation segregating in one family with four PRCA cases, average age at diagnosis 73.5 years. These data suggest that *PALB2* may be implicated in hereditary PRCA in Finnish PRCA families. However, this does not seem to be the case in similar types of PRCA families in North America. Such geographical variation has been previously seen with other genetic variants such as *CHEK2* 1100delC which was found to be at increased frequency in PRCA cases from Poland [24] and Finland [25] but not southern Sweden [26]. While it is unlikely that *PALB2* mutations play a major role hereditary PRCA, it may still be possible that deleterious *PALB2* mutations predispose to PRCA in some rare families. However, no familial PRCA studies have shown evidence of linkage to the *PALB2* locus on 16p12. It remains possible that common variants in *PALB2* could be associated with a slightly increased risk for PRCA, but this study was not designed to identify such an effect.

A number of other candidate genes have been studied in hereditary prostate cancer, but *BRCA2* is the only gene that has been shown to cause an increased risk of prostate cancer in multiple studies [7]. While the results of our study indicate that deleterious mutations in *PALB2* do not significantly contribute to hereditary PRCA, it remains possible that other genes coding for proteins associated with BRCA2 are potential PRCA-predisposition genes.

TABLE III. *PALB2* Coding Sequence Variants Identified in This Study

Exon	Variant	No of cases	Comments
2	K18R	1	SIFT: tolerated
4	L303L	1	Silent
	L337S	4	Previously reported [17]
	I309V	1	Previously reported [17]
	Q559R	15	Previously reported [17]
	S524S	1	Previously reported [17]
5	E672Q	4	Previously reported [17]
	G752G	1	Silent
8	V925L	1	SIFT: tolerated
9	G998E	2	Previously reported [17]
12	T1100T	2	Previously reported [17]

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