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Double PALB2 and BRCA1/BRCA2 mutation carriers are rare in breast cancer and breast-ovarian cancer syndrome families from the French Canadian founder population

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Abstract. French Canadian families with breast cancer and breast-ovarian cancer syndrome harbor specific BRCA1, BRCA2 and PALB2 germline mutations, which have been attributed to common founders. Mutations in these genes confer an increased risk to breast and ovarian cancers, and have been identified to play a role in and directly interact with the common homologous recombination DNA repair pathways. Our previous study described the case of a female diagnosed with breast cancer at 45 years old, who harbored the PALB2:c.2323C>T [p.Q775X] and BRCA2:c.9004G>A [p.E3002K] germline mutations, which have been found to recur in the French Canadian cancer families. As the frequency of double heterozygous carriers of breast-ovarian cancer susceptibility alleles is unknown, and due to the possibility that there may be implications for genetic counseling and management for these carriers, the present study investigated the co-occurrence of BRCA1/BRCA2 and PALB2 mutations in the French Canadian cancer families. The PALB2:c.2323C>T [p.Q775X] mutation, which is the only PALB2 mutation to have been identified in French Canadian cancer families, was screened in 214 breast cancer cases and 22 breast-ovarian cancer cases from 114 BRCA1/BRCA2 mutation-positive French Canadian breast cancer (n=61) and breast-ovarian cancer (n=53) families using a tailored polymerase chain reaction-based TaqMan® SNP Genotyping Assay. No additional PALB2:c.2323C>T [p.Q775X] mutation carriers were identified among the BRCA1/BRCA2 mutation carriers. The results suggest that carriers of the PALB2:c.2323C>T [p.Q775X] mutation rarely co-occur in French Canadian breast cancer and breast-ovarian cancer families harboring BRCA1 or BRCA2 mutations.

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Introduction

French Canadian families with breast cancer and breast-ovarian cancer syndrome present with recurrent mutations in the BRCA1, BRCA2 or PALB2 breast cancer susceptibility genes (1-3). This has been attributed to common founders in the Quebec provincial population (4,5). Carriers of the BRCA1 and BRCA2 mutations have a significantly higher risk of developing premenopausal breast cancer, and carry an estimated lifetime risk of 80-90% and 60-85%, respectively (6). It has been shown that ~4.8% of 564 French Canadian breast cancer cases not selected for a family history of cancer, and 0.4% of 6,430 French Canadian newborns harbor one of seven of the most commonly recurring BRCA1/BRCA2 mutations (7). By contrast, only one PALB2 mutation, c.2323C>T [p.Q775X], has so far been reported in the French Canadians of Quebec (1,8). This is likely to be a result of the comparatively low frequency of PALB2 mutation carriers in this population. In total, ~0.4% of the same cohort of breast cancer cases not selected for a family history of cancer, and who were also analyzed for the presence of recurrent BRCA1/BRCA2 mutations, were identified to be PALB2:c.2323C>T [p.Q775X] carriers, whereas no carriers were observed among newborns (7). These results are consistent with those of previous studies, which reported that ~40 and 1% of French Canadian breast cancer and breast-ovarian cancer families harbor germline BRCA1/BRCA2 or PALB2 germline mutations, respectively (2,3,8,9).

BRCA1, BRCA2 and PALB2 have roles in common DNA repair pathways that involve homologous repair (10). PALB2 is required for localizing and stabilizing BRCA2 to sites of DNA damage in two major DNA repair pathways that involve the homologous recombination of double-strand breaks and DNA inter-strand cross-linking of stalled replication forks (11). Previous data has indicated that PALB2 directly interacts with

Table I. Description of the cases analyzed for the PALB2:c.2323C>T [p.Q775X] mutation.

Cancer syndrome	BRCA1/BRCA2 mutation status	Number of families	Number of cases	Mean age of breast cancer, years	Age range of breast cancer, years
Hereditary breast cancer	BRCA1	18	37	43.4	29-69
Hereditary breast cancer	BRCA2	43	99	46.0	26-79
Hereditary breast and ovarian cancer	BRCA1	30	53	43.7	26-79
Hereditary breast and ovarian cancer	BRCA2	23	47	48.5	29-74
Any	BRCA1	48	90	43.6	26-79
Any	BRCA2	66	146	47.3	26-79
Any	BRCA1 and BRCA2	114	236	45.4	26-79

BRCA2 and BRCA1 to form a protein complex required for DNA repair (10). The roles of BRCA1, BRCA2 and PALB2 in common DNA repair pathways may explain the similarities in cancer phenotypes observed in breast cancer families harboring mutations in any of these genes. However, an excess of BRCA1 mutations is usually observed in triple-negative breast cancers (12). Carriers of more than one mutation in the BRCA1/BRCA2 and PALB2 genes have been reported in at least two breast cancer cases: One patient from our previous study, who harbored mutations in BRCA2 and PALB2 (13), and one patient from an independent study, who was a carrier of BRCA1 and PALB2 mutations (14). The co-occurrence of mutations in genes involved in similar molecular pathways provides insight into the biology of DNA repair pathways. Furthermore, double heterozygous mutation carriers raise questions regarding clinical management and cancer prevention strategies (6).

Our previous study described a patient with breast cancer who carried the PALB2:c.2323C>T [p.Q775X] mutation and the newly-described BRCA2:c.9004G>A [p.E3002K] mutation, which has been found to recur in the French Canadian population and was recently deemed pathogenic following an *in vitro* functional complementation assay involving DNA repair (13,15). The BRCA2-PALB2 mutation carrier was a female diagnosed with breast cancer at 45 years old who had inherited mutant parental alleles, and in which paternal (segregating the BRCA2 mutation) and maternal (segregating the PALB2 mutation) histories of breast cancer were reported (13). The frequency of double BRCA1/BRCA2 and PALB2:c.2323C>T [p.Q775X] carriers in hereditary breast and breast-ovarian cancer families of French Canadian descent is yet to be elucidated. Previous studies involving 564 breast cancer cases and 6,430 newborns did not identify any double heterozygous carriers for mutations in BRCA1/BRCA2 and PALB2 (7). In order to further investigate the occurrence of double carriers in the French Canadian population, the present study screened 236 BRCA1/BRCA2 mutation carriers from 114 French Canadian breast and breast-ovarian cancer families for the presence of the PALB2:c.2323C>T [p.Q775X] mutation.

Materials and methods

Study subjects. The study subjects consisted of female breast (n=214) or breast and ovarian (n=22) cancer patients from 114 cancer families, who had been primarily recruited through hereditary cancer clinics in Montreal, Canada, as part of research studies evaluating the contribution of BRCA1 and BRCA2 to breast cancer and breast-ovarian cancer syndrome families, as previously described (2,9). The study subjects had a family history of breast (n=61) or breast-ovarian (n=53) cancer according to the following criteria: In addition to the index case who presented with breast cancer and was <66 years old, the families must have had at least two other confirmed cases of invasive breast and/or epithelial ovarian cancer in the same familial branch. All study subjects were of self-reported grandparental French Canadian ancestry. The study subjects from BRCA1 (n=48) or BRCA2 (n=66) mutation-positive families had been subjected to a targeted mutation analysis or commercial DNA sequencing as previously described (2,3,9,13). Written consent to participate was obtained from each patient, and the study protocols were approved by the Ethics Committee of the Research Centre of The University of Montreal Hospital Centre (Montreal, Canada).

PALB2 mutation analysis. DNA from peripheral blood lymphocytes of the BRCA1/BRCA2 mutation carriers was screened for the PALB2:c.2323C>T [p.Q775X] mutation. The Custom TaqMan® SNP Genotyping Assay protocol was designed to detect the wild-type and mutant alleles. The primer and reporter sequences were as follows: Primer sequence forward, 5'-CTCAGTCTGTCTTGCCAGTGAT-3' and reverse, 5'-AGGGTGGTATGTGGTTTTGCT-3'; reporter sequence wild-type-VIC, 5'-TGAACTGTCGAATTGTTTAGT-3' and mutant-FAM, 5'-TGAACTGTCGAATTATTTAGT-3' (Life Technologies Inc., Burlington, ON, Canada). The assays were performed according to the manufacturer's instructions using the 1X TaqMan Genotyping Master Mix (Life Technologies, Burlington, ON, Canada), 1X custom primers-reporters mix (Life Technologies) and 20 ng DNA, and resolved using the Rotor-Gene 3000 real-time polymerase chain reaction cycler (Corbett Research, Mortlake, Australia). PCR conditions were as follows: Enzyme activation at 95˚C for 10 min, followed by 90 cycles of 92˚C for 15 sec and 60˚C for 1 min. Genotypes were determined using the Allelic Discrimination analysis tool, which was included in the Rotor-Gene 3000 v.6.1.93 software (Corbett Research).

Results and Discussion

A mutation screen of 236 breast cancer cases from 114 BRCA1/BRCA2 mutation-positive French Canadian

cancer families did not identify any novel carriers harboring the PALB2:c.2323 C>T [p.Q775X] mutation. Table I provides a description of the analyzed cases and their cancer syndrome context with regard to the BRCA1/BRCA2 mutation status. The findings of the present study are in accordance with the low estimated frequency of PALB2:c.2323C>T [p.Q775X] mutation carriers in the French Canadian population, which was established by previous mutation screens of breast cancer cases not selected for a family history of cancer, and newborns from the same population (7,8). The results of the present study are also consistent with the low estimated frequency of PALB2:c.2323C>T [p.Q775X] mutation carriers identified in breast cancer and breast-ovarian cancer families of French Canadian descent. For example, no PALB2:c.2323C>T [p.Q775X] mutations were previously identified following a screen of 36 BRCA1/BRCA2 mutation-negative breast cancer or breast-ovarian cancer families (16) and 91 high-risk breast cancer families (17) of French Canadian descent. It was previously reported that 1/71 (1.4%) breast cancer families and 0/23 breast-ovarian cancer families of French Canadian descent harbored a germline PALB2:c.2323C>T [p.Q775X] mutation (8). As the BRCA2-PALB2 mutation carrier from our previous study (13) is among the cancer families that were selected using the same criteria as that for the families described in the present study, it is hypothesized that double heterozygous carriers occur at a frequency of ~0.9% in 115 BRCA1/BRCA2 mutation-positive cancer families selected for a minimum of three cases of invasive breast or breast-ovarian cancer.

Due to the different selection criteria used, it is difficult to compare the results of the present study with those of previous independent studies. In addition, few studies have performed comprehensive analyses involving the genetic analysis of BRCA1, BRCA2 and PALB2, and instead have focused on the analyses of BRCA1/BRCA2 mutation-negative families (8,17). However, a mutation analysis of BRCA1, BRCA2 and PALB2 in 40 triple-negative breast cancer cases from a hospital in Germany identified a double heterozygote harboring a PALB2:c.758insT [p.S236X] and BRCA1:c.927delA mutation among eight BRCA1/BRCA2 mutation-positive cases (14). Although the double heterozygote mutation carrier did not develop breast cancer at an unusually young age (diagnosed at 65 years old), they did have a family history of premenopausal breast cancers. In addition, it was revealed that the double heterozygous patient was diagnosed with lymph node positive multifocal invasive breast cancer (ductal carcinoma), and presented with a variety of other ailments, including myoma of the uterus and meningioma (14). The multifocal occurrence of disease in this previous study would be consistent with a severe penetrance. By contrast, the PALB2-BRCA2 double heterozygous mutation carrier in our previous study was diagnosed with breast cancer at 45 years old, and other than having a maternal and paternal family history of breast and other cancer types, the disease presentation was indistinguishable from the remaining BRCA2 mutation-positive cases (13). The implications of genetic counseling for BRCA1/BRCA2 and PALB2 double heterozygous carriers is complex, as the penetrance of BRCA1 and BRCA2 has been established with clear indications for cancer prevention and surveillance strategies, whereas that of PALB2 is yet to be elucidated (6).

The biological effect of double heterozygous carriers of BRCA1/BRCA2 and PALB2 mutations, with respect to disease course, remains unknown. Due to the existence of only two double BRCA1/BRCA2 and PALB2 heterozygous carriers, it is unclear as to whether disease presentation and development are more severe than in cases where only one of the genes is implicated. Biallelic mutations in PALB2 or BRCA2 have been associated with different complementation groups of Fanconi anemia (11), which is an autosomal recessive disorder that causes a defect in DNA repair. Carriers usually present with an increased risk of cancer, and exhibit an elevated sensitivity to cytotoxic chemotherapeutic agents, such as mitomycin C, which act by crosslinking DNA (11). In the homologous recombination pathway, BRCA1 and BRCA2 protein interactions have been revealed to be mediated by the PALB2 protein (10). Patients with germline BRCA1 mutations, and possibly BRCA2 and PALB2 mutations as a consequence of their interactions, harbor a dysfunctional BRCA1 protein, which may render breast cancers more sensitive to platinum-based chemotherapy agents (18) and to inhibitors of poly(ADP-ribosyl) polymerase (PARP), which selectively target cells lacking homologous recombination DNA repair (19,20). It would be useful to determine if carriers of mutated genes involved in the homologous recombination pathway demonstrate increased sensitivity to chemotherapeutic interventions, as this would have important implications in treatment management. With the advent of whole genome exome sequencing (21), it may be possible to readily identify carriers of BRCA1/BRCA2 and other genes of the homologous recombination pathway, and therefore determine if such individuals would be more suited for emerging therapies, including the use of PARP inhibitors.

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