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FEATURE REVIEW

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Patricia N. Tonin, Ph.D.

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INTRODUCTION

According to the Canadian Cancer Statistics for the year 2001, breast and ovarian cancers will account for 19,500 and 2,500 of new cases of cancer, respectively (1). About 25% to 40% of breast cancer incidence in Canadian women can be attributed to identifiable risk factors (2), which unfortunately are not directly modifiable such as family history of disease. While the majority of breast and ovarian cancers arise 'sporadically' with no known specific etiologic cause, segregation analysis suggests that an estimated 10% of all primary cancers arise because of the inheritance of an autosomal dominant mutant allele (3,4,5,6). This mode of inheritance implies that there is a 50% chance of inheriting the disease-related allele from a carrier parent and that the inheritance of a mutated allele is sufficient to alter susceptibility to cancer (Figure 1). In addition, there is an expectation that cancer cases may 'cluster' in certain branches of the family, where the disease related allele has segregated with the affected individuals, and that the susceptibility allele can be transmitted through the male line (Figure 1) (3,7). Features of hereditary cancer families include multiple affected family members in several consecutive generations, age of cancer diagnosis younger than that in the general population, bilateral cancers in paired organs, and personal history of multiple cancers of specific sites (Table 1). The genetic analysis of families with multiple cases of breast cancer with a mean age of diagnosis before age 50 years and/or ovarian cancer facilitated the discoveries of the breast-ovarian cancer susceptibility genes, BRCA1 (8,9,10) and BRCA2 (11,12,13). A large majority of breast and/or ovarian cancer families (14) and up to 5% of all breast and ovarian cancers have been attributed to germline mutations in either of these genes. Other genes, such as TP53 in Li Fraumeni syndrome (15,16) and PTEN in Cowdens' Syndrome (17,18) confer increased susceptibility to breast cancer, but their contribution to inherited predisposition to breast cancer may be less than 1%. A review is presented on the cancer risk attributed to BRCA1 and BRCA2, options for cancer prevention and detection, the spectrum of mutations that have been described in the Canadian population and the challenges of identifying deleterious mutations in these breast-ovarian cancer susceptibility genes.

CANCER RISK IN MUTATION CARRIERS

In carriers of BRCA1 or BRCA2 mutations, estimates of the cumulative lifetime risk, by age 70 years, of developing breast cancer in females are 28% to 87% and of developing ovarian cancer are 16% to 60% (14,19,20,21,22,23). An important observation is the mutation carriers are at significantly increased risk for developing breast cancer at a young age in comparison to the general population: the risk of developing breast cancer in mutation carriers is15% to 30% by age 50 years in comparison to about 2.3% (1). The lifetime risk for developing ovarian cancer appears to dependent on the gene mutated. Unlike, breast cancer, age-specific penetrance is not significantly skewed toward early onset ovarian cancer in mutation carriers (20,24). Estimates of the life-time risk of developing ovarian cancer is 40% to 66% in BRCA1 mutation carriers and 10% to 27% in BRCA2 mutation carriers (14,19,24,25). Women with BRCA1 mutations are found to have an excess of multiple

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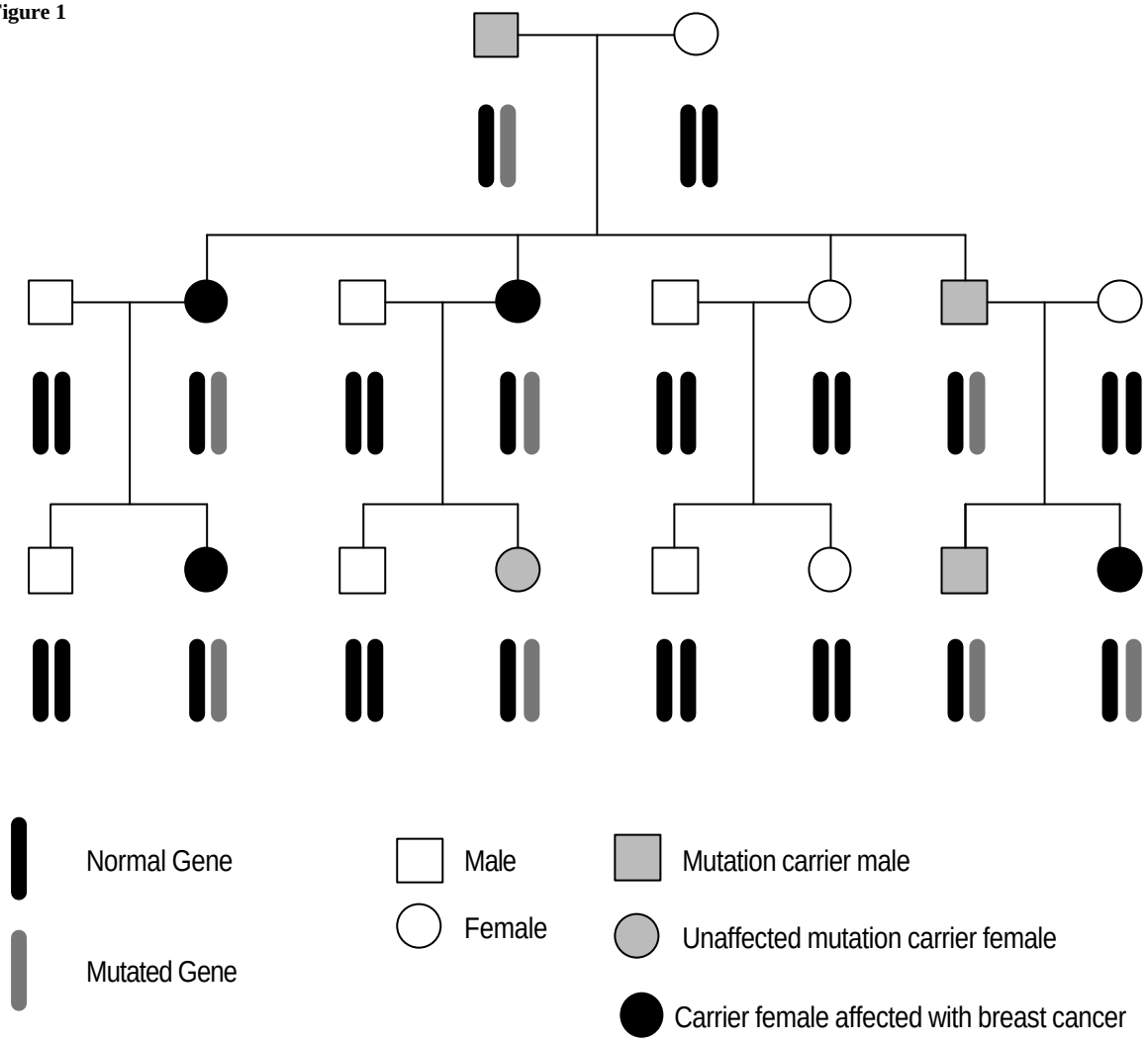
Figure 1



primary cancers of any type (of the breast or ovary) (23,26). Studies by the Breast Cancer Linkage Consortium (BCLC) have reported risk estimates for the development of contralateral breast cancer is up to 52% for BRCA1 carriers and up to 64% for BRCA2 carriers by age 70 years (19,23). Although the estimated risks for cancer in mutation carriers fall within a wide range, they are significantly higher than the lifetime risk for developing breast and ovarian cancers in the general population which are 10.6% and 1.5%, respectively (1).

Male carriers of BRCA1 and BRCA2 mutations are at increased risk of developing breast cancer (27,28,29). Breast cancer in men is a rare disease: in the United States the incidence is less than 1% of the incidence in breast cancer in women (30). Estimates for the lifetime risk (by age 70 years) for men is 0.01% (31). The observation that male breast cancer can occur in the context of family history of female breast cancer prompted researchers to investigate any association with inherited predisposition to breast cancer. Stratton et al reported the linkage of male breast cancer with BRCA2 (32). Germline BRCA2 mutations are to be more commonly reported than BRCA1 mutations (33,34). The frequency of BRCA2 mutations in male

Table 1. Features and types of hereditary breast and/or ovarian cancer families that harbour germline BRCA1/BRCA2 mutations.

Features
- Multiple affected members (first-, second- and third-degree relatives) affected with breast cancer of the same branch of a family;
- Mean age of diagnosis of breast cancers before age 50 years;
- Multiple primary breast cancers;
- Ovarian cancer diagnosed at any age (HBOC families only);
- Male breast cancer diagnosed at any age (more common in HBC families than HBOC families);
- Transmission of trait consistent with autosomal dominant mode of inheritance (Mendelian).
Types of Families
- Hereditary Breast and Ovarian Cancer Syndrome (HBOC)
- Hereditary (site-specific) Breast Cancer Syndrome (HBC)

breast cancer varies with ethnicity and geographic origins of population studied, as well as the method of ascertainment of the affected male. For example, in Iceland, 40% of male breast cancer cases were identified as carriers of a BRCA2 mutation (28). In contrast only 4% of cases were carriers in a study of male breast cancer cases ascertained in Southern California not selected for family history of cancer (33). A higher frequency (14%) was observed in another American study, and 85% of carriers reported a family history of female breast cancer (34). The rarity male breast cancer cases and variable frequency of mutation carriers in cancer cases of populations of different geographic and ethnic origins presents a challenge for establishing risk estimates and thus guidelines for genetic counselling.

Modestly increased risk estimates for cancers at other sites in carriers of BRCA1 and BRCA2 have been reported (19,23). We reported a large multi-site cancer family linked to BRCA2 that harboured cancers of the prostate, pancreas, larynx and colon in addition to 16 breast cancers where a number of cancers were shown to been mutation carriers (29,35). BRCA1 carriers are at increased risk of developing prostate cancer (RR = 4.1) and colon cancer (RR = 3.3) (19,36). BRCA2 carriers are at increased risk for developing cancers at specific sites. In a study by the BCLC, statistically significant increases in risks were observed for cancers of the prostate (RR= 4.65), pancreas (RR = 3.51), gallbladder and bile duct (RR = 4.97), stomach (RR =2.59) and skin [malignant melanoma] (RR=2.58) (23). Germline mutations have also been reported for fallopian tube cancers, a rare form of cancer found in 1% of all gynecologic malignancies (37,38,39,40). In one study of fallopian cancer cases not selected for family history about 16% cancers were shown to harbour germline mutations (41).

Guidelines for assessing cancer risk in mutation carriers is currently based on studies that have assessed penetrance in the context of individuals with a family history of breast and/or ovarian cancer as less is known about the risk of mutation carriers in the absence of a strong family history of cancer (42). The wide range in risk estimates may be due to factors that modify risk in mutation carriers, such as gene-gene and gene-environment interactions. Examples include, recent studies showing that oral-contraceptive pill use may reduce the risk of ovarian cancer in mutation carriers (43); that smoking may reduce the risk of breast cancer in mutation carriers (44); and that carriers harbouring specific variants of AIB1 that contain at least 28 or 29 polyglutamine repeats have a significant increased risk for developing breast cancer than women who carried AIB1 variant with fewer repeats (45). The smoking history of women and the AIBI variant underscores the potential steroid hormone link associated with breast cancer risk. Cigarette smoke has been found to have an anti-estrogenic effect (46). AIB1 is a transcriptional co-activator that interacts with steroid hormone receptors to enhance ligand-dependent transcription and is required for female reproductive function and mammary gland development (47). Current efforts are aimed at identifying and characterizing these (and other) variables that may modify risk in mutation carriers. However, the analysis and interpretation of the results of gene-environment interactions in known mutation carriers remains complex and has not yet been translated into guidelines for risk assessment. Thus, as the penetrance of high-risk women has been investigated based on the extensive analysis of women with a strong family history for breast and or ovarian cancer, mutation analysis and risk assessment is often limited to these women as they are most likely to harbour germline mutations with high penetrance.

OPTIONS FOR THE DETECTION OF BREAST AND OVARIAN CANCERS

The identification of known genetic factors affords the opportunity to identify those individuals at risk under the premise that this knowledge will enhance the accuracy of female breast cancer and ovarian cancer risk prediction and impacts on options for cancer screening and prevention. As the cumulative lifetime risk for developing breast and ovarian cancers is significantly higher in mutation carriers with a family history of disease than the general population, management has been focused on the detection of tumours that arise in this specific context. A number of similar guidelines (research based) have been proposed that are largely based on risk assessment in the context of a strong family history of breast and/or ovarian cancer and then were adapted to include families with proven BRCA1 or BRCA2 mutations when direct mutation detection became possible. The options for cancer detection include monthly self breast exams starting at age 20 years; annual clinical breast examination starting at age 25 years and annual mammograms starting at ages 25 to 35 years for the detection of breast cancer; and pelvic ultrasound and examination and serum CA-125 testing (a marker of primary ovarian cancer or recurrence) for ovarian cancer detection (42,48) .

Although randomized trials and population-based programs have provided evidence that breast cancer screening can be cost effective in women between 50 and 70 years of age (49,50), mammography screening in younger women that could be beneficial for mutation carriers is controversial. For ethical reasons

no randomized trials in BRCA mutations carriers are to be expected, and thus the surveillance for breast cancer in these women is likely to be evaluated by observational studies. Due to the low frequency of mutation carriers, a limited number of studies have been published (51,52,53,54). Brekelmans et al. recently reported on a combined retrospective and follow-up prospective study that analyzed the incidence and characteristics of screen-detected and interval breast cancers among 1,198 women who participated in a high risk breast cancer family clinic in the Netherlands (55). In addition to self-breast exams and clinical breast exams, they used magnetic resonance imaging (MRI) as an option for breast cancer detection. Proven BRCA mutation carriers where amongst the high-risk group of women under surveillance. The rates of both screen-detected and interval cancers were highest among the mutation carriers. The results of the study support the conclusions of earlier studies recognizing that there is a relationship between breast cancer risk and rate of cancer detection at screening, and that screening is beneficial and cost-effective in high-risk women. In addition, this study revealed a substantial risk of interval cancers in mutation carriers, which suggests that current screening protocols may be insufficient in this group of high-risk women. This observation underscores the pressing need to further evaluate why this may have occurred: is it a reflection of the inability to detect cancers by mammography or is it a reflection of the aggressivity of this variant of the disease.

Although, the benefits of mammography detection of breast cancers in women below age 50 is controversial, some studies have shown that screening between 40 and 50 years of age can also significantly reduce breast cancer mortality (56). However, due to the number of false positives and psychological toll, it may be efficacious to restrict screening to high-risk women (those with a strong positive history of breast cancer and/or BRCA mutation carriers) (57). MRI screening may improve detection of breast tumours that are undetectable by mammography either because the surrounding breast tissue is too radiodense or the tumour is insufficiently radiodense (55). A recent report by Warner et al. illustrates promising results with combination of MRI, ultrasound and mammography for the detection of breast tumours in proven BRCA mutation carriers (58). Indeed, in this study MRI was able to detect breast tumours not detectable by either ultrasound or mammography. Documentation of long-term survival in mutation carriers that would also demonstrate the long-term benefit of detection by MRI screening (or by other screening methods) of cancers presumably at their earliest stages of development and thus the most likely to respond to treatment is currently underway in the United States and Europe (59).

Effective screening protocols for ovarian cancer with the purpose of detecting primary tumours at early stage disease in mutation carriers are based on current protocols using transvaginal ultrsasonography and clinical pelvic examination (60,61,62). In the general population, the five-year survival of the disease is less than 30% despite recent improvements in treatment protocols (63) and has essentially remained unchanged for the past thirty years. Since there is a clear indication that early detection correlates with increased survival, methods to detect early stage ovarian cancer is pressing. However, the current methods have limited success for effectiveness for detecting borderline tumours, preinvasive and microscopic invasive tumours, and there has been no prospective, randomized trials to test whether screening will reduce morbidity and increase survival of women who are mutation carriers.

The low incidence of mutation positive male breast cancer cases in the context of a positive family history of female breast cancer has hindered progress in establishing guidelines for breast cancer detection in males. However, in some genetic counselling centres in Canada, male carriers of BRCA2 mutations are recommended to perform monthly self-breast examination and annual clinical breast examination, which may include mammography (64).

OPTIONS FOR THE PREVENTION OF BREAST AND OVARIAN CANCERS

In germline mutation carriers all somatic cells harbour a mutated copy of BRCA, and thus the potential for bilateral or double primary cancers is high in women who are mutation carriers in comparison non-carriers. Hence, the options for cancer prevention have included radical surgeries such as bilateral mastectomy and oophorectomy (65). Although carrier status was not determined, a recent retrospective review of Mayo Clinic experience demonstrated that bilateral prophylactic mastectomy was followed by a 90% reduction from the expected number of breast cancers in women at both moderate and high risk due to family history of disease (66).

The benefit of premenopausal oophorectomy by reducing the risk of breast cancer in the general population has been documented (67.68,69). Rebbeck et al. has shown that BRCA1 mutation carriers who underwent prophylactic oophorectomy have a significant risk reduction for breast cancer compared with mutation-positive and age matched women who

did not have oophorectomy (70). Overall, the age of diagnosis of ovarian cancer in BRCA1 mutation carrier is rare below the age of 40 years (71). While the incidence rates for ovarian cancer have been shown to be at least four-fold lower for BRCA2 mutation carriers than BRCA1 mutation carriers (23), the lowest estimate of risk, at 16% is significantly higher than that estimated for the general population. Although the overall mean age of diagnosis for ovarian cancer in mutation carriers is comparable to the mean age at diagnosis of ovarian cancer in the general population (age 56 years), there is some support from anecdotal evidence and recently from a large scale study on population series of 649 women with ovarian cancer not selected for family history, that ovarian cancer in BRCA2 mutation carriers occur later than in BRCA1 mutation (72,73). These age-specific observations could be considered when contemplating an oophorectomy. However, the long-term benefits remain to be validated.

Another consideration of surgical removal of normal ovaries is the risk for developing primary peritoneal carcinomatosis or papillary serous carcinoma or the peritoneum (PSCP) which has been documented in carriers of BRCA1 mutations (74). Based on a data from Crighton University Hereditary Cancer Institute and a review of the literature (75,76,77), Lynch and Casey estimate that fewer than 5% of women who undergo prophylactic oophorectomy because of familial ovarian cancer will develop PSCP, a risk that is significantly lower than the estimated lifetime risk for developing ovarian cancer in mutation carriers (65).

Chemopreventation is another strategy aimed at reducing risk for breast cancer. At least two recent studies have shown that tamoxifen significantly reduces the risk of primary invasive and premalignant breast cancer in women at high risk for breast cancer and of contralateral breast cancer in unselected women (78,79). Recently, Narod et al. demonstrated that tamoxifen reduces the risk of contralateral breast cancer in proven mutation BRCA1 or BRCA2 carriers (80). In women who used tamoxifen for 2 to 4 years, the risk of contralateral breast cancer was reduced by up to 75%. These results are very encouraging and in combination with the results of earlier studies where benefits were observed in high risk women (although carrier status was unknown) question the efficacy of tamoxifen use in cancer prevention in proven BRCA1 or BRCA2 mutation carriers.

GENETIC TESTING FOR BRCA1 AND BRCA2 MUTATIONS

Although a growing body of evidence implicates BRCA1 as a nuclear transcription factor with a role in response to DNA damage and BRCA2 in recombination-mediated repair of double-strand breaks, maintenance of genome integrity and chromosome segregation (81), the relationship to cancer risk and specificity of cancer sites has not been elucidated. Both genes are large and structurally complex. BRCA1 with 22 exons spans a region of approximately 80 kilobases of genomic DNA and encodes a 1,843 amino acid protein (10). BRCA2 is larger than BRCA1 spanning a region of greater than 100 kilobases of genomic DNA and encodes a 3,418 amino acid protein (12,13). Despite the similarity in nomenclature, the genes share no significant homology based on genomic sequence comparisons or no obvious homology to any known gene. However, BRCA1 encodes a protein with a sequence motif at the amino terminus that shares similarity to proteins with zinc-binding domains, and a conserved acidic carboxyl terminus (10). In contrast, BRCA2 encodes a protein that contains no identifiable functional domains based on amino acid sequence composition (12,13). Structurally they both harbour one very large exon (the 11th exon in both genes), that contains 60% of the coding region and the significance of this gene structure is not known.

The identification of carriers of germline mutations largely relies on the mutation analysis of genomic DNA or transcribed mRNA. Various methods alone or in combination for the detection of mutations have been devised based on the observations that BRCA1 and BRCA2 are large genes with many coding exons, that mutations have been identified in all coding exons, that the majority of mutations are private, and that 'deleterious' or disease causing mutations may be complex. Direct sequencing of genomic DNA or cDNA (derived from mRNA) reveals a significant proportion of sequence variants, estimated at about 85% (82). Methods such as single stranded conformation polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), and protein truncation test (PTT) assays also have been used to identify DNA segments containing putative sequence variants that are verified after DNA sequencing (83,84,85). All of these methods have limitations due to their inability to detect large deletions (86,87,88), lack in the sensitivity of detection (such as SSCP); or are technically challenging (such as DGGE). Recently, denaturing high-performance liquid chromatography (DHPLC) which is a method of comparative sequencing based on heteroduplex detection, has been shown to reliably detect a number of different BRCA1 and BRCA2 sequence variants demonstrating that this method has a high degree of sensitivity and specificity and provides a low cost

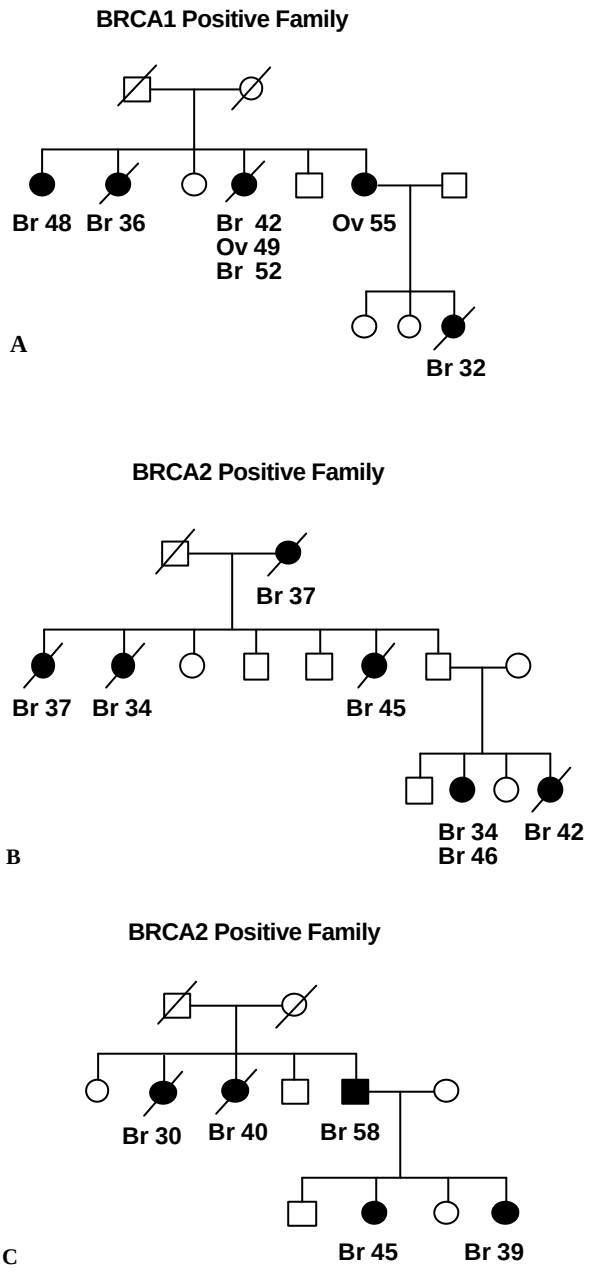


Figure 2.

alternative to direct DNA sequencing (89,90). Because of the wide-spectrum of sequence variants identified it may be necessary to use a combination of different mutation detection protocols. Another consideration that has been explored on a limited basis is that mutations, such as in promoter region(s) of BRCA genes, may alter the transcriptional activity (88, 91) and this alteration may require the use of methods that enable quantifying transcript or protein levels.

The identification of BRCA1 and BRCA2 has afforded the opportunity of identifying carriers of germline mutations by direct mutation analysis where previously the involvement of BRCA was deduced by linkage analysis to markers representative of loci harbouring the putative genes. However, the large size and complex structure of each gene and the spectrum of mutations identified has posed problems for cost-effective mutation screening for high-risk women. The large spectrum of mutations identified worldwide is exemplified by The Breast Information Core (BIC) database which is an open access-online mutation database which lists BRCA1 and BRCA2 mutations (82). A recent review of the database revealed greater than 800 entries for distinct sequence variants for each gene (83). An extraction of entries from Canadian samples is shown in Table 2. This sample is by no means complete or comprehensive as entry of sequence variants in the BIC database is on a voluntary basis and not all entries indicate country of origin of the individual samples for sequencing. The wide spectrum of sequence variations reported for Canadian samples exemplifies the complexity of sequence variants identified in the BRCA genes. Sequence variants include frameshift and nonsense mutations that both result in the introduction of a stop codon leading to chain termination of protein synthesis and thus a truncated protein. Frameshift mutations account for the majority of mutations identified in BRCA1 and BRCA2 worldwide (82). These mutations are deemed 'deleterious' or 'disease-causing' as the resulting truncated protein is presumed to affect the normal function of the gene product. The majority of sequence variants that are missense are termed unclassified variants (82) as the biological consequences of the resulting change leading to specific amino acids substitutions are not known.

The contribution of BRCA1 and BRCA2 varies with spectrum of cancers in breast cancer families (14). Germline mutations in BRCA1 are typically identified families with ovarian cancer (Figure 2A). These cancer families are often referred to as breast-ovarian cancer syndrome families (Table 1). Breast cancer families with no ovarian cancer or contain at least one male breast cancer case are more likely to harbour germline mutations in BRCA2 (Figure 2B and 2C). These families are often referred to as 'site-specific' breast cancer families. However, it is important to emphasize that these phenotypic classifications are not mutually exclusive but reflect the likelihood of identifying a mutation in a particular susceptibility gene and thus could serve as a guide to prioritizing mutation analysis.

Within defined ethnic groups specific, relatively frequent mutations have been identified. Three founder mutations have been identified in the Ashkenazi Jewish families of eastern European ancestry:

Table 2: Spectrum of BRCA1 and BRCA2 Sequence Variants Reported for Canadian Population*

Gene	Designation	Exon or intron (IVS)	Nucleotide	Base change	Codon	Amino acid change	Mutation type or effects†	Reported more than once in BIC	Recurrent mutation mutation in some populations
	185delAG	2	185	delAG	23	stop 39	F	Yes	Ashkenazi Jewish
	C61G	5	300	T>G	61	Cys>Gly	M	Yes	
	Q356R	11	1186	A>G	356	Gln>Arg	M/P	Yes	
	D369N	11	1224	G>A	369	Asp>Asn	M/UV	no	
	R496H	11	1606	G>A	496	Arg>His	M/UV	yes	
	2072insG	11	2072	insG	651	stop672	F	yes	
	S741F	11	2341	C>T	741	Ser>Phe	M/UV	no	
	2800delAA	11	2800	delAA	894	stop901	F	Yes	
	2953del3+C	11	2953	delGTAinsC	945	stop950	F	Yes	French Canadian
BRCA1	P1099L	11	3419	C>T	1099	Pro>Leu	M/UV	yes	
	3450del4	11	3450	delCAAG	1115	stop1115	F	Yes	
	3768insA	11	3768	insA	1217	stop1218	F	Yes	French Canadian
	E1250X	11	3867	G>T	1250	stop1250	N	Yes	
	R1347G	11	4158	A>G	1347	Arg>Gly	M/UV	Yes	
	4184del4	11	4184	delTCAA	1355	stop1364	F	Yes	
	R1443X	13	4446	C>T	1443	stop1443	N	Yes	French Canadian
	5221delTG	18	5221	delTG	1714	stop1714	F	no	
	IVS20+1G>A	IVS20	5396+1	G>A	na	na	S	Yes	Dutch
	5382insC	20	5382	insC	1756	stop1829	F	Yes	Ashkenazi Jewish
	IVS21-8C>T	IVS21		C>T	na	na	S/UV	no	
	983del4	9	934	delACAG	252	stop275	F	yes	
	1119del9/ins10	10	1119	delAACAGTTGT/ insGATACTTCAG	297	stop304	F	no	
	E462G	10	1613	A>G	462	Glu>Gly	M/UV	yes	
	2034insA	10	2034	insA	602	stop 615	F	yes	
	2157delG	11	2157	delG	643	stop 659	F	yes	
	2816insA	11	2816	insA	863	stop 880	F	yes	French Canadian
	4359ins6	11	4359	insTGAGGA	1378	Thr>stop	N	yes	
	D1420Y	11	4486	G>T	1420	Asp>Thr	M/UV	yes	
	G1771D	11	5540	G>A	1771	Gly>Asp	M/UV	yes	
	5699insA	11	5699	insA	1824	stop 1828	F	no	
	S1882X	11	5873	C>A	1882	stop 1882	N	yes	
	6085G>T	11	6085	G>T	1953	stop 1953	N	yes	French Canadian
BRCA2	6174delT	11	6174	delT	1982	stop 2003	F	yes	Askenazi Jewish
	R2034C	11	6328	C>T	2034	Arg>Cys	M/UV	yes	
	6503delTT	11	6503	delTT	2092	stop 2099	F	yes	French Canadian
	7297delCT	14	7297	delCT	2357	stop 2358	F	yes	
	IVS14+6G>A	IVS14	na	G>A	na	na	S/UV	no	
	C2636X	17	8136	T>A	2636	stop 2636	N	no	
	V2728I	18	8410	G>A	2728	Val>Ile	M/UV	yes	
	8474delAG	18	8474	delAG	2749	stop 2762	F	no	
	8765delAG	20	8765	delAG	2846	stop 2867	F	no	French Canadian
	Q2858R	20	8801	A>G	2858	Gln>Arg	M/UV	no	
	8904delA	21	8904	delA	2892	stop 5908	F	no	
	W2989X	23	9198	G>A	2989	Trp>stop	N	no	
	9356insA	23	9326	insA	3033	stop3034	F	yes	
	K3326X	27	10204	A>T	3326	Lys>stop	N/UV	yes	

* Extracted from Breast Cancer Information Core; †Mutation type or effects: F=framesfit, M=missense, N=nonsense, S=splice variant, UV=unknown variant.

BRCA1:185delAG, BRCA1:5382insC, and BRCA2:6173delTT (38,93,94,95,96) (see Table 2). In a study 220 North American Ashkenazi Jewish families (including families ascertained in the Montreal area) we observed that 45% of harboured one of three founder mutations (94). In another study, we have observed six common mutations in our analysis of the French Canadian population of Quebec (72,97,98) (Table 2). Two specific

mutations: BRCA1 4446C>T and BRCA2 8765delAG account for a significant fraction of mutation positive cases where 28 of 41 mutations identified in 97 families harboured one of these specific mutations (97). Haplotype analysis (genotyping of polymorphic markers adjacent or within the genes that would enable deducing parent of origin of each allele) has provided evidence for founder effects in these ethnic groups, suggesting that the mutations arose from common ancestors (99,100,101,102). We also showed that presence of ovarian cancer is a strong predictor of the presence of BRCA1 versus a BRCA2 mutation, a phenotype consistent with observations of mutation spectrum of families not selected for ethnicity (14,97). Specific mutations have also been described in other groups defined by country of origin, such as BRCA2:999del 5 which is the most prevalent mutation identified in breast cancer families in the Icelandic population (103).

The presence of founder effects, leading to a reduced heterogeneity, facilitates carrier detection and genetic counselling, for certain well-defined populations. As a first screen for mutations, the overall cost of mutation detection is significantly reduced when mutation detection is limited to mutations found at a high frequency in specific populations. Genetic counselling is also facilitated when the prevalence of specific mutations is known in the defined population harbouring recurrent mutations. For example, several studies have shown the prevalence of carriers of the common BRCA1 and BRCA2 in the Ashkenazi Jewish population is known to be high, ~2.5% (104,105,106) and the yield of other mutations in either gene is rare. These findings suggest that screening for mutations may be limited to the identification of the three common mutations identified in the Ashkenazim. In contrast to the Ashkenazi Jewish population, the frequency of the six common mutations identified in the French Canadian population is not known, although we have shown that 10% of women diagnosed with breast cancer below the age 41 years (98) and 8% of women diagnosed with ovarian cancer (72), not selected for family history of cancer, harbour one of six common mutations (97).

INTERPRETING TEST RESULTS

Prior to the discovery that germline mutations in BRCA1 and BRCA2 conferred increased risk to breast and ovarian cancers, empiric risk for hereditary cancer was computed based on both the family history of disease (breast cancer) and the age of diagnosis of the breast cancers (4,7). Figure 3 illustrates a pedigree, Family X, displaying a strong family history of breast and ovarian cancer based on the hallmark features of inherited predisposition to breast and ovarian cancers (Table 1). In Family X, the 32 year women having at least two first-degree relatives with breast cancer diagnosed before 50 years of age (in this case her mother and sister diagnosed at ages 36 and 39, respectively) has a 35%-48% cumulative lifetime risk (by age 70) of developing breast cancer. This estimate is based on estimates that derived from mathematical models that used population-based family history data (4). In the absence of a genetic test to determine carrier status she would be informed that she has at high risk for harbouring a mutation based on the 50% chance of inheriting a disease causing allele from her mother who being affected is the predicted carrier (see Figure 1). Mutation analysis of BRCA1 and BRCA2 has the potential to determine if she carriers a disease causing mutation. The results also have the potential to impact on management when considering options for cancer detection and prevention such that management procedures are concentrated on those at highest risk for developing cancer in this family.

Family X is consistent with the clinical phenotype for inherited predisposition to breast and ovarian cancer (Table 1), and thus has a high likelihood of harbouring a germline mutation in BRCA1 or BRCA2. A number of models exist to compute risk of carrier status in high-risk individuals (7,107,108,109). It is important to emphasize confirmation of pathology of cancer sites as risk assessment is based on the cancer site, age at diagnosis and number of cancers regardless of whether genetic testing is considered an option for improving risk assessment. The presence of an ovarian cancer case in a family of young onset breast cancers increases the likelihood that a BRCA1 mutation segregates with disease in this family. Preferably, genetic analysis is performed on an affected individual in the family because it is currently difficult to interpret negative test results. For example, the conclusion of a test result that yields no obvious deleterious mutation ('negative' test result) cannot be distinguished from the possibility that the mutation assay was lacking in sensitivity and thus the mutation was present but not detectable. This genetic test result would be deemed 'not informative' and would not improve risk assessment and thus risk assessment remains as computed based on family history alone. In circumstances where the frequency and spectrum of specific mutations in a defined ethnic group are known, such as the Ashkenazim for example, mutation analysis has proven useful in improving risk assessment when genetic analysis performed on unaffected individual because a clinical specimen was not available from an affected individual (94). In the event of a positive test result in an affected individual such as the women

diagnosed with breast cancer at age 39 in Family X, the genetic analysis could be extended to other family members in order to determine if they are carriers of the same mutation. The absence of a mutation, as the case of 35-year women in Family X, would suggest that she her lifetime risk for developing breast and ovarian cancers is close to population risk. This women may consider options for breast cancer detection that are open to all women in the general population beginning at age 50 years. However, the presence of mutation, as in the case of the 32-year women in Family X, would suggest that her risk for developing breast and ovarian cancers is significantly elevated above population risk. This high-risk woman may consider options for cancer detection and prevention that are available to high-risk women.

Genetic testing has the potential to improve management for women in high-risk families that have already had a breast cancer diagnosis. For example, the 39 year-old women with breast cancer deemed to be a mutation carrier in Family X is at increased risk for a second breast cancer and ovarian cancer in comparison to the women diagnosed with breast cancer at age 55 and found not to be a mutation carrier and thus likely a sporadic case of breast cancer (Figure 3). Breast cancer is the most common cancer reported in the female population (second to skin cancers) and thus it is not surprising that sporadic cases occur in the context of hereditary breast cancer families (94). As mutation carriers are more likely to develop breast cancer at a young age (prior to age 50 years), it is preferable to test the youngest breast cancer case in the family or an ovarian cancer case, as the likelihood of a sporadic ovarian cancer case in breast cancer family is rare.

THE DIFFICULTY OF INTERPRETING SEQUENCE VARIANTS

Mutations in BRCA1 and BRCA2 are deduced based on the anticipated consequence of a DNA sequence variation on the resulting protein, as there is no assay to assess the functional significance of an alteration in the amino acid sequence composition. For example, this is predicted when a sequence variation results in the production of a truncated protein (see Table 2). However, the significance of protein truncating mutations that arise in the extreme 3' end of the gene, as the case of the mutation BRCA2: K3326X, which occurs in exon 27, is not known. The BIC reports one instance where this variant was observed in the context of another frameshift mutation, BRCA2:6503delTT (92). Missense mutations, resulting in amino acid substitutions, are difficult to interpret in comparison to mutations giving rise to truncated proteins. A common missense mutation in BRCA1, 300T>G, results in an amino acid substitution of cysteine to glycine at amino acid position 61 (C61G). Although this substitution does not alter the hydrophobicity or charge it occurs in the zinc-finger-binding domain, and loss of an amino acid with a sufhydryl side chain may affect the function of the resulting Brca1 protein. The observation that this mutation segregates with disease in breast cancer families suggests that the alteration has significant functional consequences. Thus, in the absence of a biological assay for protein function or concentration, the segregation of sequence variants with disease in a family remains the most effective way to deduce the significance of sequence variants of obvious unknown significance. As segregation analysis is less feasible outside of the research facilities the importance of comparative sequence analyses such as with mutation databases, becomes evident. The rare occurrence of sequence variants in healthy women, and thus association only with breast (and ovarian) cancer cases, is also an indicator of the significance of the sequence variation (92).

CONCLUSIONS

The identification of the breast and ovarian cancer susceptibility genes has improved risk assessment of women in high risk families as the most immediate impact is the ability to distinguish women who carry high risk alleles from those that do not and thus avoiding unnecessary management procedures. Assessing risk is a complicated time consuming process involving pedigree inspection, confirmation of cancer sites and age of diagnosis, and interpretation of mutation detection results. Given the complexity of risk assessment, it is strongly recommended that risk assessment based on personal and family history disease and genetic test results is conducted in the context of highly trained personnel such as genetic counselling service specializing in inherited predisposition to adult onset cancers with management conducted in consultation with breast specialists and gynecologists/oncologists.

For the immediate future there is a need to evaluate the long-term benefits of cancer prevention and detection strategies, improve methodologies of cancer detection and risk, and determine environmental and genetic factors that may modify risk in carriers of deleterious BRCA1 and BRCA2 mutations. In addition, recent efforts have been directed towards the identification of novel breast cancer susceptibility genes. Of the estimated 10% of the all breast and ovarian cancer are due to inherited predisposition to cancer consistent with transmission of an autosomal dominant trait, an estimated 5% of these cancers are due to germline mutations in BRCA1 and BRCA2.

Either the sensitivity of detection is beyond our current means, or there are other novel cancer predisposing genes. Ford et al., presented compelling evidence that up to 67% of site-specific breast cancer families with four or five cases of breast cancer was not due to either BRCA1 or BRCA2 (14). One conclusion is that these 'negative' families may be due to mutations in novel breast cancer susceptibility genes, as the clinical phenotype of family history is not consistent with the involvement of other known breast cancer predisposing genes such as TP53 and PTEN. Earlier studies using smaller number of families have provided supportive evidence for this hypothesis (110,111,112,113). However, the identification of novel susceptibility genes has remained elusive (81) despite an intriguing loci identified on chromosomes 8 (114,115,116) and 13 (117). The identification of genetic factors that modify risk in BRCA1 and BRCA2 mutation carriers and novel susceptibility genes may lead to further improvements in risk assessment of hereditary breast and/or ovarian cancer families.

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