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Title: The Accuracy of Clinical Criteria to Predict Mutations in *BRCA1*- and *BRCA2*-Related Hereditary Breast and Ovarian Cancer Syndrome.

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Summary:

The Hereditary Breast and Ovarian Cancer (HBOC) Clinic located in Winnipeg accepts province-wide referrals to assess cancer risk and provide testing for mutations in *BRCA1* and *BRCA2* when indicated. Given the economic realities of public health care, a limited number of patients can be offered full gene testing. Manitoba has developed fourteen eligibility criteria based on personal and family history of breast and ovarian cancer that are thought to identify patients with at least a 10% chance of having a mutation. In this retrospective clinic-based study, we evaluate the association between these criteria and mutation frequency to determine which characteristics are statistically associated with *BRCA1* and *BRCA2* mutations. Information from hospital and lab records was collected for 429 probands tested between 1995 and 2010. Twenty-one percent of probands tested through the HBOC Clinic were found to have a disease-causing *BRCA1* or *BRCA2* mutation. Consistent with other studies, multiple tumor diagnoses and a strong family history of breast/ovarian cancer were the characteristics most strongly associated with the finding of mutations. Ethnicity of the Manitoba population in relation to *BRCA1* or *BRCA2* mutation frequency was also explored. The recurrent mutations found in the study population partially reflected the ethnic composition of the Manitoba population. All of the criteria examined in this study are achieving ≥10% mutation detection rate, but some re-evaluation is recommended to explore broadening criteria based on personal history of tumors and to adjust ethnic-specific screening by adding a Mennonite and dropping one of the Eastern European founder mutations.

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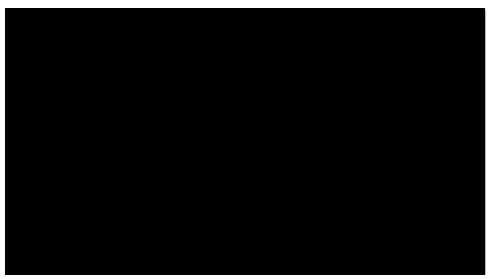

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Introduction

In recent years, many genes and subsequent mutations have been identified that are responsible for increasing the risk of developing specific cancers. These cancer susceptibility genes were originally discovered through studies of families with histories suggestive of cancer syndromes. Hereditary, breast and ovarian cancer (HBOC) syndrome is characterized by an earlier age of onset and bilateral or multiple primary diagnoses, and is seen in multiple generations. The genes, *BRCA1* (MIM 113705) and *BRCA2* (MIM 600185) were mapped using large families with multiple cases of breast and ovarian cancer[1, 2]. Both are very large genes that are inherited in an autosomal dominant pattern, and mutations in these genes are associated with a higher risk of developing breast and ovarian cancer. Current literature suggests that the lifetime risk of developing breast cancer is 40-90% in individuals with a *BRCA1* or *BRCA2* mutation as compared to 11.1% for Canadian women[3]. Having a *BRCA1* or *BRCA2* gene mutation also increases the lifetime risk of ovarian cancer from 1.4%[4] to 18-40%[5, 6].

Although the *BRCA1* and *BRCA2* genes were first discovered through the study of highly affected families, it has been recognized that these families likely "represent only a small fraction of individuals with inherited predisposition to cancer"[7]. More recent research has focused on ascertaining all breast and/or ovarian cancer cases to better appreciate the penetrance as well as variable expressivity of *BRCA1* and *BRCA2* mutations[8]. Notwithstanding the interest and research directed towards clinical assessment of the *BRCA1* and *BRCA2* genes, there has "been no clear consensus on what specific personal and family history features should prompt consideration of hereditary cancer risk assessment"[9]. Despite this lack of consensus, many medical centers around the world have developed sets of high-risk criteria based on personal and family history to determine which patients "exceed a threshold of a 10% likelihood of finding a germline genetic mutation in a cancer susceptibility gene"[10]. If a patient is considered high-risk based on these criteria, they are offered genetic testing through full gene analysis of *BRCA1* and *BRCA2*.

The Hereditary Breast and Ovarian Cancer (HBOC) Clinic located in Winnipeg Manitoba accepts referrals to assess cancer risk and provide testing for genetic mutations in the *BRCA1* and *BRCA2* genes when indicated. When the HBOC Clinic first began to offer genetic testing in the 1990's, screening was limited to mutations commonly found in the Ashkenazi Jewish population. Soon thereafter, full screening of the *BRCA1* and *BRCA2* genes using a protein truncation test[11] became available. Testing evolved once again, and currently the *BRCA1* and *BRCA2* genes are scanned using denaturing high-performance liquid chromatography (dHPLC), which allows for the detection of missense in addition to truncating mutation[12, 13]. MLPA is also used to detect large genomic rearrangements. Testing for mutations in *BRCA1* and *BRCA2* is complicated by a large number of private mutations, which necessitates the use of full gene scanning techniques to achieve appropriate sensitivity and specificity. Due to the need for full gene scanning and the large size of the *BRCA1* and *BRCA2* genes, full gene testing is a time-consuming process and patients can be expected to wait up to 12 months for their results here in Manitoba.

Given the economic realities of public health care, a limited number of patients can be offered full gene testing. Manitoba has developed its own eligibility criteria that reflect the characteristics of HBOC syndrome and are thought to identify patients with at least a 10% chance of having a *BRCA1* or *BRCA2* mutation. These criteria are similar to those used in other provinces and are based features described in the medical literature in 1999 and 2000.

As an adjuvant to the costly full screen protocol, ethnic-specific testing for individuals of Ashkenazi Jewish, Icelandic[14], Aboriginal[15] and Eastern European[16, 17] descent is also available in Manitoba. Recent data suggests there may also be a specific *BRCA2* mutation found in people of Mennonite descent[18]. Recurring founder mutations have also been described for the French Canadian populations[19].

Since carriers of a *BRCA1* or *BRCA2* mutation are at an increased risk of developing breast and ovarian cancer, surveillance, drug therapy, and preventative surgery may be feasible options to reduce the cancer risk in those patients. Prophylactic oophorectomy and has been shown to significantly decrease the risk of breast and ovarian cancer in *BRCA1* and *BRCA2* mutation carriers, while prophylactic mastectomy reduces the risk of breast cancer[20, 21]. Chemoprevention using tamoxifen has also been used to decrease cancer risk in some of these patients based on the estrogen receptor status of their tumors. Mutation carriers can also choose to pursue reproductive counseling on pregnancy options and the risk of transmission of the mutation to future children. Furthermore, once a mutation is identified in a proband, it is possible to offer their blood relatives accurate testing for HBOC syndrome.

Clearly, the current criteria need to be properly evaluated to ensure the most cost-effective approach of identifying individuals who are at risk of having *BRCA1* or *BRCA2* mutations. Beyond concerns of cost, effectual screening strategies can lead to better clinical outcomes for patients and their families.

In this retrospective chart review study, we describe the characteristics of Manitoba patients seen in the HBOC Clinic between 1995 and 2010 who qualified for full gene testing based on their personal and family histories of breast and ovarian cancer. Each eligibility criterion is examined to ensure it meets the 10% mutation detection recommendation. Based on data from previous research, we expect *BRCA1* and *BRCA2* mutations to be more strongly associated with early age of onset and multiple or bilateral primaries in probands as well as probands with strong family history of breast and/or ovarian cancer[22]. Confirmation of additional founder mutations within subpopulations of Manitoba, such as the Mennonites, is also expected. Our results may also be used to confirm the clinical criteria currently used in Manitoba to identify individuals at risk for *BRCA1* and *BRCA2* mutations, without compromising clinical sensitivity, and where appropriate to adjust the criteria to better serve our local population. Data collected from this study may be used to more accurately estimate the pre-test likelihood that a patient with a certain pattern of personal and/or family of breast and/or ovarian cancer will have a *BRCA1* or *BRCA2* mutation following genetic testing.

Methods

Proband information

Proband information was collected initially through the records of the Molecular Diagnostic Laboratory (MDL), Diagnostic Services of Manitoba; the laboratory that performs genetic testing on samples as ordered by physicians. Information from the records of probands whose specimens were sent to the MDL from 1995 to 2010 for full *BRCA1* and *BRCA2* analysis included the type of molecular analysis performed and the results of testing. Additional information on each proband was obtained from the records of the HBOC Clinic to ensure full ascertainment. These records contain letters, pedigrees, pathology reports confirming personal

and family history of cancer diagnoses, and consult notes from medical geneticists and genetic counselors that met with individuals and their families. Information on all probands was compiled in a Microsoft Excel spreadsheet and contained details including cancer type, age of diagnosis, pathology (where available), family member relationship and age at diagnosis, ethnicity, date of testing and date of final report, type of analysis performed, and results of the full gene screen including type of mutation and its clinical significance.

Criteria assignment

Once information was collected from the DSM and HBOC clinic records, the personal and family histories for each proband were considered in order to assign the criteria the proband met in order to qualify for the full gene testing as described in Table 1. Many probands met more than one eligibility criterion and were considered in the evaluation of each criterion. Crtieria 1, 2, 3, 10, and 11 are based solely on personal cancer history, and therefore these criteria were subdivided to examine the effects of family history on mutation frequency.

Mutational analysis

To detect point mutations, small deletions and insertions, the MDL employed a combination of PTT and dHPLC analysis followed by Sanger sequencing[11, 12]. MLPA was used to detect large genomic rearrangements[33].

Sequence variation classification

The Breast Cancer Information Core (BIC) database, an international collaborative central repository of *BRCA1* and *BRCA2* sequence variants that is maintained by the National Human Genome Research Institute (http://research.nhgri.nih.gov/bic/), was used as the initial source to determine if the sequence variants found in probands were previously observed and if so, whether they were classified as disease-causing mutations (DCM), unclassified variants (UCV), or benign polymorphisms. For sequence variants not reported in BIC, the clinical significance of these changes was classified based on American College of Medical Genetics (ACMG) 2007 revised recommendations for standards for interpretation and reporting of sequence variations[23] in conjunction with published data, if any, on the sequence variant. Probands were considered to be 'mutation positive' when they had a previously reported and recognized DCM, a previously unreported sequence variant expected to cause HBOC, or a variant that had multiple publications of overwhelming scientific evidence pointing to its deleterious effect. UCVs and benign polymorphisms were recorded, but not included as 'mutations' in subsequent analysis.

Statistical analysis

A statistician was consulted through the Department of Community Health Services at the University of Manitoba. Univariate analysis was performed to test each criterion individually against the outcome of mutation. The proportion of mutation carriers among those meeting each eligibility criterion is reported in Table 1. A multivariate analysis was also performed using a logistic regression model to regress the criterion simultaneously and reveal which criteria are most likely to identify mutation carriers. Some criteria were not included in the regression models due to its small sample size. Chi-square (χ^2) analysis was used to determine if the differences in the proportion of *BRCA1* to *BRCA2* DCMs for a given subgroup was statistically significant.

Results

DSM records indicated that 510 patients received full gene screens between May of 1995 and May of 2010. Eighty-one patients (approximately 16%) were excluded from the study for the following reasons. There were 25 probands whose corresponding HBOC clinic chart could not be located. Ten of these 25 were excluded from the study because there was inadequate patient and family history information provided on the DSM record alone. The remaining 15 probands were included in the study because there was sufficiently detailed personal and family history information in the DSM record to assign them to appropriate testing criteria. Another 69 probands were excluded from the study because they did not meet any of the full gene testing eligibility criteria. Many of these probands were tested either on a research basis in the earlier years, as the only surviving or available members of their family, or as an exceptional case as deemed by the geneticist. Two probands did not have their test results completed by the end of the collection period.

Of 429 probands used in this study, there were 16 males and 413 females who were between the ages of 24-74 years at the time of diagnosis of breast and/or ovarian cancer. There were 351 probands diagnosed with breast cancer only, 58 probands with ovarian cancer only, and 19 probands who were diagnosed with both breast and ovarian cancer at the time they qualified for testing. Ninety-two (21.4%) probands were found to have disease-causing mutations (DCM) (Table 2). Fifty-three probands, or 58.2% had a DCM identified in *BRCA1*, with 35 distinct *BRCA1* mutations detected. There were 28 distinct *BRCA2* mutations identified in 35 or 41.8% of the probands with a DCM. An additional 59 probands were found to have a UCV. In our population, genomic rearrangements accounted for 6% of *BRCA1* mutations and none were found in *BRCA2*.

Many of the mutations identified in the study population are known founder mutations. Five probands carried c.5266dupC, a *BRCA1* mutation found in Eastern European and Ashkenazi Jewish populations. This was the most commonly reported DCM in our study. Another 4 probands were heterozygous for the c.1387delAAAAinsGAAAG *BRCA1* mutation, which occurs commonly in people of Aboriginal descent. Two probands were identified with c.181T>G (p.Cys61Gly), a *BRCA1* missense mutation typically found in individuals of Eastern European ancestry. Recurrent mutations are listed in Table 3. Ethnicity, as reported by probands, was also analyzed and compared to the ethnic make-up of Manitoba and of Canada (Figure 1).

Data was analyzed to ascertain how frequently mutations were found in probands meeting each criterion (Table 1). All of the criteria met the suggested 10% mutation detection rate. Individuals with early onset breast cancer (<35 years of age) and no family history (criterion 1a) had the lowest frequency of approximately 13%. The relative risk associated with each criterion is listed in Table 1. Statistical analyses were performed and criteria 2b, 3b, 9, and 13 have a significant relative risk greater then 1 using a univariate analysis. Using a combined logistic regression model with a p value of 0.05, criteria 2b, 3b, 9, and 13 were again the only criteria with statistically significant results. Univariate and multivariate analyses were also performed on criterion 1, 2, 3, 10, and 11 without selecting for family history (Table 1).

Of the 16 male probands in this study, four had a DCM identified, all of which were in *BRCA2* ($\chi^2_{df=1}=5.57$, $p<0.05$). Probands with ovarian cancer only were more likely to have a *BRCA1* mutation ($\chi^2_{df=1}=6.05$, $p<0.05$).

Discussion

Although it is well-established that individuals and families with *BRCA1* and *BRCA2* mutations develop breast and ovarian cancer more often than those without mutations, medical centers must be able to decide which patients are at such an increased risk that they should undergo full gene mutation screening. Manitoba has developed a set of fourteen eligibility criteria (Table 1) based on personal and family history that are used to identify patients at an estimated 10% or greater risk of being mutation carriers. These criteria are similar to those used in other provinces and are based on features described in the medical literature in 1999 and 2000.

In this clinic-based chart review, we have retrospectively reviewed the results of 429 *BRCA1* and *BRCA2* genetic tests in men and women referred from the Manitoba HBOC Clinic. Clinical characteristics and mutation status have been examined to assess which of the testing criteria (Table 1) are most strongly associated with the presence of *BRCA1* or *BRCA2* mutations. Our data shows that 21.4% of all probands tested are mutation carriers and that all of the Manitoban criteria are associated with at least a 10% mutation frequency and should, therefore, continue to be used.

Although all criteria lead to the detection of *BRCA1* or *BRCA2* mutations in a proportion of probands, a few criteria in our study are significantly better than others at identifying probands with such mutations. As expected, probands diagnosed with multiple breast cancer primaries or both breast and ovarian cancer were significantly more likely to be carriers of *BRCA1* and *BRCA2* mutations (Table 1). Seventy-five percent (10/15) of probands diagnosed with both breast and ovarian cancer were found to have a mutation (criterion 2). Of probands diagnosed with bilateral breast cancer or multiple breast primaries (criterion 3), 30.7% (23/75) were found to be mutation carriers. Our study found a strong association between multiple cancer diagnoses (criteria 2 and 3) and mutation status, especially when probands were selected for family history. In an Ontario population-based study, only 19.4% of probands with breast and ovarian cancer or multiple breast primaries were found to be mutation carriers[22]. However, one striking difference between our study and that of Ontario was the age of diagnosis. Our study required that the first tumour be diagnosed by age 50 whereas the Ontario study alluded to diagnosis before 55 years of age.

As demonstrated by multiple studies, individuals with a strong family history of breast and/or ovarian cancer are more likely to be carriers for a *BRCA1* or *BRCA2* mutation than individuals with a negative or weak family history[5, 9, 24, 25]. This proved to be true in this study as probands diagnosed with breast cancer under the age of 50 years with more than 3 first or second-degree relatives with breast cancer or ovarian cancer (criterion 9) had a statistically significant greater risk of being *BRCA1* or *BRCA2* mutation carriers as compared to other criteria.

Probands diagnosed with ovarian cancer at any age with at least 1 first- or second-degree relative diagnosed with breast cancer at the age of 50 years or younger (criterion 13) were identified as mutation carriers in 41% (16/39) of cases. In the United States, 49.7% of women

with the same characteristics were mutation positive[9]. In addition, when criteria 1, 2, 3, and 11 were subdivided to compare mutation frequency in patients without and with family histories of breast or ovarian cancer, the presence of cancer in primary or second-degree relatives was associated with an increased mutation frequency of at least 10%.

It is possible to now counsel those probands with multiple tumor diagnoses and strong family histories of breast and/or ovarian cancer (criteria 2b, 3b, 9, and 13) that they are at an even higher risk of carrying a *BRCA1* or *BRCA2* mutation than those probands meeting other criteria prior to genetic testing. While our results show that family history is a significant factor to consider, this feature cannot be relied upon absolutely. Probands may lack a strong family history due to unrelated issues such as estrangement, adoption, small family size, segregation of mutation through predominately male relations, or family deaths at a young age from other causes. Therefore, basing eligibility criteria strongly on family history will exclude many probands, including those that are mutation positive, from testing.

Characteristics other than cancer history have been shown to increase the likelihood that an individual carries a *BRCA1* or *BRCA2* mutation. Recent evidence shows that *BRCA1*-related tumors are often triple-negative: these tumors are more likely than sporadic tumors to be estrogen- and progesterone-receptor negative and are less likely to overexpress Her2/neu[26]. Estimations of the frequency of *BRCA1* and *BRCA2* mutations in triple-negative breast cancers range from 10% to 70%[27]. In our study there were only 9 probands with pathology reports confirming triple-negative breast cancer. Of these probands, 4 (44.4%) were mutation carriers. It is important to note that testing for all three features was not routinely performed locally until recently. Given the limited sample size in our study, it is not possible to evaluate the statistical significance of the triple negative phenotype in relation to *BRCA1* or *BRCA2* carrier status. However, Kwon et al. concluded that testing individuals with triple-negative breast cancer was a cost-effective way to identify mutation carriers, even without family history. Our preliminary findings support adding this feature as a new criterion where probands with triple-negative breast cancer diagnosed by 50 years of age qualify for full gene testing[28].

Estimates of the frequency of *BRCA1* or *BRCA2* mutations in women diagnosed with breast cancer under the age of 35 years range from 9.4%[25] to 13.2%[22] in population-based North American studies. Our results demonstrate that 21.3% (29/136) of probands with breast cancer diagnosed under the age of 35 years have a *BRCA1* or *BRCA2* mutation (criterion 1). As expected, when this criterion was subdivided for the absence or presence of a primary or second-degree relative with breast or ovarian cancer, the mutation frequency was notably different 13.2% to 26.5%, respectively.

The rarity of male breast cancer results in small sample sizes and wide confidence intervals in many studies attempting to examine the *BRCA1* and *BRCA2* mutation frequency in the male breast cancer population. It has been estimated that *BRCA2* mutations account for 4-38% of male breast cancer cases[29]. In our study, *BRCA2* mutations were detected in four of sixteen male probands with a personal history of breast cancer (criterion 4). For probands with a personal history of breast cancer diagnosed under the age of 50 years with a case of male breast cancer in a relative (criterion 7), 25% (2/8) were *BRCA2* mutation carriers. No *BRCA1* mutations were identified in probands meeting criteria 4 and 7 in our study. These results are consistent with other Canadian studies: in a hospital-based study from the Maritimes, 25% of male probands

and probands with male relatives diagnosed with breast cancer had *BRCA1* or *BRCA2* mutations[30]. In a Toronto hospital, 14.3% (2/14) of males diagnosed with breast cancer were *BRCA2* mutation carriers[29]. While male breast cancer is rare, it appears that this criterion is, as expected, reaching the recommended 10% mutation detection rate.

The most commonly fulfilled criterion was met by probands who had been diagnosed with breast cancer under the age of 50 years with at least one relative also diagnosed with breast cancer under the age of 50 years (criterion 5). In the 203 probands who met this criterion, 47 (23.2%) were found to be *BRCA1* or *BRCA2* mutation carriers. Frank et al. found that 30.9% of women with identical personal and family history had a *BRCA1* or *BRCA2* mutation[9]. Of the probands diagnosed with breast cancer under the age of 50 years with at least one first or second-degree relative diagnosed with ovarian cancer, (criterion 6) 30% (15/50) were mutation carriers. These results are very similar to what was expected; 26.9% in the Maritime provinces[30] and 33.2% in a large American study[9].

Probands with ovarian cancer (criterion 10-12) had a *BRCA1* or *BRCA2* mutation identified between 18.2% and 41.7% of the time. It is known that over 90% of ovarian tumors in women with a *BRCA1* mutation are serous, compared to approximately 50% in women without[31]. In our study, 34.8% (8/23) of mutation-positive probands were diagnosed with confirmed serous ovarian cancer (criterion 10). However, not all of the probands with ovarian cancer in this study had pathology reports available and therefore it is difficult to knowingly compare the effects of this tumor type in our population. Interestingly, the presence of positive family history had minimal effect on mutation frequency for criterion 10. Probands with ovarian cancer diagnosed under the age of 60 years (criterion 11) had *BRCA1* or *BRCA2* mutations in 27.7% (18/65) of cases, similar to other North American studies with mutation frequencies between 32.4% and 38.1%[5, 22] in women with ovarian cancer diagnosed under the age of 50 years. When this criterion was examined with respect to the presence or absence of family history, it was found that mutation frequency increased from 18.18% to 29.63% when breast and ovarian cancer were seen in a primary or second-degree relative. For those probands diagnosed with ovarian cancer at any age with at least 1 first- or second degree relative with ovarian cancer (criterion 12), the mutation frequency was 41.7% (10/24). These results are consistent with those reported by other studies. Frank et al. found that 44.1% of nearly 10,000 women studied in the United States[22] with the same personal and family histories were mutation carriers. A more recent study on the frequency of *BRCA1* and *BRCA2* mutations among ovarian cancer patients unselected for family history found mutations in 14.3% of patients with non-mucinous cancer. Based on these findings, we should consider expanding criterion 10 so that all patients with non-mucinous ovarian cancer qualify for full gene screening[6].

In summary, it is evident that the Manitoba HBOC Clinic should continue to use these criteria as a means of classifying patients who are at an increased risk of carrying a *BRCA1* or *BRCA2* mutation. Some criteria, specifically those associated with multiple tumor diagnoses and strong family histories are more likely to identify probands with a mutation. While no criteria should be eliminated, it may be beneficial to consider the findings of this study and of others to ensure most *BRCA1* and *BRCA2* carriers are identified by including broader personal cancer history criteria. As full gene sequence analysis becomes more rapid and less costly, it will be possible to broaden our testing criteria to make them more inclusive.

One of the challenges of molecular analysis for *BRCA1* and *BRCA2* is the lack of mutation hotspots. As expected, mutations were dispersed throughout both genes, with no hotspots identified. *BRCA1* and *BRCA2* mutations represented 58.2% and 41.8%, respectively, of the disease-causing mutations detected in probands tested at the HBOC Clinic with a predominance of point mutations. Other Canadian studies have described similar results. Of the 65 mutations detected in a study from the Maritime provinces[30], *BRCA1* and *BRCA2* accounted for 68% and 32%, respectively. In an Ontario study, 17 *BRCA1* mutations and 14 *BRCA2* mutations were detected, accounting for 55% and 45%, respectively[22].

The difference in methodologies used in similar studies may also be leading to variations in the mutation detection rates. In this Manitoba study, it is expected that the combination of PTT and dHPLC would detect at least 95% of point mutations. Furthermore the consistent use of MLPA allowed for large genomic rearrangements to be identified, which are known to account for approximately 0-35% of *BRCA1* mutations[33]. As the overall mutation detection rate is much greater than the expected 10%, and greater than or similar to those reported in other Canadian studies, it is highly suggestive that the molecular approach used in this study is very comparable.

Mutations in *BRCA1* have been more strongly associated with ovarian cancer than *BRCA2* mutations[5, 6]. Zhang et al. found that 8.1% (107/1342) of Ontario patients with ovarian cancer had *BRCA1* mutations, while 4.1% of patients had *BRCA2* mutations. The frequency of *BRCA1* and *BRCA2* mutations increased to 11.6% and 4.8% respectively when the sample was limited to patients diagnosed under the age of 60 years[6]. As expected, our study also demonstrated a prevalence of *BRCA1* mutations in ovarian cancer patients. In Manitoba 28.7% (48/167) of patients diagnosed with ovarian cancer had *BRCA1* mutations, compared with 9% (15/167) who had *BRCA2* mutations. Only 2 probands had mutations in the *BRCA2* ovarian cancer cluster region[6]. As probands in our study had to meet strict personal and/or family history criteria to be offered full gene testing, it is expected that the overall frequency of mutations would be higher compared to studies with more inclusive criteria.

Perhaps the wide range of reported mutation frequencies in the literature can be at least partially explained by the fact that *BRCA1* and *BRCA2* mutation frequencies are influenced by the ethnic composition of the study population[6]. To account for this, studies based on North American populations were primarily used where possible as comparisons against our study. However, the Manitoban population does differ from that of Canada (Figure 1). A greater percentage of the Manitoban population, as compared to the Canadian population, is composed of ethnicities that have previously been reported to have founder mutations, namely Icelandic, Eastern European and Aboriginal. These three ethnic groups account for 2.7%, 23.4% and 16.5%, respectively, of the Manitoba population as compared to <1%, 9%, and 5% for the Canadian population.

Currently, Manitoba offers ethnic-specific screening where individuals of Icelandic, Ashkenazi Jewish, Aboriginal and more recently Eastern European descent are directly tested for the limited number of corresponding founder mutations. Individuals who screen negative for these founder mutations can only go on to a full screen if they met one of the 14 criteria and consented to such testing. However, it was recognized as this study come to an end that individuals who tested positive for a founder mutation may have been those who would have qualified for a full screen. A quick review of the 37 patients who tested positive for one of these

seven founder mutations over the past decade identified a total of 13 probands with 4 Aboriginal, 4 Icelandic, 2 Eastern European (*BRCA1* c.181T>G and *BRCA1* c.5266dupC) and 3 Ashkenazi Jewish individuals (*BRCA1* c.68_69delAG (2x) and *BRCA1* c.5266dupC) that would have met full gene testing criteria.

It is well-known that the Ashkenazi Jewish population has one of the highest prevalence of *BRCA1* and *BRCA2* mutations and hence it is not surprising to note that the study population contains a greater proportion of probands who are of Ashkenazi Jewish descent. In probands reported to be of Aboriginal descent, 42.9% (18/42) were found to be mutation carriers. Only eight of the mutations were the founder mutation *BRCA1* 1387_1390delAAAAinsGAAAG. The high frequency of mutations in probands who identify as Aboriginal may have contributed to the high frequency of mutations in this study. Of concern, the proportion of Aboriginal probands being screened is less than what is estimated based on the Manitoban population. In many cases, these probands have striking personal and family cancer histories. We can speculate that this might be due to access restrictions, as many Aboriginal communities are located in remote and rural centers where access to health care is limited and perhaps only those families with very strong personal and family histories of cancer are presenting for genetic testing. Other Canadian studies have noted high frequencies in certain ethnic groups. For example, Zhang et al. commented that the Italian population had a mutation detection frequency of 43.5%[6].

Having ethnic specific tests available allows for targeted analysis making genetic testing more readily available. All of the founder mutations currently offered have been identified in multiple probands, with the exception of one Eastern European *BRCA1* mutation, c.4035delA which to date has not been yet identified in the Manitoba population. There were eight mutations found in three or more of the 92 probands that had a DCM identified after full gene analysis (Table 3). Two of these mutations were known founder mutations and the ethnicities of the probands carrying these mutations corresponded accordingly. Two of the recurrent DCMs are reported to be founder mutations in the French Canadian population, but in our study only one third of the patients found to have these mutations reported any French Canadian background. The remaining three are not known to be founder mutations. One striking feature noted was that the predicted inframe deletion, *BRCA1* c. 4186-?_5193+?del, was identified in three probands, all with Ukrainian ancestry. Ticha et al. recently identified this in-frame deletion of exons 13-19 in a Czech study[32]. Further research is needed to elucidate the nature of its ethnic association in the Manitoba Ukrainian population.

Based on results from this study as well as those 13 probands who were found to be positive for ethnic-specific mutations, it is worth considering some adjustments to the ethnic-specific panel, namely dropping the *BRCA1* c.4035delA as part of the Eastern European panel and adding an ethnic-specific testing option, *BRCA2* c.5238dupT, for Mennonite individuals.

Conclusion

In this clinic-based retrospective chart review of Manitoba's HBOC Clinic, we examined the ethnic and breast and ovarian cancer history characteristics for association with *BRCA1* and *BRCA2* mutations in 429 probands. Consistent with other studies, multiple tumor diagnoses and a strong family history of breast and/or ovarian cancer were the characteristics most strongly associated with the finding of *BRCA1* and *BRCA2* mutations. Most of the recurrent mutations found in this study reflected the ethnic composition of the Manitoba population. All of the criteria

examined in this study are achieving a 10% mutation detection rate, but some reevaluation is recommended to explore broadening criteria to include ethnic-specific screening for the Mennonite founder mutation, inclusion of triple-negative breast cancers, as well as all non-mucinous ovarian cancers, based on current literature and the findings of this study.

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Table 1: Eligibility criteria used in Manitoba to determine which patients receive full gene testing for BRCA1 and BRCA2 mutations based on personal and family history of breast and ovarian cancer. Criteria 1, 2, 3, 10, 11 are split into a (no family history of breast or ovarian cancer) and b (positive family history where at least one first or second degree relative diagnosed with breast or ovarian cancer at any age). Results of statistical analysis, namely relative risk from univariate analysis and odds ratio from multivariate analysis are shown along with corresponding 95% confidence intervals.

Criterion number and description		**Probands meeting criterion**	**BRCA1** mutation No. (%)	**BRCA2** mutation No. (%)	**Total** mutation No. (%)	**Odds ratio (95% CI)**
1a	Breast cancer at age ≤35	53	5 (9.4)	2 (3.8)	7 (13.2)	0.82 (0.33-2.05)
1b	Breast cancer at age ≤35	83	15 (18.1)	7 (8.4)	22 (26.5)	1.47 (0.81-2.67)
2a	Breast cancer and ovarian cancer with the first tumor occurring at age ≤50	5	3 (60.0)	0 (0)	3 (60.0)	N/A
2b	Breast cancer and ovarian cancer with the first tumor occurring at age ≤50	10	6 (60.0)	1 (10.0)	7 (70.0)[1]	7.33 (1.74-30.84)[5]
3a	Bilateral breast cancer or multiple breast primaries with the first diagnosis at age ≤50	21	0 (0)	3 (14.3)	3 (14.3)	0.94 (0.26-3.43)
3b	Bilateral breast cancer or multiple breast primaries with the first diagnosis at age ≤50	54	12 (22.2)	8 (14.8)	20 (37.0)[2]	2.47 (1.28-4.77)[6]
4	Male proband diagnosed with breast cancer at any age	16	0 (0)	4 (25.0)	4 (25.0)	1.76 (0.53-5.85)
5	Breast cancer at age ≤50 with ≥1 first- or second-degree relative diagnosed with breast cancer at age ≤50	203	27 (13.3)	20 (9.9)	47 (23.2)	0.95 (0.56-1.60)
6	Breast cancer at age ≤50 with ≥1 first- or second-degree relative diagnosed with ovarian cancer at any age	50	11 (22.0)	4 (8)	15 (30.0)	1.15 (0.55-2.37)
7	Breast cancer at age ≤50 with a first- or second-degree male relative diagnosed with breast cancer at any age	8	0 (0)	2 (25.0)	2 (25.0)	N/A
8	Breast cancer at age ≤50 with ≥3 first- degree relatives with breast cancer diagnosed at any age	10	4 (40.0)	1 (10.0)	5 (50.0)	2.51 (0.63-10.13)
9	Breast cancer at age ≤60 with ≥3 first- or second-degree relatives with breast cancer diagnosed at age ≤50 or ovarian cancer diagnosed at any age	31	8 (25.8)	5 (16.1)	13 (41.9)[3]	2.49 (1.08-5.74)[7]
10a	Ovarian cancer at any age with pathology confirming serous type	6	2 (33.3)	0 (0)	2 (33.3)	N/A
10b	Ovarian cancer at any age with pathology confirming serous type	17	4 (23.5)	2 (11.8)	6 (35.3)	1.63 (0.47-5.63)
11a	Ovarian cancer at age ≤60	11	2 (18.2)	0 (0)	2 (18.2)	0.92 (0.19-4.33)
11b	Ovarian cancer at age ≤60	54	11 (20.4)	5 (9.3)	16 (29.6)	0.56 (0.20-1.58)
12	Ovarian cancer at any age with ≥1 first- or second-degree relative diagnosed with ovarian cancer at any age	24	9 (37.5)	1(4.2)	10 (41.7)	2.12 (0.75-6.00)
13	Ovarian cancer at any age with ≥1 first- or second-degree relative diagnosed with breast cancer at age ≤50	39	11 (28.2)	5 (12.8)	16 (41.0)[4]	3.06 (1.16-8.09)[8]
14	Ovarian cancer at any age with a first- or second-degree male relative diagnosed with breast cancer at any age	1	0 (0)	1 (100)	1 (100)	N/A

[1-4] **Significant results of univariate analysis** – relative risk with 95% confidence intervals: 2.66 (1.03-6.86)[1], 1.28 (1.04-1.58)[2], 1.38 (1.02-1.87)[3], 1.37 (1.05-1.78)[4]. [5-8] **Significant results of multivariate analysis** – p-values are all <0.05 with p=0.01[5], 0.01[6], 0.03[7], 0.02[8]

Combined results (a and b for each criterion) and corresponding multivariate analysis – 1a and 1b: 21.32% mutation, p>0.05 • 2a and 2b: 66.67% mutation, p<0.05 • 3a and 3b: 30.67% mutation, p<0.05 • 10a and 10b: 34.78% mutation, p>0.05 • 11a and 11b: 27.69% mutation, p>0.05

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Table 2: List of BRCA1 and BRCA2 disease-causing mutations identified in 92 unrelated probands in this study

Exon	Sequence Change (c. or HGVS)	Predicted Amino Acid Change	No. Times Observed	Previously Reported
BRCA1				
2	1A>G	p.Met1?	1	Yes
5	181T>G[1]	p.Cys61Gly	2	Yes
Intron 5	212+3A>G	IVS5+3A>G[Sp]	1	Yes*
6	269_281delTTTGTGCTTTTCA	p.Ile90ThrfsX25	1	Yes
7	427G>T	p.Glu143X	1	Yes
11	1016dupA	p.Val340GlyfsX6	1	Yes
11	1252dupG	p.Glu418GlyfsX4	1	No
11	1387_1390delAAAAinsGAAAG[2]	p.Lys463GlufsX17	4	Yes
11	1510delC	p.Arg504ValfsX28	1	Yes
11	1687C>T	p.Gln563X	3	Yes
11	1953_1956delGAAA	p.Lys653SerfsX47	1	Yes
11	2035A>T	p.Lys679X	1	Yes
11	2059C>T	p.Gln687X	1	Yes
11	2389G>T	p.Glu797X	2	Yes
11	2908A>T	p.Lys970X	1	No
11	3254_3255dupGA	p.Leu1086AspfsX2	1	Yes
11	3331_3334delCAAG	p.Gln1111AsnfsX5	1	Yes
11	3607C>T	p.Arg1203X	1	Yes
11	3756_3759delGTCT[5]	p.Ser1253ArgfsX10	1	Yes
11	4065_4068delTCAA	p.Asn1355LysfsX10	1	Yes
13	4327C>T[5]	p.Arg1443X	1	Yes
16	4689C>G	p.Tyr1563X	4	Yes
17	5030_5033delCTAA	p.Thr1677IlefsX2	1	Yes
17	5074G>A	p.Asp1692Asn[Sp]	2	Yes
18	5080G>T	p.Glu1694X	1	Yes
18	5096G>A	p.Arg1699Trp	1	Yes
20	5251C>T	p.Arg1751X	1	Yes
20	5266dupC[1, 4]	p.Gln1756ProfsX74	5	Yes
Intron 20	5278-2A>G	IVS20-2A>G[Sp]	1	Yes
22	5346G>A	p.Trp1782X	2	Yes
24	5503C>T	p.Arg1835X	1	Yes
1A-B, 2	-200-?_80+?del	p.0?	1	Yes
13	4186-?_4357+?dup	p.Ala1453fs?	1	Yes
13-19	4186-?_5193+?del	p.Gln1396_Glu1731del?	3	No
21-24	5278-?_5592+?del	p.Ile1760fs?	1	Yes
BRCA2				
Intron 2	67+1G>A	IVS2+1G>A[Sp]	1	Yes
Intron 7	631+2T>G	IVS7+2T>G[Sp]	1	Yes
8	658_559delGT	p.Val220IlefsX4	1	Yes
9	755_758delACAG	p.Asp252ValfsX24	1	Yes
9	756_757delCA	p.Asp252GlufsX2	1	Yes
9	774_775delAA	p.Glu260SerfsX18	1	Yes
11	3170_3174delAGAAA[5]	p.Lys1057ThrfsX8	3	Yes
11	3199_delA	p.Thr1067LeufsX10	1	Yes
11	3599_3600delGT	p.Cys1200X	1	Yes

11	4780delA	p.Met1594CysfsX23	1	Yes
11	4936_4939delGAAA	p.Glu1646GlnfsX2	1	Yes
11	4965C>A	p.Tyr1655X	1	Yes
11	5238dupT[3]	p.Asn1747X	3	Yes
11	5621_5624delTTAA	p.Ile1874ArgfsX23	1	Yes
11	5682C>G	p.Tyr1894X	1	Yes
11	5720_5723delCTCT	p.Ser1907X	1	No
11	5857G>T[5]	p.Glu1953X	2	Yes
11	5864C>A	p.Ser1955X	2	Yes
11	5946delT[4]	p.Ser1982ArgfsX22	1	Yes
11	6275_6276delTT	p.Leu2092ProfsX7	1	Yes
11	6491_6494delAGTT	p.Gln2164ArgfsX3	1	Yes
13	7007G>A	p.Arg2336His[Sp]	2	Yes
14	7069_7070delCT	p.Leu2357ValfsX2	1	Yes
15	7558C>T	p.Arg2520X	1	Yes
18	7988A>T	p.Glu2663Val[Sp]	2	Yes*
18	8024_8025delTA	p.Ile2675AsnfsX5	1	No
20	8537_8538delAG[5]	p.Glu2846GlyfsX22	3	Yes
23	9027delT	p.His1030IlefsX18	1	Yes

*Mutation listed in the widely used online BRCA1 and BRCA2 mutation database, the BIC or Breast Cancer Information Core (http://research.hhgri.nih.gov/bic/) as an UCV. It is well known amongst the hereditary breast/ovarian cancer scientific and medical community that there is a significant delay in BIC with re-classification of VUS as either disease-causing or benign polymorphism as new scientific and/or medical evidence is published. Therefore, all VUS were further explored using published literature and only when there was overwhelming scientific data from more than one source, the VUS was reclassified by the molecular geneticist as a disease-causing mutation.

[Sp] Mutation predicted to interfere with proper splicing

Founder mutations for individuals with the following origins: [1]Eastern European, [2]Aboriginal, [3]Mennonite, [4]Ashkenazi Jewish, [5]French Canadian population in Quebec

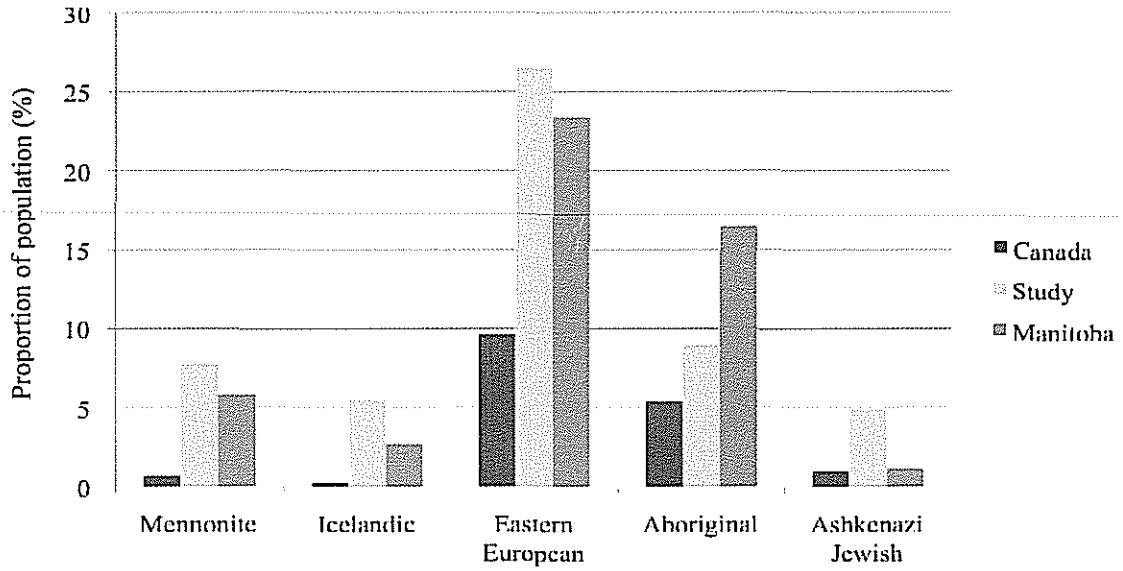

Figure 1: Proportion of Canadian and Manitoban population reporting to belong to selected ethnic groups (in percentage of total population). Data was derived from the 2006 Census (Statistics Canada http://www40.statcan.ca/l01/cst01/demo26h-eng.htm) and included both single and multiple responses. Also shown are the relative percentage of probands in this study, who reported to have descendants belonging to these ethnic groups.

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Table 3: Recurrent disease-causing mutations and proband ethnicities

No. of times observed	Exon	Sequence Change (c. or HGVS)	Predicted Amino Acid Change	Ethnicity of probands (maternal:paternal)	Known founder mutation?
BRCA1					
4	11	1387_1390delAAAAinsGAAAG	p.Lys463GlufsX17	- Aboriginal - Aboriginal - Unknown - Aboriginal/ Metis:Polish	Yes: Aboriginal
3	11	1687C>T	p.Gln563X	- Croatian - Finnish/Austrian: Russian - Polish/German-English	No
3	13-19	4186-?_5193+?del	p.Gln1396_Glu1731del?	- Ukranian/English/ Ukranian - Ukranian - Ukranian/Polish: Icelandic	No
4	16	4689C>G	p.Tyr1563X	- Polish:Russian - German - German:Russian - German:Hungarian	No
5	20	5266dupC	p.Gln1756ProfsX74	- Swedish/ German/Dutch:Irish - Hungarian - Ukranian:Polish/ Welsh - German - German:Scottish/ French Canadian	Yes: Ashkenazi Jewish and Eastern European
BRCA2					
3	11	3170_3174delAGAAA	p.Lys1057ThrfsX8	- English:Scottish - French Canadian: Icelandic - Aboriginal	Yes: French Canadian
3	11	5238dupT	p.Asn1747X	- German/Mennonite - German/Mennonite - Dutch Mennonite	Yes: Mennonite
3	20	8537_8538delAG	p.Glu2846GlyfsX22	- French Canadian - Unknown - Czech/German	Yes: French Canadian