No Evidence of BRCA1/2 Genomic Rearrangements in High-Risk French-Canadian Breast/Ovarian Cancer Families

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ABSTRACT

The discovery of deleterious mutations in the breast and ovarian cancer susceptibility genes, BRCA1 and BRCA2, has facilitated the identification of individuals at particularly high risk of these diseases. There is a wide variation between populations in the prevalence and related risks of various types of BRCA1/2 mutations, so estimates cannot be extrapolated to Canadians, especially not founder populations such as French-Canadians. Polymerase chain reaction (PCR)-based methods were used to detect the majority of these mutations. These approaches usually failed to detect large DNA rearrangements, which have been claimed to be involved in other populations in 5% to up to 36% of BRCA1-positive families. There is very little information about the contribution of this type of mutation in BRCA2-positive families. To investigate if our available mutation spectrum of BRCA1 and BRCA2 in high-risk French-Canadian breast/ovarian cancer families has been biased by PCR-based direct sequencing methods, we first used Southern blot analysis to test DNA samples from 61 affected/obligate carrier individuals from 58 families in which no BRCA1/2 deleterious mutation was found. Finally, 154 individuals from 135 BRCA1/2 nonconclusive families, including all those tested previously by Southern blot analysis, were tested with the new multiplex ligation probe amplification (MLPA) technique. These approaches failed to detect any rearrangement. Moreover, if the frequency of MLPA-detectable rearrangements in our cohort of 135 BRCA1/2 nonconclusive families was 2.2% or higher, we would have had a 95% or greater chance of observing at least one such rearrangement. As no rearrangements were identified, such large rearrangements must be quite rare in our population.

INTRODUCTION

In many developed countries, breast cancer is the most common malignancy among women with nearly 1,000,000 new cases per year (Key et al. 2001; Lacey et al. 2002; Pisani et al. 1999). In the race to decrease breast cancer mortality, early detection and improved treatment have emerged as the most critical strategies. The potential for early detection using genetic indicators is crucial, because positive family history is second only to age as the strongest epidemiologic risk factor for breast cancer. Twin studies indicate that inherited susceptibility, rather than lifestyle or environmental factors, is responsible for most...
familial aggregation (Lichtenstein et al. 2000; Peto 2001). In part, this is explained by specific familial cancer syndromes in which variants of single genes confer a high risk of disease. BRCA1 (MIM 113705) and BRCA2 (MIM 600185) are the two major known genes that cause a genetic susceptibility to breast and ovarian cancer (Miki et al. 1994; Tavtigian et al. 1996; Wooster et al. 1995).

There are wide variations between populations in the prevalence and related risks of BRCA1/2 mutations so estimates cannot be extrapolated to Canadians, especially to the founder French-Canadian population, which possesses unique genealogical characteristics. Furthermore, the identification of founder mutations in specific populations has been shown to dramatically reduce the cost and technical limitations of testing. One of the main objectives of the Interdisciplinary Health Research International Team on Breast Cancer susceptibility (INHERIT BRCAs) research program was to estimate the prevalence of deleterious BRCA1 and BRCA2 mutations in this founder population in order to improve predictive genetic testing utility and genetic counseling to affected as well as asymptomatic individuals (Simard et al. 2004, 2005).

In other populations, several large genomic rearrangements were detected in BRCA1, which have frequently been attributed to unequal homologous recombination within Alu repeats (for a review see Mazoyer 2005); in contrast, only 8 rearrangements have been reported in BRCA2. For instance, the BRCA1 gene contains 129 Alu elements, which is equivalent to 41.5% of its sequence (Puilick et al. 2004; Smith et al. 1996). This relatively high density of Alu repeats appears to be the main source of large genomic rearrangements identified so far, involving at least 26 different high-risk Alu elements that have been remarkably stable throughout the hominoid primates (Puilick et al. 2004). The presence of the BRCA1 pseudogene upstream of BRCA1, in which exons 1a, 1b, and 2 are duplicated, represents an additional source of rearrangements caused by unequal homologous recombination (Puget et al. 2002). The proportion of all pathogenic BRCA1/2 mutations arising from genomic rearrangements has been estimated to be approximately 10% to 15%, but founder effects have been reported in some populations, thus resulting in higher frequencies of such mutations (Gad et al. 2002; Petrij-Bosch et al. 1997).

To assess the importance of BRCA1 and BRCA2 alterations that could be missed by standard polymerase chain reaction (PCR)-based screening techniques, we looked for germline rearrangements in high-risk French-Canadian breast and/or ovarian cancer families. We searched for rearrangements using both Southern blot and multiplex ligation-dependent probe amplification (MLPA) methods.

MATERIALS AND METHODS

Ascertainment of families

The recruitment of high-risk French-Canadian breast and/or ovarian families started in 1996 through a research project, which thereafter evolved into a large ongoing interdisciplinary research program designated INHERIT BRCAs (Avard et al. 2005; Simard et al. 2005; J. Simard et al., unpublished data, 2006). A major component was to identify and characterize BRCA1 and BRCA2 mutations in the French-Canadian population and to determine the role of the demographic history in their origin and diffusion. This integrated clinical research program was composed of a network of referring clinicians across the province of Québec. After pretest education sessions and detailed analysis of familial history, a total of 251 (counted as 256 in total because 5 families had a history of cancer on both sides that met the criteria of eligibility mentioned below) high-risk French-Canadian breast/ovarian cancer families were recruited according to strict criteria: (1) families with at least four individuals with breast and/or ovarian cancer diagnosed in first- or second-degree relatives; (2) families presenting three individuals with breast and/or ovarian cancer in first-degree relatives; and (3) families bearing a mutation already identified in the BRCA1 or BRCA2 gene. These families have been classified in two groups. A subset of 191 families (subset A) for which at least one DNA sample from a woman affected with breast and/or ovarian cancer was available for genetic testing and a subset consisting of 65 families (subset B), for which only asymptomatic individuals were available. All participants had to be at least 18 years of age and mentally capable. In most instances, the diagnoses of breast and/or ovarian cancer were confirmed by obtaining a pathology report. clinicians involved in this research program were responsible for BRCA1/2 test result disclosure to participants. Approval was obtained from eight ethics committees corresponding to the different institutions participating in the research program.

Prior mutation testing by partial and full-length sequencing

Participants entering the study were first tested for a panel of mutations known to occur in the French-Canadian population. At the present time DNA samples from participants have been tested for a panel of 29 mutations including 26 truncating mutations and 3 unclassified variants. This first step approach led to the discovery of deleterious mutations in 54 different families (Simard et al. 2004; J. Simard et al., unpublished data, 2006). For each family that received a BRCA1/2 nonconclusive result (i.e., no mutation was found by screening for the panel of mutations analyzed in the first step), at least one DNA sample from a woman affected with breast and/or ovarian cancer (with the exception of four DNA samples from an unaffected parent from whom the familial breast/ovarian cancer history derived) was sent to Myriad Genetic Laboratories, Salt Lake City, Utah, for full-length BRCA1/BRCA2 sequencing following their Comprehensive BRACAnalysis®-BRCA1 and BRCA2 gene sequence analysis for susceptibility to breast and ovarian cancer test. Testing services were performed according to the Memorandum of Understanding (MOU) with the National Cancer Institute (NCI) for NCI-funded research testing services for BRCA1 and BRCA2 (project no. NCI 173). The second step screening approach led to the discovery of eight novel mutations found in eight different families (Simard et al. 2004). For each individual belonging to a BRCA1/2-positive family, a confirmation test has been performed on a second blood sample by the Molecular Diagnostic Laboratory, Alberta Children’s Hospital, Calgary. More details concerning the results obtained in this cohort will be described elsewhere (J. Simard et al., unpublished data, 2006). From subset A, including 191 families
in which at least one DNA sample was available from an affected woman, 135 families remained $BRCA1/2$ nonconclusive after the two-step analyses described above, whereas 59 were $BRCA1/2$ nonconclusive in the subset B including 65 families in which only asymptomatic individuals were available for testing (Simard et al. 2004; J. Simard et al., unpublished data, 2006). All DNA samples analyzed in the present study belong to subset A. The breast and ovarian cancer history of the 135 $BRCA1/2$ nonconclusive families are described in Table 1.

DNA and RNA samples

After obtaining signed informed consent from each participant, 40 ml of blood was drawn and genomic DNA was extracted using the guanidine hydrochloride-protease K method and the QIAmp Maxi blood kit following the manufacturer’s instructions (QIAGEN, Mississauga, Ontario, Canada). Epstein-Barr virus (EBV)-transformed B-lymphoblastoid cell lines were established. DNA from lymphoblastoid cell lines was extracted using the cetyltrimethylammonium bromide (CTAB) protocol (Del Sal et al. 1989). RNA was extracted using TRIzol (Invitrogen, Gibco BRL, Carlsbad, CA) according to the manufacturer’s instructions and reverse transcription of RNA was performed using 10 $\mu$g of total RNA with SuperScript™ II Reverse Transcriptase and an oligo dT12–18 primer (Invitrogen) as directed by the supplier’s protocol. PCR was done using primers (available on request) to verify the presence of small exons 3 and 20 for $BRCA1$ and 2–6, 8, 12, 13, 17, 21, and 26 for $BRCA2$.

Southern blot analysis

We first performed Southern blot analysis with genomic DNA from lymphoblastoid cell lines of 61 individuals belonging to 58 $BRCA1/2$ nonconclusive families as well as 30 individuals from 28 $BRCA1/2$-positive families as control. Digestions were performed with 15 $\mu$g of genomic DNA from lymphoblastoid cell lines overnight with approximately 100 units of AvaI, PstI and TaqI for $BRCA1$ and AvaI, HindIII, and TaqI for $BRCA2$. Following a standard NaOAc precipitation, digestions were run overnight on a 0.8% agarose gel at 45V in 1× tris-borate EDTA buffer (TBE). Gels were partially depurinated in 0.25N HCl for 15 min and denatured in 0.4N NaOH/0.6N NaCl for 30 min with gentle shaking. DNA was transferred to a GeneScreen Plus nylon membrane (NEB, Boston, MA) overnight in the 10× SSC alkaline buffer. The blots were prehybridized at 42°C for 2 hr with 50% formamide, 2× SSC, 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate, and 5× Denhardt’s solution. Thereafter, denatured salmon sperm DNA (0.2 mg/ml) was included with 1× 10$^6$ cpm/ml of a 32P-labeled cDNA probe in the solution for an overnight hybridization. The probes were obtained by digestion of plasmid DNA containing the entire $BRCA1$ or $BRCA2$ coding region (Figs. 1 and 2). Fifty nanograms of cDNA fragment corresponding to EcoRV-BamHI fragment covering $BRCA1$ exons 2–11 (nt 120–4058); BamHI-SacI fragment covering $BRCA1$ exons 11–24 (nt 4058–7140) according to U14680; NdeI fragments of $BRCA2$ covering exons 2–10 (nt 229–1796); exon 10–17 (nt 1796–8110); exon 17–27 (nt 8110–10,775) according to U43746, were labeled using Random Primed DNA Labeling kit following the supplier’s protocol (Roche Applied Science, Mannheim, Germany). After hybridization, the membrane was washed at room temperature for 10 min in 2× SSC, 30 min at 65°C in 2× SSC/0.1% SDS and 30 min at 65°C in 0.1× SSC/0.1% SDS. Filters were exposed for 24 h and scanned with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA), and thereafter exposed for 10 days to x-ray single emulsion film (Kodak BioMax MR Film, Rochester, NY) with an intensifying screen (REN) at −80°C and developed.

| TABLE 1. DISTRIBUTION OF THE 135 $BRCA1/2$ NONCONCLUSIVE FAMILIES FOLLOWING THEIR BREAST AND OVARIAN CANCER HISTORY AT 2ND, 3RD, OR 4TH DEGREE FROM THE INDEX CASE |
|----------------|----------------|----------------|----------------|----------------|
| Breast cancer cases (n) | Ovarian cancer cases (n) | Degree from the index case* |
|----------------|----------------|----------------|----------------|----------------|
| ≤2 | 0 | 11 | 3 | 1 |
| ≥1 | 11 | 9 | 7 |
| 3 | 0 | 29 | 19 | 17 |
| ≥1 | 5 | 4 | 5 |
| 4 | 0 | 35 | 21 | 17 |
| ≥1 | 4 | 6 | 7 |
| 5 | 0 | 23 | 28 | 28 |
| ≥1 | 3 | 6 | 6 |
| 6 | 0 | 6 | 13 | 18 |
| ≥1 | 1 | 2 | 4 |
| ≥7 | 0 | 6 | 18 | 19 |
| ≥1 | 1 | 5 | 7 |
| 135 | 135 | 135 |

*Number of breast and/or ovarian cancer cases at most 2nd, 3rd, or 4th degree from the index cases, which are the first recruited and tested individuals of a family.

MLPA analysis

We performed MLPA analysis with genomic DNA samples from 154 individuals belonging to the 135 $BRCA1/2$ nonconclusive families as well as 39 individuals who belong to 32 $BRCA1/2$-positive families as control, using the SALSA P002 human $BRCA1$ and P045 human $BRCA2$ test kits, respectively (MER-Holland, Amsterdam, The Netherlands). Moreover, we have used as positive controls for MLPA analysis, DNA from individual carrying rearrangements in $BRCA1$, namely, deletion of exons 1a–1b-2 (Puget et al. 2002), deletion of exon 17 (Puget et al. 1997), and duplication of exon 13 (Puget et al. 1999a), which were kindly provided by Dr. Olga Sinilnikova from Centre Léon Bérard, Lyon, France. One hundred nanograms of genomic DNA were diluted with 1× TE in 5 ul and were heated at 98°C for 5 min. After adding 1.5 $\mu$L salt solution (1.5 M KCl, 300 mM Tris-HCl pH 8.5, 1 mM EDTA), samples were mixed with 1.5 $\mu$L probe mix, heated for 1 min at 95°C, and then incubated for 16 hr at 60°C. Ligation of annealed primers was performed by adding 32 $\mu$L ligation mix and incubating for 15 min at 54°C. The ligase enzyme was inactivated by heating 5 min at 98°C. A 10-μl aliquot of the ligation reaction was mixed with 30 μl of PCR buffer. While the samples were at 60°C, a mix of primers X and Y (Fam labeled), dNTPs, and SALSA polymerase was added. PCR was performed for 33 cycles (30
sec at 95°C, 30 seconds at 60°C, and 1 min at 72°C). Samples were analyzed using a capillary electrophoresis system (ABI 3100 genetic analyzer; Applied Biosystems).

Sequencing of genomic DNA samples

Genomic DNA was extracted from blood samples obtained from individuals analyzed previously by the MLPA method, in order to confirm the presence of a silent polymorphism at codon 1436 (TCT) encoding Ser to TCC in exon 13 of BRCA1. A 503-bp fragment from BRCA1 was amplified using primers (available on request). Sequencing of this genomic region corresponding to exon 13 was performed with the BigDye® Terminator Cycle Sequencing Kit and analysed on a 3730 automated DNA sequencer (Applied Biosystems, Foster, CA).

RESULTS

The aim of this study was to estimate the prevalence of rearrangements in the BRCA1 and BRCA2 genes in BRCA1/2 non-

FIG. 1. Southern blot analyses of the BRCA1 gene. A: Restriction sites are indicated on the gene structure and BRCA1 probes used in the Southern blot analyses and sizes (in base pairs [bp]) of restriction fragments expected to be revealed by the probes are indicated in grey boxes. Open boxes represent the fragments not detectable using this Southern blot analysis strategy. B: Results from typical Southern blot analyses of the BRCA1 gene. Fifteen micrograms of genomic DNA was digested by three restriction enzymes for each probes and exposed for 10 days to x-ray single emulsion film with an intensifying screen at −80°C.
conclusive high-risk French-Canadian breast and/or ovarian cancer families. Initially, using Southern blotting, we analyzed genomic DNA samples isolated from lymphoblastoid cell lines from 91 affected individuals from 86 families, including 61 individuals from 58 families in which no \textit{BRCA1/2} deleterious mutation was detected by sequencing approach. The strategy used and typical results obtained from the Southern blot analyses of the \textit{BRCA1} (Fig. 1) and \textit{BRCA2} (Fig. 2) genes, as well as from reverse transcriptase-polymerase chain reaction (RT-PCR) analysis (Fig. 3) are shown in the figures.

In \textit{BRCA1}, a known polymorphism in exon 16, S1613G (Durocher et al. 1996), creating an \textit{AvaII} restriction site, is responsible for the restriction fragment length polymorphism (RFLP) observed in 65 chromosomes from 51 individuals (Fig. 4). Screening using cDNA-probes containing \textit{BRCA1} exons 11–24, led to the detection of two bands of 4.1 kb and 3.8 kb in all tested samples digested with \textit{AvaII} and \textit{BglII}, respectively. These fragments do not fit the predicted restriction map of \textit{BRCA1}, and may be explained by the high sequence homology between the 3' end of \textit{BRCA1} exon 24 and a 348-bp segment.
on chromosome 4q28.3 (Stec et al. 2001) (Fig. 5A and 5B). Moreover, supplementary bands of 7.3 kb and of 10 kb were observed after digestion with AvaI and BglII, respectively, in two individuals (one BRCA1 carrier and one from a BRCA1/2 nonconclusive family); these fragments are considered to result from the loss of these restriction sites on the homologous segment on chromosome 4q28 (see Fig. 5A and B).

We performed MLPA analysis for BRCA1 with DNA samples from 154 individuals belonging to the 135 BRCA1/2 nonconclusive families as well as 39 individuals who belong to 32 BRCA1/2-positive families as control. This analysis did not reveal any genomic rearrangements. Nevertheless, a reduction in relative copy number of exon 13 was observed (Fig. 6A) in several individuals. The presence of a previously reported silent polymorphism Ser1436Ser (TCT to TCC) (Durocher et al. 1996) located near the ligation-junction of the two probes of exon 13 was confirmed by direct sequence analysis in each samples in which this false positive from exon 13 was observed. It is of interest to note that currently, a new MLPA kit for BRCA1 is now available (#P087) and the probe mix contain two sets of probes for exon 13, where one of these is located upstream of this polymorphism in order to avoid such an artefact.

For BRCA2, Southern blot analysis revealed the presence of a frequent polymorphism abolishing an AvaII restriction site in intron 16 (the 6.1-kb band became a 7.5-kb band), which was observed in 28 heterozygous individuals (Fig. 7A). Another rare RFLP has been detected in one heterozygote by using BRCA2 exons 17–27 probe caused by the creation of a new TaqI site 2388 nucleotides following the stop codon that converts the 5.3-kb band into a 4.9-kb band (Fig. 7B). An AvaII RFLP was found with the same probe in three samples, as revealed by an additional 2.5-kb band (data not shown). This RFLP is most likely not pathogenic, because (1) no RFLP was found with TaqI and HindIII when using the same probe (2) no evidence of exon deletion/duplication was detected by MLPA in these individuals (Fig. 6E–6G), and (3) two of these samples were isolated from noncarrier individuals who belong to two different BRCA1-positive families, which were used as reference control group, thus supporting that the familial aggregation of cancer cases is not caused by this RFLP, but is...
rather explained by the presence of BRCA1 R1443X or 2080insA deleterious mutations.

An RFLP generating an additional 10 kb band observed with HindIII digestion using a probe covering BRCA2 exons 2–10 was detected in one affected individual from a BRCA1/2 non-conclusive family (data not shown). No RFLP was found with TaqI and AvaII. Extensive PCR-based analysis using a cDNA sample and combination of 11 different primers (19 amplicons) failed to detect deletion/duplication in the region covering exons 11–24. The first lane represents a homozygous individual; the second lane a heterozygous individual; the third an individual homozygous for the polymorphism.

**FIG. 4.** An AvaII restriction fragment length polymorphism (RFLP) observed with the BRCA1 cDNA probe covering exons 11 to 24 due to the known polymorphism S1613G. The additional 1.54 kb (●) band is generated because of the creation of AvaII restriction site in exon 16 of BRCA1. Genomic DNA, isolated from a lymphoblastoid cell line, was digested with AvaII and hybridized with a cDNA probe containing exons 11–24. The first lane represents a homozygous individual; the second lane a heterozygous individual; the third an individual homozygous for the polymorphism.

**FIG. 5.** The high sequence similarity between the 3′ end of BRCA1 and a segment on chromosome 4q28 is responsible for the additional AvaII/BglII restriction fragment length polymorphisms (RFLPs) observed using the BRCA1 exon 24 probe. Genomic DNA, isolated from a lymphoblastoid cell line, was digested with AvaII and BglII and hybridized with a cDNA probe covering exon 24. The aberrant 3.8 kb and 4.1 kb bands in all tested samples digested with AvaII and BglII did not fit the predicted restriction map of BRCA1 and may be explained by the high sequence similarity between the 3′ end of BRCA1 exon 24 and a 348-bp segment on chromosome 4q28. The supplementary bands (●) of 7.3 kb (A) and of 10 kb (B) observed after digestion with AvaII and BglII, are caused by the loss of these restriction sites on the homologous segment on chromosome 4q28 (GenBank reference sequence AF231509.1) and were confirmed by sequencing this genomic region (data not shown).

**DISCUSSION**

We have analyzed 154 individuals from all the 135 high-risk BRCA1/2 French-Canadian breast and/or ovarian cancer families for large deletions/duplications in BRCA1 and BRCA2 genes, in
which no mutations in these genes were found by a previous two-step PCR-based sequencing approach. Because no rearrangements were identified in either gene, such large rearrangements must be quite rare in the French-Canadian population.

The lack of large deletions in BRCA2 French-Canadian families is consistent with previous results from other populations, such as the Finnish and Dutch (Lahti-Domenici et al. 2001; Peelen et al. 2000). To our knowledge, only 11 BRCA2 rearrange-
ments have been reported: 9 deletions (Agata et al. 2005; Nordling et al. 1998; Tournier et al. 2004; Wang et al. 2001; Woodward et al. 2005), 1 duplication (Tournier et al. 2004) and an insertion of an Alu element into exon 22, which resulted in alternative splicing that skipped exon 22 (Miki et al. 1996). Two of these studies have analyzed families with at least one male breast cancer and found rearrangements in 7.7% and 12% (Tournier et al. 2004; Woodward et al. 2005). Because BRCA2 defects are more frequent in families with cases of male breast cancer, it may be useful to test for BRCA2 gene rearrangements in these families.

However, for BRCA1, inherited large genomic rearrangements appear to be relatively common. Indeed, more than 30 different BRCA1 rearrangements have been reported and 29 have mapped breakpoints (Mazoyer 2005). Two of them account for 36% of all BRCA1 mutations found in high-risk Dutch breast-cancer families (Petrij-Bosch et al. 1997) and genomic rearrangement represent 11.6%, 15%, and 40% of all BRCA1 deleterious mutations in a group of French, American, and Italian breast and ovarian cancer families, respectively (Gad et al. 2002; Montagna et al. 2003; Puget et al. 1999a, 1999b). It is important to note that although some studies have reported a high percentage of families with chromosomal rearrangements in BRCA1, this proportion should be taken with caution because some of these families were selected based on a positive linkage to the BRCA1 locus or by using a highly selective operational criteria (Montagna et al. 2003; Puget et al. 1999b).

One of the most well-known founder mutations is probably the BRCA1 exon 13 duplication (ins6kbEx13), distributed mainly in English-speaking countries with historical links to Britain (BRCA1 Exon 13 Duplication Screening Group 2000). This founder mutation was observed six times in Canadian English-speaking families and absent in 1749 families who were screened in non-English-speaking countries, including 45 from our French-Canadian cohort. All exon 13-duplication carriers share the same haplotype supporting the founder effect. A recent study characterized the prevalence of five specific rearrangement mutations in a large North American patient population (Hendrickson et al. 2005). The five mutations accounted

FIG. 7. Characterization of the AvaiI and the TaqI restriction fragment length polymorphisms (RFLPs) observed with the BRCA2 cDNA probe covering exons 10 to 17 and 17 to 27, respectively. A: cDNA probe covering exons 10 to 17 of BRCA2 showing a supplementary 7.5-kb band (★) caused by the loss of the AvaiI restriction site in intron 16 (IVS16-1750; G→A). The first lane represents a homozygous individual; the second lane a heterozygous individual; the third a homozygous individual for the polymorphism. B: An additional TaqI restriction site located 2388 nucleotides following the stop codon of BRCA2 modifies the length of the 5.3-kb band to a 4.9-kb (★) fragment. Only found in one heterozygous individual.

FIG. 8. Additional anomalous AvaiI and TaqI bands with BRCA2 cDNA probe covering exons 17 to 27 are only observed using a lymphoblastoid cell line. Lane 1 represents the digestion of genomic DNA extracted from fresh leukocytes compared to lane 2, which represents digestion of the DNA isolated from a lymphoblastoid cell line of the same individual. When digested with AvaiI, an additional band at 6.5Kb appears, while the TaqI digestion revealed an additional 2.5-kb band only in the lymphoblastoid cell line of the same individual.
for 4.34% of deleterious BRCA1 mutations identified in 20,712 hereditary breast/ovarian cancer patients undergoing genetic testing in North America.

New complementary and/or alternative strategies have been developed over past few years that allow detection of rearrangements missed by one or another techniques because of their respective limitations, including: quantitative multiplex PCR of short fluorescent fragments (QMPSF) (Casilli et al. 2002), DNA array-based method (Favis et al. 2000; Frolov et al. 2002), color bar code on combed DNA (Casilli et al. 2002), semiquantitative multiplex PCR (Hofmann et al. 2003), MLPA (Schouten et al. 2002), real-time PCR (Barrois et al. 2004), and single nucleotide polymorphism (SNP) haplotype analysis (Ward et al. 2005). Knowing limitations of Southern and MLPA analyses used in the present study, we cannot exclude the possibility that rearrangement(s) could have been missed in our tested population. For example, the false-negative results would involve small portions of exons because only short sequence stretches are targeted in each exon with MLPA and very small rearrangements might potentially be missed by Southern blot analysis if they occurred in a large restriction fragment. In order to avoid such an event, long-range PCR (LR-PCR) could be used to confirm the integrity of the genomic sequence as well as RT-PCR analysis to confirm the integrity of transcripts. Note that when using cDNA, it should be relevant to inhibit nonsense-mediated mRNA decay prior to RNA extraction.

In this study using French-Canadian families, no large deletion/duplication or other rearrangements were found in BRCA1 and BRCA2, thus suggesting that the frequency of such mutations is quite low in our population. This implies that the PCR-based sequencing approach for BRCA1/2 predictive testing in this population will be highly sensitive. Nevertheless, the possibility of occurrence of large deletions/duplications or other rearrangements in French-Canadian families from other regions in Canada should be kept in mind (Carson et al. 1999). Moreover, it should be mentioned that although index cases were referred from almost all regions of the province of Quebec, the majority of high risk families tested in the present study have been recruited from East of Quebec (Simard et al., unpublished data); thus the existence of some recurrent mutations more frequent in other regions of Quebec, such as the Montreal region, remains a possibility. The demographic history of Quebec helps to explain why the distribution of mutation spectra for several diseases is unique to the province of Quebec (Scriber 2001).

Taking into consideration the numerous advantages of MLPA analyses, it would seem appropriate to use this approach to complement PCR-based sequencing to decrease as much as possible the occurrence of false-negative results, thus improving the clinical validity of the predictive BRCA1/2 test.

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