Complete mutation screening and haplotype characterization of the \textit{BRCA1} gene in 61 familial breast cancer patients from Norway

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Abstract. Mutations in the \textit{Breast-Cancer-1 (BRCA1)} gene are the major cause of familial breast/ovarian cancer. Among familial breast cancer only, 15–20% have been suggested to have a deleterious mutation in \textit{BRCA1}. A highly sensitive method (REF-SSCP) was applied to screen the open reading frame and the 5'UTRs of \textit{BRCA1} for mutations. The patient cohort comprised 61 unrelated moderate to high risk breast cancer patients from Western-Norway. Only one known deleterious \textit{BRCA1} mutation (c.816-817delGT) was found in two of the 61 patients (3.3%). Four haplotypes were established based on nine known single nucleotide polymorphisms. Two patients had a novel deletion (c.-33\_29delAAAAA) in the 5'UTR, and a novel amino acid substitution (L523W) was found in one patient. Size variations analysis in the 5'UTR was repeated in a cohort of 159 unrelated familial breast/ovarian cancer patients and 94 healthy blood donors. Two patients were identified with 5'UTR (c.30 to -60) variations (CAAAA)\textsubscript{5} and (CAAAA)\textsubscript{7}, instead of the (CAAAA)\textsubscript{6}-repeat. All of the identified 5'UTR size variations were localized between the start codon and the most stable secondary structures previously proposed for the exon 1b transcript. No such alterations were found among the healthy blood donors but association studies of the 5'UTR variations within the respective families were not conclusive.

Keywords: Familial cancer, breast cancer only, \textit{BRCA1}, 5'UTR, genetic screening, haplotypes

1. Introduction

Approximately 10% of all breast cancer cases cluster in families, and germline mutations in the breast-cancer susceptibility genes \textit{BRCA1} and \textit{BRCA2} account for 20–60% of these cases [24]. \textit{BRCA1} and \textit{BRCA2} mutations also predispose for ovarian cancer, but increased risk for cancer at sites other than the ovary and breast appears to be relatively small [35]. Among women with a family history of both breast and ovarian cancer, \textit{BRCA1} mutations have been identified in 60–80% of the cancer cases compared to 15–20% among women with a family history of breast cancer only [3, 25]. At present, approximately 1500 distinct \textit{BRCA1} mutations and sequence variants have been reported to the breast cancer information core database (BIC, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/). In genetically homogeneous groups, however, the number of mutations appears to be relatively small, with only a few highly dominating founder mutations [5,8,23].

\textit{BRCA1} contains two alternative first exons – exons 1a and 1b – that give rise to two alternative 5'UTRs through the selective use of promoters $\alpha$ and $\beta$ [37, 38]. Previous reports have indicated that the exon 1b transcript is dominant in the placenta, whereas the exon
1a transcript is the dominant one in normal mammary glands [31,37]. However, both mRNAs have been detected in breast cancer tissues. Decreased BRCA1 transcription has been shown to accelerate growth of both normal and malignant mammary cells, and this increased growth is also apparent during sporadic breast cancer progression[34]. Furthermore, BRCA1 is likely to be translationally downregulated by strong secondary mRNA structures present in the 5'UTR, especially in the exon 1b transcript [31]. Thus, when exon 1b transcripts appear in breast cancer tissues, translational downregulation may contribute to the decreased level of the BRCA1 protein observed in breast cancer [33]. Although germline sequence variations in the 5’UTR of BRCA1 have not yet been published, a somatic point mutation in the 5’UTR (position -3) detected in sporadic breast cancer has been reported to down-modulate translation efficiency [29].

With the exception of a few studies on genomic deletions and rearrangements [12,16,26], most mutation reports in BRCA1 have been at the single nucleotide level within protein-coding regions only. Moreover, the majority of such reports have been in high-risk families with a mixed breast and ovarian cancer history. Fewer attempts have been made to address the effect of mutations in the 5'UTR of BRCA1, although the process by which mutations in the 5'UTR affect translation efficiency has been described as a molecular mechanism in disease causation [2,18]. Further, only a handful of studies have specifically looked at patients with familial breast cancer only as opposed to patients with familial breast/ovarian cancer.

We have previously reported the application of a highly sensitive mutation screening method, restriction endonuclease fingerprinting–single-strand conformation polymorphism (REF-SSCP), for detecting sequence variations in BRCA1 exon 11 [14]. Here we report the development of additional REF-SSCP fragments for screening the entire ORF and both alternative 5'UTRs of BRCA1. Further, we present the results of a complete BRCA1 screening of 61 moderate to high risk familial breast cancer only patients from Western-Norway.

2. Patients and methods

2.1. Patients

A consecutive series of 61 women (27–61 years) with breast cancer only (bc) attending our familial cancer clinic in the period 1996–1997 were enrolled in this study (Table 1). Their families satisfied the criteria used for risk of inherited cancer [20]. By applying the European Consensus Criteria 1999 [21], 22 families were classified into the high-risk and 39 into the moderate-risk categories. Of these 61 patients, 51 had unilateral breast cancer only (ulbc) and 10 had bilateral breast cancer (blbc). In 2003, fifty-four families still had breast cancer only, while seven had at least one family member with ovarian cancer (oc) (but not satisfying the criteria for classification into bc/oc families). The breast cancer phenotypes are outlined in Table 1. In addition, 94 healthy blood donors and 159 unrelated bc/oc patients with familial cancer (tested in advance for the Norwegian BRCA1 founder mutations 1675delA, 1135insA, 816delGT, and 3347delAG) were also subjected to 5’UTR fragment analysis.

2.2. Isolation of genomic DNA and mRNA

Whole blood was drawn from the bc patients and collected in tubes with EDTA-anticoagulant. Lymphocytes for mRNA isolation were grown in vitro by adding 0.4 ml of freshly-sampled EDTA-blood to culture tubes containing 8 ml of lymphocyte growth medium (Gibco Medium 199 supplemented with 18% FBS, 1 × Glutamax™-1 Supplement 200 mM, 1 × Antibiotic-Antimycolyc (Penicillin, Streptomycin and Amphotericin B), 2% Phytohaemagglutinin/NaCL (Gibco BRL) and 1% Heparin (10 IE/ml, Nycomed) and the mixture incubated at 37°C for three days. The rest of the blood sample was frozen at −20°C until it was further processed for genomic DNA. The cultured lymphocytes were harvested by centrifugation (190 × g for 10 min) and washed in 8 ml cold PBS. Following a second centrifugation and wash, the supernatant was discarded and the cells were frozen at −80°C. Genomic DNA was extracted by standard phenol/chloroform extraction methods using a DNA extractor (Applied Biosystems, 341 Nucleic Acid Purification System GenePure) according to manufacturer’s instructions, and stored at −20°C. mRNA was isolated from cultured lymphocytes using the QuickPrep® Micro mRNA purification kit (Amersham Pharmacia Biotech) according to manufacturer’s instructions, and kept precipitated in ethanol at −80°C until cDNA was synthesized. Precipitated mRNA (approximately 1 µg) was resuspended in 20 µl DEPC-treated ddH₂O, and 2.5 µl of this was used in a 20 µl reaction mixture for cDNA synthesis using the random hexamer-primed method (GeneAmp® RNA-PCR core kit, Applied Biosystems).
Table 1
Distribution of breast cancer phenotypes by age among 61 patients with familial breast cancer from Western-Norway

| Age at the time of initial breast cancer (bc) diagnosis | All patients (n = 61) | Patients with unilateral bc (n = 51) | Patients with bilateral bc (n = 6) | Patients initially diagnosed with unilateral bc and later with bilateral bc (n = 4) |
|--------------------------------------------------------|----------------------|--------------------------------------|-----------------------------------|--------------------------------------------------------------------------------|
| < 40 years                                             | 14                   | 14                                   | 0                                 | 0                                                                               |
| 40–49 years                                            | 34                   | 29                                   | 3                                 | 2                                                                               |
| 50 years or >                                          | 13                   | 8                                    | 3                                 | 2                                                                               |
| Mean age                                               | 43.7                 | 42.6                                 | 48.8                              | 50.25                                                                           |

Table 2
REF-SSCP fragments for detection of sequence variants in BRCA1

| PCR fragment* | PCR primers (5’–3’) | Direction | Annealing temperature (°C) | PCR fragment location** | PCR fragment size (bp) | RE used | Restriction fragment length (bp) in REF-SSCP (5’–3’*) |
|---------------|----------------------|-----------|----------------------------|-------------------------|-----------------------|---------|-----------------------------------------------------|
| G             | AGC TCG CTG AGA      | Forward   | 58                         | Exon 1a-1b, (nt g3366–3748) | 383                   | BstNI   | 17, 228, 138                                        |
|               | CTT CCT G            | Reverse   |                            |                         |                       | HinfI   | 106, 214, 63                                        |
|               | GCC GAC GTT TTT      |           |                            |                         |                       | RsaI    | 149, 234                                            |
|               | AAA GAC ATA G        |           |                            |                         |                       |         |                                                     |
| H             | TCC TCG TGA TAG      | Forward   | 58                         | Exon 1b 5’UTR-exon 11 (nt g3688–c873) | 1084                  | AluI    | 114, 153, 166, 349, 302                             |
|               | GAA CTG G            | Reverse   |                            |                         |                       | DdeI    | 164, 9, 220, 391, 39, 193, 60, 8                    |
|               | GCT TCT CAG TGG      |           |                            |                         |                       | MboII   | 338, 30, 137, 88, 286, 26, 179                      |
|               | TGT TCA A            |           |                            |                         |                       |         |                                                     |
| I             | CAG GGA GTT GGT      | Forward   | 59                         | Exon 11–18 (nt c4110–5218) | 1109                  | MboII   | 43, 21, 15, 69, 161, 242, 77, 84, 39, 35, 109, 93, 66, 55 |
|               | CTG AGT GAC          | Reverse   |                            |                         |                       | MnlI    | 43, 124, 24, 130, 114, 144, 27, 72, 39, 83, 309    |
|               | GTC CGT TCA CAC      |           |                            |                         |                       | NlaIII  | 227, 28, 37, 298, 33, 148, 81, 78, 179             |
|               | ACA AAC TCA          |           |                            |                         |                       |         |                                                     |
| J             | TGC AAT GGA AGA      | Forward   | 55                         | Exon 16–24 (nt c4997–5690) | 694                   | MnlI    | 242, 84, 239, 36, 93                                |
|               | AAG TGT GAG          | Reverse   |                            |                         |                       | Adal    | 43, 223, 211, 27, 165, 25                           |
|               | CTG GGG TAT CAG      |           |                            |                         |                       | NlaIII  | 76, 103, 138, 128, 142, 107                         |
|               | GTA GGT GTC          |           |                            |                         |                       |         |                                                     |

*Fragments A-F (for exon 11) were subjected to REF-SSCP analysis according to Jugessur et al. [14].
**Genomic and cDNA sequences are according to L78833 and U14680 respectively.

2.3. REF-SSCP analysis

REF-SSCP screening of BRCA1 exon 11 was performed as previously described [14] using six overlapping genomic PCR fragments (named A-F). In addition to exon 11, exons 1a/1b-10 and exons 12–24 were screened using one genomic PCR (fragment G) and three RT-PCR fragments (fragments H-J), respectively (Table 2). PCR on genomic template (fragment G) was performed as described by Jugessur et al. [14], but changes included the use of 1.25 mM Mg2+ and annealing and denaturing steps of 30 sec each. The RT-PCR was performed with 15 µl of cDNA template in a 60 µl reaction, with annealing temperatures provided in Table 2. The RT-PCR cycles were as previously described, with 30 sec annealing/denaturing steps and 35 cycles, except for fragment H where 40 cycles and 1.75 mM Mg2+ were used instead. REF-SSCP analyses, including restriction enzyme selection and digestion were as reported [14].

2.4. DNA sequencing

PCR products were purified using the Qiaquick PCR purification kit (Qiagen) and sequenced using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). In addition to both PCR primers, internal sequencing primers were used for the sequencing of longer fragments (primer sequences are available upon request from the authors). Sequencing reactions were purified using Sephadex G-50 columns and analyzed on an ABI PRISM 377 or 3100 Sequence Analyzer, using the accompanying software for sequence analysis (Applied Biosystems).

2.5. Fragment analysis of the 5’UTR (CAAAA)n-repeat in BRCA1 exon 1b

Thermal cycling consisted of an initial denaturing step at 95°C for 10 min followed by 35 cycles at 94°C for 30 sec, annealing at 52°C for 30 sec and primer extension at 72°C for 30 sec, and a final elongation for
Table 3

BRCA1 sequence variants among 61 breast cancer patients from Western Norway

| Sequence variant | Location            | No. of alleles/total no of alleles | Linked to haplotype no. | Reports in BIC |
|------------------|---------------------|-----------------------------------|-------------------------|---------------|
| c.-33,-29 delAAAAA | 5' UTR of BRCA1-1b  | 2/122                             | H1                      | –             |
| c.816,817delIGT   | p.V233fsdelGT       | 2/122                             | H1                      | 18            |
| c.1687T>G         | p.L523W             | 1/122                             | H1                      | –             |
| c.3238G>A         | p.S1040N            | 2/122                             | H1                      | 25            |
| c.4158A>G         | p.R1347G            | 1/122                             | H1 or H2                | 41            |
| c.5002T>C         | p.M1628T            | 1/122                             | H1 or H2                | 21            |
| c.5075C>A         | p.M1652I            | 1/122                             | H1 or H2                | 34            |

7 min at 72°C. 500 nM of the primers 5'-GCG-AGA-CTG-TCT-CAA-AAC-AA-3' (labeled with fluorescent dye 6-FAM) and 5'-GGT-CCC-ATC-CTC-TCA-TAC-ATA-3'. PCR products were diluted 1:10, and 1 µl of this was added to 1 µl Gene Scan® 400 HD (ROX) size standard (Applied Biosystems) and 12 µl Hi-Di Formamide (Applied Biosystems). After denaturation for 2 min at 95°C and cooling on ice for 5 min, capillary electrophoresis was carried out on an ABI PRISM 3100 Sequence Analyser using the POP-4 polymer. Fragment analysis was performed using GeneScan® software (Applied Biosystems). All changes in fragment length were subjected to DNA sequencing as described above.

3. Results

3.1. BRCA1 sequence variants in 61 moderate to high risk breast cancer only (bc) patients

A novel deletion in the 5'UTR of the BRCA1-1b transcript (c.-33,-29delAAAAA) and one known deleterious mutation (c.816,817delIGT) were each identified in two patients (Table 3). Further, a previously unreported variant in exon 11 (p.L523W) was identified in a single patient. Two patients were carriers of the known variant p.S1040N, while the three variants p.R1347G, p.M1628T, and p.M1652I were all identified in a single patient. In addition, nine known single nucleotide polymorphisms were detected, six of which cause a change in amino acid (Table 4).

The two patients carrying the c.-33,-29delAAAAA variant were diagnosed at the age of 30 and 44, while the patients carrying c.816,817delIGT were diagnosed at the age of 35 and 45. In one of the two families with c.-33,-29delAAAAA, the deletion was detected in one 47-year-old healthy sister, but neither in a sister diagnosed with bc at age 51 nor in another 52-year-old healthy sister.

3.2. Haplotypes

The nine known polymorphisms detected in this study could be arranged into two major (H1 and H2) and two minor (H4 and H6) haplotypes (Table 4). The most common haplotype (H1), which corresponds to the consensus sequences [19,30], was found in 65.6% of the alleles (Table 4). H2, found in 23.8% of the alleles, is characterized by sequence variants at seven of the nine polymorphic sites. No other sequence variant was identified in H2 following the complete sequencing of all ten REF-SSCP fragments in two patients homozygous for either the H1 or the H2 haplotype. Furthermore, no additional sequence variant was detected in the patient homozygous for H1 when compared to the BRCA1 consensus sequences U14680 and L78833 [19, 30]. The low-frequency haplotype H4 (4.9% of alleles) contains one additional sequence variant compared to H1. On the other hand, haplotype H6 (5.7% of alleles) was identical to H2 except for one additional variant.

3.3. Variations in the BRCA1 5' UTR (CAAAA)n sequence among 94 controls and 159 breast/ovarian cancer (bc/oc) families

Screening of 159 unrelated bc/oc patients revealed no additional c.-33,-29delAAAAA carriers. However, two new size variations – (CAAAA)5 and (CAAAA)7 – were identified that were different from the normal (CAAAA)6 repeated sequence. The 5- and 7-repeats were identified separately in patients without any of the four common Norwegian BRCA1 founder mutations (see Patients and methods). Family analysis of the 5- and 7-repeats revealed no correlation to other women with bc/oc in the family. Results of screening for size variations in the BRCA1 5'UTR (CAAAA)n sequence of exon 1b among the 94 blood donor controls were negative.
Table 4
Nine BRCA1 variants defining four haplotypes (H1–H4) among 61 breast cancer patients from Western-Norway

| Sequence variant | Exon | Haplotype |
|------------------|------|-----------|
|                  |      | H1 | H2 | H4 | H6 |
| c.1186A>G (p.Q356R) | 11  | −− | + | − | − |
| c.2196G>A (p.D693N)  | 11  | −− | − | + | + |
| c.2201C>T (p.S694S)  | 11  | −+ | + | − | + |
| c.2430T>C (p.L771L)  | 11  | −+ | − | + | + |
| c.2731C>T (p.P871L)  | 11  | −+ | − | + | + |
| c.3232A>G (p.E1038G) | 11  | −+ | − | + | + |
| c.3667A>G (p.K1183R) | 11  | −+ | − | + | + |
| c.4427T>C (p.S1436S) | 13  | −+ | − | + | + |
| c.4956A>G (p.S1613G) | 16  | −+ | − | + | + |

Haplotype frequency: 80/122, 29/122, 6/122, 7/122

4. Discussion

In a previous study, we reported a REF-SSCP mutation detection sensitivity of close to 100% when the method was applied to the large exon 11 of BRCA1 [14]. In the present study, the entire ORF and the two alternative 5'UTRs of BRCA1 were screened for sequence variants by REF-SSCP, using a combined genomic/cDNA based strategy. Using this approach, we analyzed 61 breast cancer only (bc) patients from Western-Norway, and found one previously known deleterious mutation (c.816_817delGT) in two patients (3.3%). A novel missense mutation (p.L523W) was also detected in one patient, but the clinical significance of this mutation remains unknown until family-based and/or functional analyses can be performed. Further, we found a novel deletion (c.-33_29delAAAAA) in the 5'UTR exon 1b in two patients, but not among any of the 94 healthy blood donors tested.

4.1. 5'UTR variants in BRCA1

To our knowledge, no germline mutation in the 5'UTR of BRCA1 has yet been published, most likely because this region of the gene is rarely screened for mutations. The c.-33_29delAAAAA deletion in exon 1b was not detected among any of the controls, and might therefore stand for something more than just a silent variant. However, our family analyses were inconclusive and no additional c.-33_29delAAAAA deletion was detected among the 159 unrelated patients with moderate or high risk familial bc/oc.

In mammary glands, exon 1b transcripts appears to be detected only during cancer development [31, 37]. In this study, screening of exon 1b for c.-33_29delAAAAA among unrelated bc/oc patients revealed single cases of two additional size variations, (CAAAA)$_5$ and (CAAAA)$_7$, in contrast to the normal (CAAAA)$_6$. However, we could not show any segregation with bc/oc. Therefore, the relevance of these size variations to the development of bc or oc remains undetermined at this time.

In BRCA1, the CAAAA repeats are localized between the start codon and the regions that generate secondary mRNA structures suggested to be involved in translational downregulation of the exon 1b-transcript [31]. By changing the mRNA secondary structure, sequence variations within the 5'UTR in either the exon 1a or exon 1b transcript can significantly affect translational regulation. The CAAAA repeats are located within a stretch of 232 nucleotides, which belongs to an Alu-Sx subfamily of repetitive sequences detected in about 4% of fully-spliced human mRNA [31]. This again points to a general mechanism for translational regulation.

There may also be other mechanisms at work. For instance, (CAAAA)$_{5–7}$ repeats have been found within a human promoter [10], and in plants, a CAAAA motif is believed to be involved in breakage-reunion mechanisms involving repeated sequences [15,17]. The CAAAA$_n$ motif could also be involved in somatic alterations (commonly observed in BRCA1) through a mechanism involving rearrangements between repetitive elements [36]. Furthermore, a new type of BRCA1 rearrangement in families with bc/oc was recently suggested to be due to the existence of repeats that constitute recombination hotspots [27].

4.2. Mutations in familial breast cancer vs. familial breast/ovarian cancer

Our results suggest that the prevalence of BRCA1 mutations in early-onset familial bc is considerably lower than that in familial bc/oc. In a Norwegian bc/oc
material with two c.816_817delGT carriers, as identified in the present study, detection of more than 10 cases of 1675delA or 1135insA would have been expected according to findings in Borg et al. [1]. With regard to 1675delA, this discrepancy is unlikely to be due to geographical differences, since families with 1675delA and families with c.816_817delGT aggregate in the same Southwestern part of Norway [22]. In a recent report, the risk for ovarian cancer was found to be twice as high among 1675delA carriers than among those harboring c.816_817delGT [11]. Our results suggest that the opposite might be the case for bc, and support a suspected lower prevalence of 1675delA and 1135insA in a bc-only material as opposed to previously examined bc/oc materials [1,4,5]. Moreover, the c.816_817delGT mutation appears to be frequently associated with Norwegian patients diagnosed with bc in their thirties ([11]; present study). By contrast, the Norwegian bc/oc founder mutations 1675delA and 1135insA appear to be associated with later-onset disease [1]. This is consistent with the observation that familial bc – but not oc – is skewed towards early-onset [7,9,32].

4.3. Haplotypes

The nine known high-frequency polymorphisms identified in this study (Table 4) have been reported previously by other authors to have no significant differences in allele frequencies between familial bc/oc cancer patients and the population at large [6]. Consequently these haplotypes are normal variants that do not associate with breast cancer risk. Although eight of these polymorphisms are actually part of the same haplotype, they are usually reported individually. In so doing, the reported degree of variation in the BRCA1 sequence may appear to be more substantial than it is.

In a seminal study, Shattuck-Eidens et al. [28] described a total of 10 haplotypes among 1590 alleles from a US population. The reported haplotypes include those identified in our study (i.e., haplotypes H1, H2, H4, and H6). In the US study, haplotypes 3, 9 and 10 had a frequency of less than 1%, and are absent among our 61 patients. Haplotype 5 (allele frequency 1.8%) corresponds to the haplotype carrying the S1040N variant, which features among the low-frequency unclassified variants in the present study (Table 3). Haplotype 7 reported by Shattuck-Eidens et al. [28] is identical to haplotype 6 as reported here, except for a variant in exon 4. Haplotype 2 and the closely related haplotype 6 have a combined allele frequency of 29.5%, compared to 32% among the 1590 US alleles. A combined allele frequency of approximately 30% for haplotypes 2 and 6 has also been reported in other US study populations [6]. In our study, haplotypes H1 and H2 appear to be made up of ancient alleles that have independently acquired additional variants with time.

The mutations and low-frequency variants in this study are invariably detected on any of the two major haplotypes, H1 or H2, albeit more frequently on the dominant haplotype H1. This is consistent with the observation that human BRCA1 may be subjected to adaptive evolution [13]. For instance, the c.2196G>A variant is identified only on H2, giving haplotype 6, whereas the c.1186A>G is identified exclusively on H1, giving haplotype 4 (Table 4). The rare haplotypes 3, 8, 9, and 10 reported in Shattuck-Eidens et al. [28] comprise different combinations of the H2 polymorphisms, which may reflect recombination and/or independent nucleotide substitution events.

To conclude, we performed a follow-up study of 61 bc families from Western-Norway in order to have an improved assessment of their inherited bc only status. These 61 familial bc only patients were subjected to mutation analysis in BRCA1 by REF-SSCP, which was engineered to allow the screening of sequence variants in the entire BRCA1 open reading frame as well as both alternative 5'UTRs. Relying upon this well-defined bc-only patient cohort and REF-SSCP as a sensitive tool for mutation detection, we were able to 1) arrange 122 alleles in as few as four haplotypes based on nine single nucleotide polymorphisms, 2) identify one deleterious BRCA1 (c.816_817delGT) mutation in two of the 61 patients (3.3%), 3) identify a novel deletion in the 5'UTR in two patients, as well as a novel amino acid substitution in a single patient, and 4) identify two additional changes in the 5'UTR among 159 bc/oc cancer patients, but not among any of the 94 healthy blood donors tested.

Repetitive detection of clinically irrelevant known polymorphisms, often many within the same sample, dramatically increases the workload in a mutation screening effort. This underscores the potential benefit of using reference samples with relevant haplotypes during comparative mutation screening, in order to circumvent the repetitive detection of silent sequence variants. Our results also confirm previous observations that only a very small fraction of familial bc is accounted for by mutations in BRCA1. The major genetic cause for inherited bc, therefore, still remains to be explored.
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