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SHORT REPORT

Haplotype analysis of *BRCA1* gene reveals a new gene rearrangement: characterization of a 19.9 KBP deletion

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Germ-line mutations in the *BRCA1* gene cause hereditary predisposition to breast and ovarian cancer. *BRCA1* and *BRCA2* mutations account for about 40% of high-risk families. Mutation-screening methods generally focus on genomic DNA and are usually PCR based; they enable the detection of sequence alterations such as point mutations and small deletions and insertions. However, they do not allow the detection of partial or entire exon(s) loss, because the presence of the homologous allele results in a positive PCR signal, giving rise to a false-negative result. Identification of unusual haplotypes in patient samples by an expectation maximisation algorithm has recently been suggested as a method for identifying hemizygous regions caused by large intragenic deletions. Using a similar approach, we identified a novel *BRCA1* genomic rearrangement in a breast/ovarian cancer family negative at the first mutation screening; we detected a deletion encompassing exons 14–19, probably due to replication slippage between *Alu* sequences.
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Germ-line mutations in *BRCA1* and *BRCA2* genes cause hereditary predisposition to breast and ovarian cancer. *BRCA1* and *BRCA2* mutations account for about 40% of high-risk families.[1] The predisposition to develop cancer is transmitted as an autosomal dominant trait. For such reason, it is very important to detect single-allele inactivation events caused by intragenic deletions leading to hemizygosity. Methods employed for mutation screening usually focus on genomic DNA and are PCR based (ie, direct sequencing, single-strand conformation polymorphism analysis, denaturing gradient gel electrophoresis or heteroduplex analysis). These approaches enable the detection of sequence alterations, such as point mutations, and small deletions and insertions. Depending on the breakpoint location, the loss of partial or entire exon(s) will not be detected by these methods because the presence of the homologous normal allele results in a positive PCR signal. This mechanism is considered as an important cause of false-negative results.[2]

It has been estimated that not more than the 70% of mutations are detected among the *BRCA1*-linked families screened by one or the other of the methods described above; this relative low sensitivity can be due to a substantial

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fraction of *BRCA1* mutations occurring outside the coding region and splice sites and also to large deletions.[3]

The human genome contains up to 1 million copies of interspersed *Alu* elements (one *Alu* repeat every 5 kb) that are thought to mediate chromosomal rearrangements and homologous recombination events that result in translocations, duplications, inversions or deletions.[4] Analysis of the genomic structure of the *BRCA1* gene has revealed an high density of *Alu* repeats, with 41.5% of the genomic sequence being composed of *Alu* sequences or one *Alu* repeat every 0.65 kb.[5] At present, 22 different large genomic rearrangements have been characterised, they include both deletions and duplications of one or more exons in *BRCA1*.[6–10]

Only two rearrangements of the *BRCA2* gene have been detected to date: a 5 kb deletion leading to exon 3 skipping[11] and a 6.2 kb deletion removing exons 12–13.[12] Since the *BRCA2* genomic sequence contains fewer *Alu* repeats than *BRCA1*, it is presumed that genomic rearrangements involving *Alu*-mediated recombination events are less frequent.

In the present work, the genomic DNAs from 47 individuals belonging to 47 families with a strong history of breast and/or ovarian cancer (33 HBC, one HOC and 13 HBOC) were analysed for point mutation of *BRCA1* and *BRCA2* genes by direct sequencing of all coding exons and intron–exon boundaries. Seven pathogenic mutations in *BRCA1* and four in *BRCA2* and several allelic variants were detected. In particular, seven SNPs of the *BRCA1* gene were used to build the haplotypes of the entire sample: four were intronic (IVS8−58delT, IVS14−63C/G, IVS16−68A/G, IVS18 + 72G/A) and three were exonic (2731 C/T in exon 11; 4427 T/C in exon 13; 4956 A/G in exon 16) (Table 1). Three frequent multilocus genotypes were detected as follows: 18 subjects (38.3%) were homozygous for the consensus allele at all loci, 10 (21.3%) were homozygous for the variant allele at all loci and 17 (36.2%) were heterozygous at all loci. In addition, two subjects carried a unique genotype (Table 1); it thus appeared that these SNPs were in strong linkage disequilibrium, confirming previous reports.[13,14] This feature greatly facilitates the construction of haplotypes; in fact, inferring the haplotype composition of the entire sample was straightforward. Only two haplotypes exist at high frequency, the 'A' haplotype ('1111111') and the 'B' haplotype ('2222222'), respectively, whereas two haplotypes are present once ('2222221' and '1112222'). We suspected that these unique haplotypes were spurious, being instead the consequence of loss of heterozygosity due to large intragenic deletions. Therefore, we carried out the molecular characterisation by RT-PCR using primer pairs located in the presumed deletion flanking regions of the only sample for which RNA was available. The tested sample showed evidence of rearrangement in *BRCA1* gene: as a matter of fact two bands (1.5 kb band corresponding to the wild-type mRNA product and an additional 0.7 kb band of unknown meaning) were detected using a forward primer located in exon 11 and a reverse primer located in exon 24 (Figure 1a). Sequence analysis of a 0.7 kb fragment showed that this alteration encompasses exons 14–19. The same deletion was detected at mRNA level in mother's proband affected by ovarian cancer (data not showed).

Table 1 Seven diallelic polymorphisms in *BRCA1* gene considered in the present study and multilocus genotypes with their frequencies detected in a sample of 47 subjects at high risk of carrying *BRCA1* or *BRCA2* mutations

Nucleotide change	*Multilocus genotypes*				
IVS8−58delT	11	22	12	12	12
2731C/T (exon 11)	11	22	12	12	12
4427T/C (exon 13)	11	22	12	12	12
IVS14−63C/G	11	22	12	12	12
4956A/G (exon 16)	11	22	12	12	12
IVS16−68A/G	11	22	12	12	12
IVS18+72G/A	11	22	12	12	12
Number of cases	18	10	17	1	1
Frequency (%)	38.3	21.3	36.2	2.1	2.1

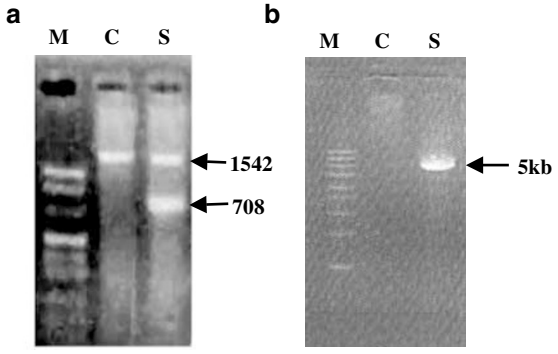

Figure 1 (a) RT-PCR analysis with primers located in exons 11 and 24. (b) Long PCR with primers in intron 13 and in intron 19/exon 20. M = marker; C = control DNA or cDNA; S = patient DNA or cDNA.

To confirm the novel rearrangement detected and to define the boundaries of the deletion, long-range PCR was performed on genomic DNA using primers located in intron 13 (5′-CCAGAACAAAGCACATCAGAAAAA-3′) and in intron 19/exon 20 (5′-ATGCTGAAAGAAACCAAACA-CAAC-3′). From the patient's DNA a 5 kb PCR product was observed, whereas the 25 kb expected product was not obtained (Figure 1b.). This 5 kb product was gel extracted and sequenced using various combination of PCR primers in introns 13 and 19 designed to identify the exact localisation of junction on *BRCA1* gene. It turned out that a segment of 19 886 bp was deleted starting at nucleotide 51 482 in intron 13 and ending at nucleotide 71 368 in intron 19 (Accession number GenBank L78833). An Alu sequence is located near both positions (Alu *Sq* in intron 13 and Alu *Sx* in intron 19), making it likely that the deletion was the consequence of replication slippage (Figure 2).

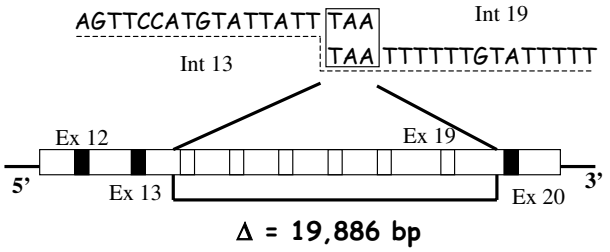

Figure 2 The recombination site is located between nucleotide 51 482–51 484 in intron 13 (near *AluSq*) and nucleotide 71 368–71 370 in intron 19 (*AluSx*) of BRCA1 gene (Accession number GenBank L78833).

We also considered whether the inactivation of the wild-type allele in the patient's breast tumour was due to somatic deletion. DNA was extracted from pure neoplastic cells obtained by laser capture microdissection (AS-LMD, Leica, Germany) of paraffin-embedded formalin-fixed tissues and exon 16 (which maps in the deleted region) was amplified. A PCR product compatible with the exon 16 length was obtained, indicating that the total inactivation of the *BRCA1* gene was not due to allelic deletion (data not shown).

To date, methods able to identify large genomic rearrangements are Southern blot based and, more recently, QMPSF (quantitative multiplex PCR of short fluorescent fragments)[9] and MLPA (multiplex ligation probe amplification) based.[13] Identification of unusual haplotypes in patient samples by an expectation maximisation algorithm has recently been suggested as a method to identify hemizygous regions caused by intragenic deletions.[14] In fact, the existence of only two major haplotypes segregating at high frequency (0.64 and 0.33, respectively) in the Caucasian population for the entire length of the *BRCA1* gene[15] makes this approach feasible by simple inspection of the multilocus genotypes at a small number of intragenic SNPs.[16] We identified a novel *BRCA1* deletion in a breast/ovarian cancer family using this approach, so it results that in our family collection, the 12% (1/8) of *BRCA1* mutations may be due to large genomic rearrangements. In conclusion, examining the SNPs, which actually are a pitfall of the gene screening, could instead be useful for the identification of patients harbouring large intragenic deletions; this reading do not need any additional work. A drawback of the method is that it can work for heterozygous individuals only (44.7% in our series), meaning that about 50% of patients carrying large deletions can be diagnosed with this approach; in practice, however, the chance of missing a mutation is higher, because even in heterozygous only those deletions that encompass a polymorphic nucleotide can be detected. Therefore, other methodologies should be considered for detecting deletions in patients who are homozygous and also heterozygous for either haplotypes.

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