Journal of Human Genetics (2013) 58, 78–83

www.nature.com/jhg

ORIGINAL ARTICLE

Genomic characterization of two large Alu-mediated rearrangements of the *BRCA1* gene

Ana Peixoto[1,3], Manuela Pinheiro[1,3], Lígia Massena[2], Catarina Santos[1], Pedro Pinto[1], Patrícia Rocha[1], Carla Pinto[1] and Manuel R Teixeira[1,2]

To determine whether a large genomic rearrangement is actually novel and to gain insight about the mutational mechanism responsible for its occurrence, molecular characterization with breakpoint identification is mandatory. We here report the characterization of two large deletions involving the *BRCA1* gene. The first rearrangement harbored a 89 664-bp deletion comprising exon 7 of the *BRCA1* gene to exon 11 of the *NBR1* gene (c.441 + 1724_o*NBR1*:c.1073 + 480del). Two highly homologous Alu elements were found in the genomic sequences flanking the deletion breakpoints. Furthermore, a 20-bp overlapping sequence at the breakpoint junction was observed, suggesting that the most likely mechanism for the occurrence of this rearrangement was nonallelic homologous recombination. The second rearrangement fully characterized at the nucleotide level was a *BRCA1* exons 11–15 deletion (c.671-319_4677-578delinsAlu). The case harbored a 23 363-bp deletion with an Alu element inserted at the breakpoints of the deleted region. As the Alu element inserted belongs to a still active AluY family, the observed rearrangement could be due to an insertion-mediated deletion mechanism caused by Alu retrotransposition. To conclude, we describe the breakpoints of two novel large deletions involving the *BRCA1* gene and analysis of their genomic context allowed us to gain insight about the respective mutational mechanism.
Journal of Human Genetics (2013) **58,** 78–83; doi:10.1038/jhg.2012.137; published online 6 December 2012

Keywords: Alu retrotransposition; *BRCA1*; homologous recombination; rearrangement

INTRODUCTION

Several hundred distinct *BRCA1* and *BRCA2* mutations have been identified in hereditary breast/ovarian cancer (HBOC) families, including large genomic rearrangements (LGRs). The high content of Alu elements in the intronic sequences of both *BRCA1* and *BRCA2* genes contributes to the occurrence of rearrangements in these genes.[1,2] These genomic rearrangements include primarily deletions and duplications of one or more exons and represent 10–15% of all deleterious germline mutations in the *BRCA1* gene and 1–7% in the *BRCA2* gene.[3,4] The contribution of these LGRs to *BRCA1*/2 mutation-positive families varies in a population-dependent manner and numerous *BRCA1* founder rearrangements have been identified in particular ethnic groups.[5–7] On the other hand, the proportion of rearrangements in the *BRCA2* gene is generally much smaller than that in the *BRCA1* gene, which can be explained by the lower content of repetitive Alu sequences in the former compared with the latter. The only exception to this rule occurs in Portugal, where the founder rearrangement *BRCA2* c.156_157insAlu (an Alu insertion in exon 3[8]) accounts for more than one fourth of HBOC families with deleterious *BRCA1*/*BRCA2* mutations and about half of those with *BRCA2* mutations.[9]

The detection of LGRs requires specific techniques, like multiplex ligation probe amplification (MLPA) or quantitative PCR, which only give information of the exonic region involved in the rearrangement and not on the exact genomic breakpoints. Therefore, rearrangements with distinct breakpoints but involving the same exons may be described similarly, making it difficult to assess whether a given rearrangement is new or even specific of a given population.[10] To determine whether a LGR is actually novel and to gain insight about the mutational mechanism responsible for its occurrence, molecular characterization with breakpoint identification is mandatory. About 81 distinct *BRCA1* LGRs have been described, the majority of them being deletions, followed by rearrangements with both an insertion and a deletion, duplications, one insertion and one triplication.[4] We here report the characterization of the genomic breakpoints of two *BRCA1* LGRs detected in Portuguese HBOC families, namely, a deletion involving *BRCA1* to the nearby gene *NBR1* and an intragenic *BRCA1* deletion.

MATERIALS AND METHODS

Patients and MLPA analyses

Two patients from two HBOC families, initially shown to be negative for *BRCA1* and *BRCA2* point mutations, were included in this study after being shown to carry *BRCA1* exonic rearrangements by MLPA. This study was approved by the Institutional Review Board of the Portuguese Oncology

[1]Department of Genetics, Portuguese Oncology Institute, Rua Dr António Bernardino de Almeida, Porto, Portugal and [2]Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira, Porto, Portugal
[3]These authors contributed equally to this work and both should be considered first authors.
Correspondence: Professor MR Teixeira, Department of Genetics, Portuguese Oncology Institute, Rua Dr. António Bernardino de Almeida, Porto 4200-072, Portugal.
E-mail: manuel.teixeira@ipoporto.min-saude.pt
Received 3 August 2012; revised 12 October 2012; accepted 25 October 2012; published online 6 December 2012

Institute-Porto, and written informed consent was obtained from all patients after genetic counseling. DNA was isolated from peripheral blood samples using the salt–chloroform extraction method. Routine screening for *BRCA1* and *BRCA2* exonic rearrangements was performed by MLPA using the P002 kit and the P045 kit, respectively (MRC-Holland, Amsterdam, The Netherlands), according to the manufacturer's instructions. One index patient presenting a deletion from exon 1 to exon 7, c.-232-?_441+?del (NM_007294.3) according to the Human Genome Variation Society nomenclature, had bilateral breast cancer at age 32 and 36, gastric cancer at age 38 and ovarian cancer at age 61, besides having a first-degree relative with breast cancer diagnosed at age 37. The second index patient presented a *BRCA1* deletion including exon 11 to exon 15, described as c.671-?_4675+?del (NM_007294.3) according to the Human Genome Variation Society nomenclature and initially reported in Peixoto *et al.*,[11] had an ovarian borderline tumor at age 38 and breast cancer at age 46, with a first-degree relative presenting breast cancer diagnosed at age 50. These two genomic rearrangements account for two of 48 HBOC families with deleterious *BRCA1* mutations so far identified at the Department of Genetics of the Portuguese Oncology Institute, Porto, Portugal.

Breakpoint identification of the *BRCA1* exons 1–7 deletion

To map the 5′ breakpoint region of this *BRCA1* c.-232-?_441+?del mutation, we used the MLPA kit P239 (MRC-Holland) to evaluate the *BRCA1* promoter region, the *BRCA1* pseudogene (*ΨBRCA1*), and the *NBR2* (exons 1, 3 and 5) and *NBR1* (exons 3, 7 and 11) genes, followed by a semi-quantitative multiplex PCR method spanning intron 11 to exon 22 of the *NBR1* gene (NM_005899.3). Four multiplex reactions were designed, each one containing four controls (exons 10 and 24 of the *BRCA1* gene and exons 11 and 27 of the *BRCA2* gene) and three different *NBR1* regions (Table 1). In addition, all these *NBR1* fragments were designed to include single nucleotide polymorphisms (SNPs) to question, at the same time, their heterozygosity status. The primers for multiplex amplification were designed using the online Primer-BLAST tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). AutoDimer (http://www.cstl.nist.gov/strbase/NIJ/AutoDimer.htm) was used to test for potential hairpin structures and primer dimers. Subsequently, primers were designed spanning the putative breakpoints and PCR was carried out using standard conditions. PCR fragments containing the suspected weight were sequenced with BigDye Terminator cycle sequencing chemistry on an ABI PRISM 310 automatic sequencer (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's recommendations.

Breakpoint identification of the *BRCA1* exons 11–15 deletion

The strategy for breakpoint identification of the *BRCA1* c.671-?_4675+?del mutation was based on the heterozygosity status of a set of SNP markers localized in introns 10 and 15 of *BRCA1*. The selected SNPs were rs7503154, rs8176214, rs77152443 and rs72130855. Subsequently, primers were designed flanking the putative breakpoints and PCR was carried out using standard conditions. PCR fragments were sequenced with BigDye Terminator cycle sequencing chemistry on an ABI PRISM 310 automatic sequencer (Applied Biosystems), according to the manufacturer's recommendations.

Breakpoint sequence context analysis

Breakpoints were defined as a set of coordinates in the genome spanning the genomic sequence of the deletion. Bioinformatic analyses were carried out to assess the genomic context of the region. Both Censor Server (http://www.girinst.org/)[12] and the RepeatMasker software (http://www.repeatmasker.org) were used to search for low-complexity DNA sequences and interspersed repeats in both breakpoint regions. All sequence alignments were performed using the complementary strands to match the Alu consensus sequence.[13]

RESULTS

Characterization of the *BRCA1* exons 1–7 deletion

Analysis by the MLPA kit P002 revealed a genomic deletion encompassing *BRCA1* exons 1–7. Analysis with the MLPA kit P239 of the *BRCA1* promoter region, *ΨBRCA1* and the *NBR2* (exons 1, 3 and 5) and *NBR1* (exon 3, 7 and 11) genes revealed that all these regions were also deleted. To analyze the remaining *NBR1* exons, we performed a semi-quantitative multiplex PCR method spanning intron 11 to exon 22 of the *NBR1* gene. All these *NBR1* fragments included SNPs to question, at the same time, their heterozygosity status (Table 1). The results showed that the *NBR1* region encompassing rs112693602 (intron 11/ exon 12) to rs13119 (exon 22) was not deleted, narrowing the distance to the breakpoint. After long-range PCR with putative primers spanning intron 7 of the *BRCA1* gene and intron 11 of the *NBR1* gene, we obtained an ~1300-bp fragment in the sample with the *BRCA1* rearrangement. Sequence analysis of this PCR product revealed that the case harbored a 89 664-bp deletion, comprising exon 7 of the *BRCA1* gene to exon 11 of the *NBR1* gene, with the 5′ and 3′ breakpoints located 1724-bp upstream of *BRCA1* exon 7 and 480-bp upstream of *NBR1* exon 11, respectively, corresponding to the mutation *BRCA1* c.441+1724_o*NBR1*:c.1073+480del (Figure 1). The 5′ and 3′ breakpoint flanking regions presented a complete homology sequence of 20 bp. Two Alu elements were found in the genomic sequences flanking the deletion breakpoints in *BRCA1* intron 7 (AluSc) and *NBR1* intron 11 (AluSg), and sequence alignment indicated that these two elements were highly homologous (Figure 2a).

Table 1 *NBR1* gene analysis using SNPs and a semi-quantitative multiplex PCR method to characterize the *BRCA1* exons 1–7 deletion

Multiplex	*SNP ID*	NBR1 *gene location*	*Semi-quantitative region information*	*Heterozygosity status*
1	rs35995789	Exon/intron 18	Not deleted	Heterozygous
	rs3744243	Intron 20	Not deleted	Not informative
	rs71379212	Intron 21	Not deleted	Heterozygous
2	rs13119	Exon 22	Not deleted	Heterozygous
	rs35166890	Intron 15	Not deleted	Heterozygous
	rs34743495	Exon/intron 17	Not deleted	Heterozygous
3	rs111808644	Intron 14/exon 15	Not deleted	Not informative
	rs17527933	Exon/intron 20	Not deleted	Heterozygous
	rs112693602	Intron 11/exon12	Not deleted	Not informative
4	rs8482	Exon 22	Not deleted	Heterozygous
	rs111691171	Intron 21	Not deleted	Not informative
	rs2306829	Exon/intron 19	Not deleted	Heterozygous

Characterization of the *BRCA1* exons 11–15 deletion

The breakpoint identification of the *BRCA1* exons 11–15 deletion was based on the information obtained through the study of SNPs located in introns 10 (rs7503154) and 15 (rs8176214, rs77152443 and rs72130855) of the *BRCA1* gene. Markers rs7503154 and rs72130855 were heterozygous, indicating that these regions were not deleted. Markers rs8176214 and rs77152443 were not heterozygous, possibly suggesting that they were located within the deleted region. After long-range PCR with the forward primer of marker rs7503154 and the reverse primer of marker rs72130855, we obtained a fragment of ~1100-bp in the sample with the *BRCA1* rearrangement. Sequence analysis of this PCR product revealed the breakpoint region in the mutated allele. The case harbored a 23 363-bp deletion, comprising exons 11–15 of the *BRCA1* gene with the 5′ and 3′ breakpoints located 319-bp upstream of exon 11 and 578-bp upstream of exon 16 (Figure 1). In addition, at the deletion breakpoint, a sequence of ~250-bp was inserted (Figure 1). Although

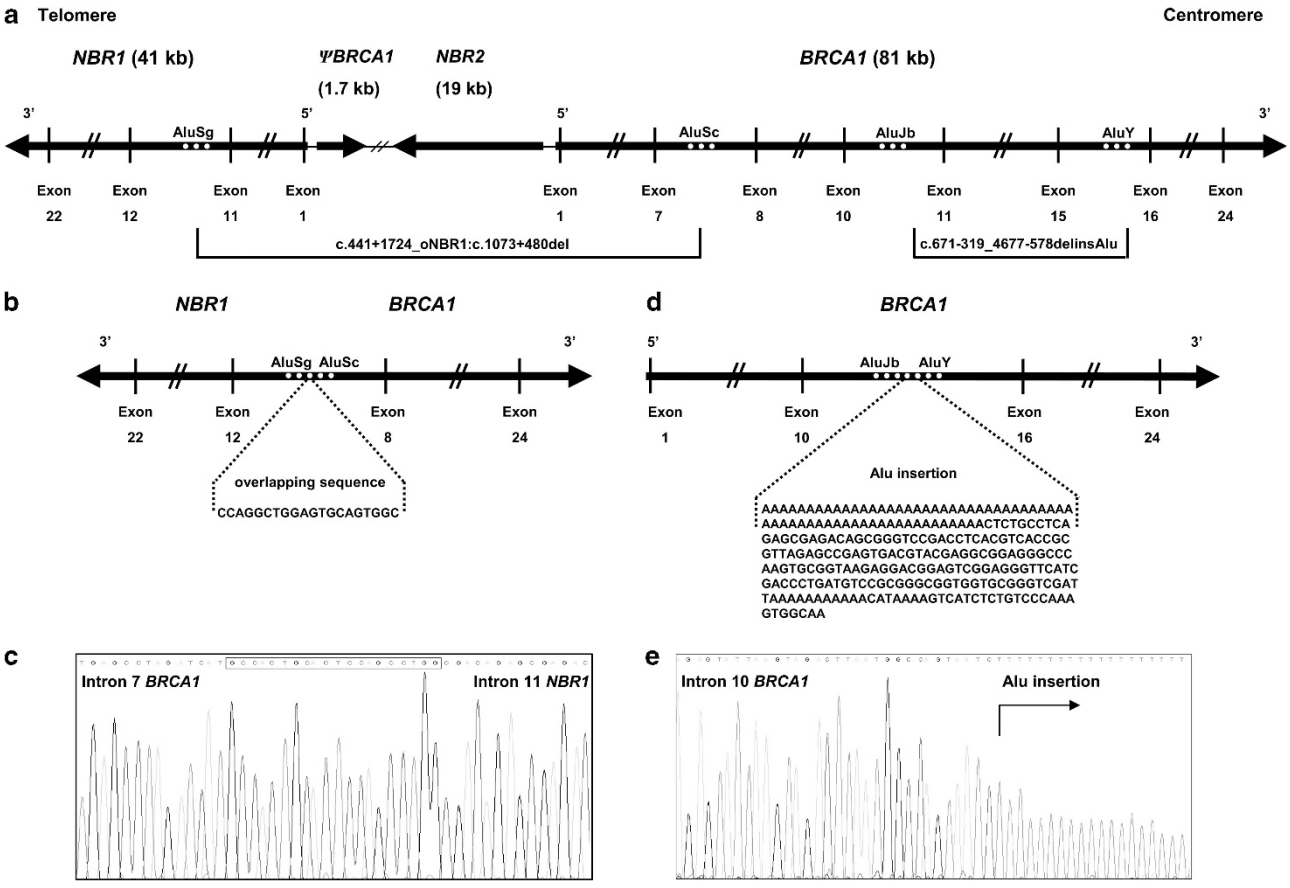

Figure 1 Scheme representing the molecular characterization of the *BRCA1* c.441 + 1724_oNBR1:c.1073 + 480del and c.671-319_4677-578delinsAlu mutations. (**a**) Genomic region encompassing the *NBR1* and *BRCA1* genes, with the deleted regions and flanking Alu elements represented for both mutations. (**b**) *BRCA1* c.441 + 1724_oNBR1:c.1073 + 480del mutation with the 5′ and 3′ breakpoints located in intron 7 and 11 of *BRCA1* and *NBR1*, respectively. The deletion breakpoints are flanked by two AluSc/AluSg elements with a complete homology sequence of 20 bp within these Alu repeats. (**c**) Sequence electrophorogram of the *BRCA1* c.441 + 1724_oNBR1:c.1073 + 480del breakpoint with the overlapping sequence highlighted. (**d**) *BRCA1* c.671-319_4677-578delinsAlu mutation, with the breakpoints in introns 10 and 15 of the *BRCA1* gene and presenting an Alu insertion. The 5′ breakpoint of the deletion was near an AluJb element, whereas the 3′ breakpoint occurred in an AluY element. (**e**) Sequence electrophorogram of the 5′ breakpoint region of the *BRCA1* c.671-319_4677-578delinsAlu, showing a stretch of the poly-A inserted Alu element. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

the exact length could not be assigned because of the inherent difficulties in sequencing long poly-A tracts, this fragment contained ~60-bp poly-A. The Censor Server and RepeatMasker software showed that this sequence was an Alu element belonging to the AluY family, which allowed us to describe this rearrangement as *BRCA1* c.671-319_4677-578delinsAlu. The 5′ breakpoint of the deletion was 120-bp downstream from an Alu element (AluJb), whereas the 3′ breakpoint occurred in another Alu element (AluY). Sequence alignment of the three Alu elements supposedly involved in this rearrangement is represented in Figure 2b. The inserted Alu sequence presented a higher homology to the intron 15 breakpoint region of the *BRCA1* gene.

DISCUSSION

According to Sluiter and van Rensburg,[4] 81 and 17 different LGRs have been characterized for the *BRCA1* and *BRCA2* genes, respectively. One of the mechanisms causing major gene rearrangements in humans involves Alu elements, which are retrotransposable interspersed repetitive sequences that use the L1-encoded transposition machinery and constitute ~10% of the human genome.[14] Alu sequences comprise three major subfamilies, namely, the J (oldest) and S (intermediate) subfamilies that are responsible for the large majority of Alu copies currently present in the human genome, and the Y subfamily (youngest) that is responsible for all of the recently integrated Alu sequences in the human genome.[15,16] These repetitive sequences are responsible for creating genetic variation and also human diseases, not only because Alu sequences are known to mediate the occurrence of genetic recombination events as a direct result of their abundance and sequence homology, but also due to the occurrence of insertion mutations as a consequence of Alu retransposition events.[17]

We characterized two large deletions involving the *BRCA1* gene, which account for about 4% (2/48) of all *BRCA1* HBOC families identified in our population. The first rearrangement we here report harbored a deletion comprising exon 7 of the *BRCA1* gene to exon 11 of the *NBR1* gene (c.441 + 1724_o*NBR1*:c.1073 + 480del). Two highly homologous Alu elements were found in the genomic sequences flanking the deletion breakpoint, namely, an AluSc in *BRCA1* intron 7 and an AluSg in *NBR1* intron 11. The alignment of these intronic sequences suggested that the most likely mechanism for the occurrence of this rearrangement was nonallelic homologous recombination between these Alu repeats, substantiated by the presence of a 20-

a

```
AluSc (INT7 BRCA1) GGCTGGGTGC GGCGGCTAAC ACCTGCAATC CCAGCACCTT
AluSg (INT11 NBR1) ---CA..C.. A.T....C.. .....TT... T..C..TT..

AluSc (INT7 BRCA1) GGGAGGCTGA GGCCAGTGGA T-CATGAGGT CAGGAGATTG
AluSg (INT11 NBR1) .......CA. ...GG.C... .T........ ......T.C.

AluSc (INT7 BRCA1) AGACCACCCT GGCCAACATG GTGAAACCCG GTCTCTACT-
AluSg (INT11 NBR1) ......G... .A......G. A........C A........G

AluSc (INT7 BRCA1) -AAAAATACA AAAATTAGCT GGGTGTGGTG GCACGTGCCT
AluSg (INT11 NBR1) A......... .........C ...CA..... ...T.CA...

AluSc (INT7 BRCA1) GTAATCCCAG CTACTCCAGA GGCTGAGGAT GGAGAATCAC
AluSg (INT11 NBR1) .......T.. ......AG.. ........CA ..........

AluSc (INT7 BRCA1) TTGAACCTGA GAGTCGGAGG TTGCAGTGAG CCGAGATTGC
AluSg (INT11 NBR1) .......C.G ...G.A.... .......... ..T....CAT

AluSc (INT7 BRCA1) GCCACTGCAC TCCAGCCTGG -CGACAGAGC GAGACTCCGT
AluSg (INT11 NBR1) .......... .......... G......... ........A.

AluSc (INT7 BRCA1) CTCAAAAAAA AAAAAAAAAA A
AluSg (INT11 NBR1) .......... .......... .
```

b

```
AluJb (INT10) GGCCAGGCAC AGTGGCTCAA GCCTGTAACT GCAGCACTTT GGGAGGCTGA
AluY (INT15)  ....GA.... G........C A.......TC C......... .......C..
Alu insertion ---------- ---------- ---------- ---------- ----------

AluJb (INT10) AGTGGGCAGA CTGCTTGAGT CCAGGAGTTT GAGACCAGCC TCGGTAACAT
AluY (INT15)  GAC...TG.. TCA.--...G T......A.C A......T.. .G.C.....C
Alu insertion ---------- ---------- ---------- ---------- -------A.C

AluJb (INT10) GGTGAAACCC CATCTCTA-T AAAACCACAA A-----AAAT TAACCGGGTG
AluY (INT15)  A......... TG......C. G...AT.... .AAAAA.... ..G.T...C.
Alu insertion .......... TG......C. G...AT.... .AAAAA.... ..G.T...C.

AluJb (INT10) TGGTGGCATG CGCCTGTAGT CCCAGCTACT CAGGAGGCTG AAGTGGGAGG
AluY (INT15)  .......GG. .......... ...G...... AG........ .G.CA..G.A
Alu insertion .......GG. .......... .......... TG........ .G.CA....A

AluJb (INT10) ATAGCTTGAG CCTTGGAGGT GGAGGTTGCA GTGAGCCAAG ATTGCATCAC
AluY (INT15)  ..G..G..TA ..CG.....C A...C..... ......TG.. ....AGC..T
Alu insertion ..G..G...A ..CG.....C ....CA.... .......G.. .....GC...

AluJb (INT10) TGCACTCCAG CCTGGGCAAC AGAGTGAGAC CCCATCTCAA AAAAAAACAA
AluY (INT15)  ....T..... .......... ...AC..... T..G...... .....T.A.T
Alu insertion .......... .......G.. ....C..... T..G...... .......A..

AluJb (INT10) ACAAACAA-- ---------- ---------- ---------- --------
AluY (INT15)  .A.T.A..-- ---------- ---------- ---------- --------
Alu insertion .A...A..AA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAA
```

Figure 2 Alignment of the Alu elements in the BRCA1 rearrangements. (**a**) Alignment of the Alu elements involved in the *BRCA1* c.441 + 1724_oNBR1:c.1073 + 480del rearrangement, namely, an AluSc in *BRCA1* intron 7 (INT7) and an AluSg *in NBR1* intron 11 (INT11). (**b**) Alignment of the Alu elements involved in the *BRCA1* c.671-319_4677-578delinsAlu rearrangement, an AluJb in *BRCA1* intron 10 (INT10), an AluY in intron 15 (INT15) and the Alu element inserted (Alu insertion). Each dot stands for the same nucleotide and dashes indicate gaps introduced so as to maximize alignment.

bp overlapping sequence at the breakpoint junction. We hypothesize that this sequence contains a recombinogenic hotspot as assumed for other sequences in Alu elements, as also postulated before.[18,19] Rüdiger *et al.*,[19] suggested that four different rearrangements in the low density lipoprotein receptor gene presented a 26-bp common sequence that functions as a recombinational hotspot. This core sequence contains a pentanucleotide motif (CCAGC), which is also part of *chi*, an 8-bp sequence known to stimulate *recBC*-mediated recombination in *Escherichia coli*.[19] We compared our 20-bp overlapping sequence with the sequences involved in the 81 *BRCA1* rearrangements described by Sluiter and van Rensburg.[4] This 20-bp sequence was observed (up to a maximum of three mismatches without affecting the pentanucleotide motif) at the breakpoint region in four rearrangements (NG_005905.2: g.114552_121641del, g.148011_151126del, g.153110_161767dup and g.166375_170153delins), supporting the possibility that these rearrangements were induced by a specific sequence with a recombinogenic nature (Figure 3).[4,19] However, this 20-bp sequence is highly conserved as it is present in the consensus sequence of the three major Alu subfamilies (J, S and Y), which alone can explain the high incidence of homologous recombination involving these sequences.[15] A deletion of exons 1–7 of the *BRCA1* gene was previously described by Engert *et al.*,[20] but the genomic breakpoint was not determined. However, they confirmed by comparative genomic hybridization that the minimum size of the deletion was about 200 kb and the maximum size was about 285 kb encompassing the entire *NBR1* region, so one can assume that the *BRCA1* c.441 + 1724_o*NBR1*:c.1073 + 480del we here report is a novel genomic rearrangement. This *BRCA1* LGR encompasses three more *loci*, namely, *NBR1*, *ΨBRCA1* and *NBR2*. No recognizable biological function has been assigned to *ΨBRCA1* and *NBR2*,[21] whereas the *NBR1* gene encodes a highly conserved multidomain scaffold protein involved in selective autophagy.[22] The family here reported does not show any remarkable phenotypic trait apart from being predisposed to breast and ovarian cancers, indicating that the relevant genetic defect is the inactivation of the *BRCA1* gene. A contiguous gene deletion syndrome was also excluded by Gad *et al.*,[23] in one family presenting a *BRCA1* deletion of exons 1–22 that also encompassed the *NBR1*, *ΨBRCA1* and *NBR2* genes. The absence of mutations in the *NBR1* and *NBR2* genes in breast and ovarian cancer patients also indicates that *BRCA1* inactivation is the relevant consequence of this LGR.[24,25]

The second rearrangement fully characterized at the nucleotide level was the *BRCA1* mutation c.671-319_4677-578delinsAlu, previously described as EX11_15del by our group.[11] The 3′ breakpoint occurred in an AluY element and the 5′ breakpoint was 120 bp downstream from a second Alu element (AluJb) in the *BRCA1* gene. In addition, an ~250-bp Alu fragment was inserted at the breakpoints of the deleted region. This Alu fragment presents a high homology to the intron 15 breakpoint region of the *BRCA1* gene and, according to the Censor Server and RepeatMasker software, this sequence is an Alu element belonging to the AluY family. Alu-mediated unequal homologous recombination is the most common mechanism causing *BRCA1* LGRs. The deletion/insertion event here described could be due to homologous recombination with exchange of genetic material.[26] However, another possible explanation for the observed rearrangement could involve an insertion-mediated deletion mechanism caused by the retrotransposition of Alu elements at this genomic site leading to the deletion of adjacent genomic regions.[26–29] This hypothesis is corroborated by the fact that the inserted Alu element belongs to a still active AluY family. There are only a few reports of large deletions caused by the retrotransposition of an Alu element: for instance, a complex deletion–insertion rearrangement has been reported in a patient with lipoprotein lipase deficiency disorder caused by an Alu retrotransposition-mediated mechanism[29] and the *TP53* gene has been shown to be rearranged by a complex rearrangement resulting in deletion of exons 2–4 with an insertion of a truncated Alu fragment.[26] Only eight different deletion–insertion rearrangements have been reported in *BRCA1*, but none attributable to an Alu-mediated retrotransposition mechanism,[4] so this would be

***BRCA1* exons 8-9 deletion (g.114552_121641del)**

AluSx INT7

tctttttttttttttttttgagacagagtctcgctctgtcgcccaggctggag
cgcagtgacacgatgtcagctcactgcaacctccgcctcccaggttcaagtgat
tctcctgcctcagcctcctgagtagttgggactacaggggtacgacaccagacc
tggctaatttttgtaatttagtagagtcggggtttcaccatattggtcaggct
ggtctcgaactcctgacctcaggtgatccaccctccttggcctcccagagtgct
gggattacaggcgtgagccaccaagcccggcca

AluSx INT9

tctttttttttttttttttgagacagagtcttgctctgttgcccaggctggag
tacagaggtgtgatctcacctctccgcaacgtctgcctcccaggttgaagccat
actcctgcctcagcctctctagtagctgggactacaggcgcgcgccaccacacc
cggctaatttttgtatttttagtagagatggggtttcaccatgttggccaggct
ggtcttgaactcatgacctcaagtggtccacccgcctcagcctcccaaagtgct
ggaattacaggcttgagccaccgtgcccagc

***BRCA1* exons 18-20 duplication (g.153110_161767dup)**

AluY INT17

gccaagcgtggtggctcaggcctgtaatcccagcgctttgggggccaaggcgg
gcagatcacgaggtcaggagatcgagaccatcctggctaacacggtgaaaccccc
atctctagtaaaaatacaaaaaattagccgggcatggtggcgggcatctgtagt
cccagctactcaggaggctgaggcaggagaatggcatgaacccaggaggcagag
cttgccgtgagctgagatccagccactgcactccagcctggctgacagagcaag
actgcatctcaaaaaaaaaaaaaaaaaaaa

AluJb INT20

ggccaggcacagcggctcgttcctgttatcccagcactttgggaagctgaggca
ggcagatcgcttgagccaaggagttcgataccagcctgggcaacatggcaaaac
cccatctctaccaaaaaaaatatacaaaaattagccaggcgtggtggcatgtac
ttgtagttccagctactcggaaggctgagttgagagtatctcttgagcccaaga
agaggggactacagtgaacggagattgcgccactgcactccagcctagacgaca
gacagaagatctcaaaagaaaaaaaaaaaaaa

***BRCA1* exon 17 deletion (g.148011_151126del)**

AluSp INT16

ggccgggcatggtggctcaagcctgtaatcccagcactttgggaggctgaggtg
gtcagatcacctaaggtcaggagatcaagaccagcctgaccaacatgaagaaac
cccatctttactaaaaatacacaattagccgggcgtggtggcgcatgcctataa
tcccagctactcaggaggctgaggcaggagaattgcttgaacccgggaggcgga
ggttgcggtgagccgagattgcaccattgcactccagcctgggcaacaagagcg
aaactctgtctcaataataagaagaagaa

AluSq INT17

ggccaggcacggtggctcacacctgtaatctcagcactttgggaggccgaggcg
ggtggatcacctgaggtcaggagttcgagaccagcctggccaacgtgttgaaac
cccgtctctactaaaaatataaaaattaggtgggcgtggtggcaggcacctgta
atcccagctactcaggaggctgaggcagcagaatcgcttgaacccaggaggtgg
aggttgcagtgacccaagatcgccaccattgcactccagcctgggacaagagcg
agattcttgtctcaaaaaaaaaaaaaaaaaaaa

***BRCA1* exons 21-22 delins (g.166375_170153delins)**

AluJb INT22

tgctgggtgcggtggctcatgcctataatcccgggggtgaagttgagcccagga
gtttgagaccagcctgggcaacatggcaaaaccctgtctctaccaaaaatacaa
aaaaattagccaggggtggtggtacgtgtctgtagttccagctacttaggaggc
tgagatggaaggattgcttgagcccaggaggcagaggtggcagtgagctgagat
cacaccactgcactccagcctgggtgacagagcaagaccctgtctcaaaaacaa
acaaaaaaaa

AluSx INT20

tctttgttaatttatttttcgggccagagtcttactctgtcaccaggctggag
tgcagtgccactatctctgctcactgcaaccttcacctcccaagttcaaacctt
gttcaattcttgtgccttggcctcccaagtggctaggattacaggcatgtgcca
caacaactagctaattttt

Figure 3 Flanking sequences of four different *BRCA1* gene rearrangements presenting the 20-bp overlapping sequence (gray colored boxes) near or at the breakpoint region, which have been previously reported. The genomic breakpoints (underlined) were obtained from Sluiter and van Rensburg using the RefSeqGene record, NG_005905.2.[4]

the first description of such a mechanism of mutation of the *BRCA1* gene. The deletion of exons 11–15 of the *BRCA1* gene was also described by de la Hoya *et al.*;[30] however, the mutational mechanism and the genomic breakpoint (L78833:g.33450_56562del) are different from those we here report, emphasizing the importance of breakpoint characterization to determine whether rearrangements involving the same exons are related or have occurred *de novo*.

In conclusion, we have identified the breakpoints of two novel large deletions involving the *BRCA1* gene, one encompassing exons 1–7 in addition to the two adjacent genes *NBR2* and *NBR1* (c.441 + 1724_o*NBR1*:c.1073 + 480del) and another comprising exons 11–15 (c.671-319_4677-578delinsAlu). Both rearrangements most likely were mediated by Alu elements, either by homologous recombination between Alu repeats or by the retrotransposition of an Alu element.

ACKNOWLEDGEMENTS

This work was funded by a grant awarded by Fundação Professor Ernesto Morais.

1 Smith, T. M., Lee, M. K., Szabo, C. I., Jerome, N., McEuen, M., Taylor, M. *et al.* Complete genomic sequence and analysis of 117kb of human DNA containing the gene BRCA1. *Genome Res* **6,** 1029–1049 (1996).
2 Welcsh, P. L. & King, M. C. BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. *Hum. Mol. Genet* **10,** 705–713 (2001).
3 Mazoyer, S. Genomic rearrangements in the BRCA1 and BRCA2 genes. *Hum. Mutat* **25,** 415–422 (2005).
4 Sluiter, M. D. & van Rensburg, E. J. Large genomic rearrangements of the BRCA1 and BRCA2 genes: review of the literature and report of a novel BRCA1 mutation. *Breast Cancer Res. Treat* **125,** 325–349 (2011).
5 Hogervorst, F. B., Nederlof, P. M., Gille, J. J., McElgunn, C. J., Grippeling, M., Pruntel, R. *et al.* Large genomic deletions and duplications in the BRCA1 gene identified by a novel quantitative method. *Cancer Res* **63,** 1449–1453 (2003).
6 Petrij-Bosch, A., Peelen, T., van Vliet, M., van Eijk, R., Olmer, R., Drusedau, M. *et al.* BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients. *Nat. Genet* **17,** 341–345 (1997).
7 Weitzel, J. N., Lagos, V. I., Herzog, J. S., Judkins, T., Hendrickson, B., Ho, J. S. *et al.* Evidence for common ancestral origin of a recurring BRCA1 genomic rearrangement identified in high-risk Hispanic families. *Cancer Epidemiol. Biomarkers Prev* **16,** 1615–1620 (2007).
8 Teugels, E., De Brakeleer, S., Goelen, G., Lissens, W., Sermijn, E. & De Greve, J. De novo Alu element insertions targeted to a sequence common to the BRCA1 and BRCA2 genes. *Hum. Mutat* **26,** 284 (2005).
9 Peixoto, A., Santos, C., Rocha, P., Pinheiro, M., Principe, S., Pereira, D. *et al.* The c.156_157insAlu BRCA2 rearrangement accounts for more than one-fourth of deleterious BRCA mutations in northern/central Portugal. *Breast Cancer Res. Treat* **114,** 31–38 (2009).
10 van den Ouweland, A. M., Dinjens, W. N., Dorssers, L. C., van Veghel-Plandsoen, M. M., Bruggenwirth, H. T., Withagen-Hermans, C. J. *et al.* Deletion of exons 1a-2 of BRCA1: a rather frequent pathogenic abnormality. *Genet. Test. Mol. Biomarkers* **13,** 399–406 (2009).
11 Peixoto, A., Salgueiro, N., Santos, C., Varzim, G., Rocha, P., Soares, M. J. *et al.* BRCA1 and BRCA2 germline mutational spectrum and evidence for genetic anticipation in Portuguese breast/ovarian cancer families. *Fam. Cancer* **5,** 379–387 (2006).
12 Jurka, J., Kapitonov, V. V., Pavlicek, A., Klonowski, P., Kohany, O. & Walichiewicz, J. Repbase update, a database of eukaryotic repetitive elements. *Cytogenet. Genome Res* **110,** 462–467 (2005).
13 Kariya, Y., Kato, K., Hayashizaki, Y., Himeno, S., Tarui, S. & Matsubara, K. Revision of consensus sequence of human Alu repeats-a review. *Gene* **53,** 1–10 (1987).
14 Prak, E. T. & Kazazian, Jr H. H. Mobile elements and the human genome. *Nat. Rev. Genet* **1,** 134–144 (2000).
15 Batzer, M. A., Deininger, P. L., Hellmann-Blumberg, U., Jurka, J., Labuda, D., Rubin, C. M. *et al.* Standardized nomenclature for Alu repeats. *J. Mol. Evol* **42,** 3–6 (1996).

16 Kapitonov, V. & Jurka, J. The age of Alu subfamilies. *J. Mol. Evol* **42,** 59–65 (1996).
17 Cordaux, R. The human genome in the LINE of fire. *Proc. Natl Acad. Sci. USA* **105,** 19033–19034 (2008).
18 Palanca, S. S., Esteban, C. E., Barragán, G. E., Oltra, S. S., de Juan, J. I., Chirivella, G. I. *et al.* Identification of a novel BRCA1 large genomic rearrangement in a Spanish breast/ovarian cancer family. *Breast Cancer Res. Treat* **112,** 63–67 (2008).
19 Rüdiger, N. S., Gregersen, N. & Kielland-Brandt, M. C. One short well conserved region of Alu-sequences is involved in human gene rearrangements and has homology with prokaryotic chi. *Nucleic Acids Res* **23,** 256–260 (1995).
20 Engert, S., Wappenschmidt, B., Betz, B., Kast, K., Kutsche, M., Hellebrand, H. *et al.* MLPA screening in the BRCA1 gene from 1,506 German hereditary breast cancer cases: novel deletions, frequent involvement of exon 17, and occurrence in single early-onset cases. *Hum. Mutat* **29,** 948–958 (2008).
21 Jin, H., Selfe, J., Whitehouse, C., Morris, J. R., Solomon, E. & Roberts, R. G. Structural evolution of the BRCA1 genomic region in primates. *Genomics* **84,** 1071–1082 (2004).
22 Johansen, T. & Lamark, T. Selective autophagy mediated by autophagic adapter proteins. *Autophagy* **7,** 279–296 (2011).
23 Gad, S., Bieche, I., Barrois, M., Casilli, F., Pages-Berhouet, S., Dehainault, C. *et al.* Characterisation of a 161 kb deletion extending from the NBR1 to the BRCA1 genes in a French breast-ovarian cancer family. *Hum. Mutat* **21,** 654 (2003).
24 Campbell, I. G., Nicolai, H. M., Foulkes, W. D., Senger, G., Stamp, G. W., Allan, G. *et al.* A novel gene encoding a B-box protein within the BRCA1 region at 17q21.1. *Hum. Mol. Genet* **3,** 589–594 (1994).
25 Xu, C. F., Brown, M. A., Nicolai, H., Chambers, J. A., Griffiths, B. L. & Solomon, E. Isolation and characterisation of the NBR2 gene which lies head to head with the human BRCA1 gene. *Hum. Mol. Genet* **6,** 1057–1062 (1997).
26 Slebos, R. J., Resnick, M. A. & Taylor, J. A. Inactivation of the p53 tumor suppressor gene via a novel Alu rearrangement. *Cancer Res* **58,** 5333–5336 (1998).
27 Chen, J. M., Stenson, P. D., Cooper, D. N. & Ferec, C. A systematic analysis of LINE-1 endonuclease-dependent retrotranspositional events causing human genetic disease. *Hum. Genet* **117,** 411–427 (2005).
28 Gilbert, N., Lutz-Prigge, S. & Moran, J. V. Genomic deletions created upon LINE-1 retrotransposition. *Cell* **110,** 315–325 (2002).
29 Okubo, M., Horinishi, A., Saito, M., Ebara, T., Endo, Y., Kaku, K. *et al.* A novel complex deletion-insertion mutation mediated by Alu repetitive elements leads to lipoprotein lipase deficiency. *Mol. Genet. Metab* **92,** 229–233 (2007).
30 de la Hoya, M., Gutierrez-Enriquez, S., Velasco, E., Osorio, A., Sanchez de Abajo, A., Vega, A. *et al.* Genomic rearrangements at the BRCA1 locus in Spanish families with breast/ovarian cancer. *Clin. Chem* **52,** 1480–1485 (2006).