MUTATION IN BRIEF

De Novo Alu Element Insertions Targeted to a Sequence Common to the BRCA1 and BRCA2 Genes

Erik Teugels[1,2*], Sylvia De Brakeleer[1], Guido Goelen[2], Willy Lissens[3], Erica Sermijn[2], and Jacques De Grève[1,2]

[1] Laboratory of Molecular and Medical Oncology, AZ-VUB, Vrije Universiteit Brussel, Laarbeeklaan 101, B1090 Brussels, Belgium; [2] Familial Cancer Clinic, AZ-VUB, Laarbeeklaan 101, B1090 Brussels, Belgium; [3.]Laboratory of Medical Genetics, AZ-VUB, Laarbeeklaan 101, B1090 Brussels, Belgium

*Correspondence to: Erik Teugels, Laboratory of Molecular and Medical Oncology (EMGE), Vrije Universiteit Brussel (AZ-VUB), Laarbeeklaan 101, 1090 Brussels; E-mail: eteugels@az.vub.ac.be

Grant sponsor: FWO; grant number: G.0502.05

Communicated by Haig H. Kazazian, Jr.

Linkage analysis suggests that mutations in the BRCA1 and BRCA2 genes are responsible for cancer predisposition in more than 80% of the families with high incidence of breast/ovarian cancer. However, pathogenic mutations in the BRCA1/2 genes are generally identified in much less than half of the families investigated in a diagnostic setting with the currently used PCR-based screening protocols. Here we report the identification of two different de novo Alu element insertions within the BRCA1/2 coding sequences in three out of the 50 families in which we found a cancer predisposing mutation, suggesting that this type of mutation is much more common than suggested by their occurrence in mutation databases. The Alu insertion in the BRCA2 gene resulted in the removal of the targeted exon from the corresponding mRNA molecule. Unexpectedly the Target Site Duplications generated by both Alu element insertions contained a specific 9 bp long segment, which might eventually serve as a recognition site for the transposition machinery. Finally, in contrast to the disease causing Alu insertions reported to date, the transposon identified in the BRCA1 gene does not belong to a "young" AluY but to an AluS subfamily, indicating that some of these "old" Alu elements, which are supposed to be non-functional fossil relics, are still able to retrotranspose in vivo. © 2005 Wiley-Liss, Inc.

KEY WORDS: retrotransposition; Alu element; BRCA1; BRCA2

INTRODUCTION

Repetitive sequences are dispersed throughout the human genome and were acquired by transposition (reviewed in Prak and Kazazian 2000). The bulk of human repetitive sequences consists mainly of Line-1 (L1) and Alu elements, and a detailed analysis has revealed the presence of more than one million Alu copies, which corresponds to more than 10% of the genome (International Human Genome Sequencing Consortium, 2001). Only a very small fraction of these repetitive sequences are still able to actively transpose. Full length L1 elements are about 6kb long and encode the proteins necessary for autonomous retrotransposition (Scott et al. 1987). Alu elements are much smaller, 300-350bp long, and lack any protein-coding capacity. It has been demonstrated that

Received 27 January 2005; accepted revised manuscript 18 May 2005.

DOI: 10.1002/humu.9366

Alu elements parasitize the L1-encoded transposition machinery (Dewannieux et al. 2003). Jurka (1997) proposed a molecular mechanism for L1 and Alu elements insertion, which is mainly based on the model for R2Bm retrotransposition in Bombyx mori (Luan et al. 1993). R2Bm elements insert at a very specific site in the 28S rRNA genes. In contrast, L1 and Alu elements are believed to insert randomly (Ovchinnikov et al. 2001) although Jurka (1997) pointed to the existence of a (relatively poorly) conserved first nick signal (5'-TTAAAA-3') that is cleaved between the A and the T on the bottom strand.

These "jumping genes" can cause disease by altering the genome in different ways (Prak and Kazazian 2000). Because Alu and L1 elements are very abundant in the human genome, mispairing of homologous sequences may frequently occur, eventually generating unequal crossing-overs resulting in deletions or duplications of genomic sequences. The intronic sequences of the breast cancer susceptibility gene BRCA1 (MIM# 113705; GenBank: U14680; GDB: 126611) are very rich in Alu elements (Smith et al. 1996), and several examples of Alu element mediated deletions or duplications, including whole exons, have been reported (e.g. Puget et al. 1997; Swensen et al. 1997). In the Netherlands, 36% of the families with a BRCA1 mediated breast cancer predisposition carry a mutation of this type (Petrij-Bosch et al. 1997). De novo insertion of an Alu element into (or at proximity of) the coding region of a gene can also have functional implications, but until now only about 20 such examples have been reported worldwide in the germ cell lineage (Deininger and Batzer 1999; Ostertag and Kazazian 2001; http://129.81.225.52).

We could identify a BRCA1/2 cancer predisposing mutation in 50 families (Goelen et al. 1999, and unpublished results). Among the 35 different mutations we isolated, 2 resulted from an Alu element insertion, one in BRCA1 and one in BRCA2 (MIM# 600185; GenBank: U43746; GDB: 387848). Here we describe the unusual characteristics of these 2 particular mutations.

MATERIALS AND METHODS

DNA was extracted from EDTA blood samples using the QIAamp DNA blood midi kit (Qiagen). Control samples from BRCA1 mutation carriers were obtained from our own patients (c.1687C>T) or kindly provided by Dr. M. Ligtenberg (c.1881_1884del).

The standard screening protocol for BRCA1/2 consisted of a Protein Truncation Test (PTT, see Hogervorst et al. 1995) on exon 11 of BRCA1 and exons 10 & 11 of BRCA2 (TNT-T7 coupled reticulocyte lysate system from Promega). In addition, all remaining small exons as well as the 5' and 3' borders of the larger exons were analyzed by Conformation Sensitive Gel Electrophoresis (CSGE, see Ganguly et al. 1998). The primers used for PCR amplifications are described in the BIC. PCR fragments generating abnormal migration patterns after electrophoresis were purified (high pure PCR product purification kit from Roche) and submitted to a direct sequence analysis (sequenase version 2.0 DNA sequencing kit from USB) to identify the putative cancer predisposing mutation.

To PCR amplify the exon 3 region from BRCA2 the reaction conditions were 35 cycles of 1 min. at 94°C, 1 min. at 55°C and 1 min. at 72°C. To PCR amplify the 5' half of exon 11 we used BR11F1 as forward primer and BR11R2 as reverse primer (Hogervorst et al. 1995). The reaction consisted of 5 cycles of 1 min. 94°C, 1 min. 50°C and 3 min. 72°C followed by 35 cycles of 1 min. 94°C, 1 min. 60°C and 3 min. 72°C. To specifically amplify (by nested PCR) the BRCA1 allele bearing the Alu element we used in the first step primers BR11F1 and BR11R2, and the second step primer set 5' CAAACGGAGCAGAATGGTCA 3' and 5' TTTTTAGTAGAGACGGGGTTTC 3'. All PCRs were initiated with 1 min. incubation at 94°C and concluded by incubation at 72°C for 10 min. The Taq DNA polymerase was from Qiagen.

For Southern blot analysis of BRCA2, the protocol described by Petrij-Bosch et al. (1997) was followed with small modifications. DNA was digested with BglII or EcoRI (from Amersham). As probe we used a RT-PCR fragment obtained with the following nested primer sets: F1 5' CGCGAGCTTCTGAAACTAGG 3', R1 5' GCAATAGGGGTATTTTCTCCATC 3', F2 5' ATGCCTATTGGATCCAAAGAG 3' (target site located within exon 2) and R2 5' CACATTCATCAGCGTTTGCTTC 3' (target site located in the 5' region of exon 11). The reaction conditions in the first PCR were 35 cycles of 1 min. 94°C, 1 min. 59°C and 3 min. 72°C and for the second PCR 30 cycles of 1 min. 94°C, 1 min. 60°C and 3 min. 72°C. Purified fragments were ^{32}P labeled using the Random primed DNA labeling kit (Boehringer) according to the manufacturer's protocol. Membranes were exposed with intensifying screens to Kodak X-omat autoradiograms for at least 4 days at –80°C.

The nucleotide sequence of the two Alu elements was determined using an ABI3130 Genetic Analyser (Applied Biosystems, Foster City, Ca)

RESULTS

Alu element insertion in the BRCA2 gene

Index patient from family 059 is a Caucasian of Portuguese origin who belongs to a family with 6 cases of breast cancer and 1 ovarian cancer. She developed breast cancer at age 46. In addition, an abnormally high incidence of other cancers (including intestinal, brain, skin and several tongue cancers) was observed in male relatives. A BRCA1/BRCA2 mutation screen was initiated on the index patient but no sequence abnormalities were detected with the standard screening protocol (see materials and methods). However, by Southern blot analysis we observed an insertion (about 350 bp long) within or close to exon 3 of BRCA2 (Fig. 1a). The PCR product covering exon 3 on which a CSGE analysis had been performed did not reveal an additional band when analysed on agarose gel. However, when adjusting the PCR conditions (by shifting the

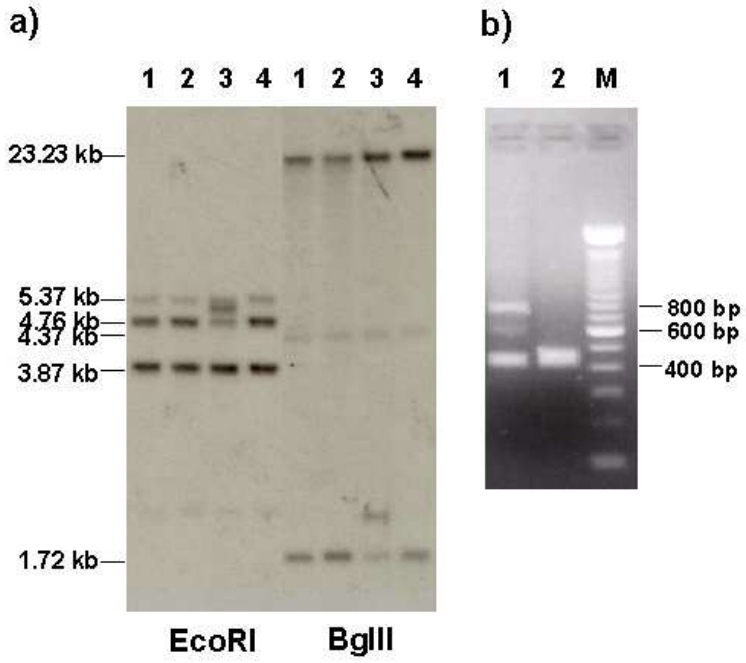

Figure 1. (**A**) Southern blot analysis of genomic DNA samples from four patients with a probe covering exons 2-9 of *BRCA2*. In lane 3 an additional band is observed resulting from the insertion of a DNA fragment about 350 bp long within or close to exon 3 of *BRCA2*. (**B**) Agarose gel electrophoresis of the PCR products obtained after amplification of exon 3 and flanking regions from *BRCA2*. The same DNA template obtained from patient 3 (see Southern blot) was used both in lane 1 and 2, but the elongation time during the PCR was 4 minutes in lane 1 and 1 minute in lane 2.

elongation time from 1 to 4 minutes) both the mutant and wild type allele could be observed (Fig. 1b). Amplicons corresponding to the mutant allele were purified from the agarose gel and subsequent PCR/sequence analysis led to the identification of the inserted DNA as an Alu element of the AluYa5 subtype (Fig. 2) that was located within exon 3 of BRCA2 (c.156_157insAlu). Subsequent RT-PCR analysis revealed that the Alu insertion resulted in the removal of exon 3 from the spliced transcript (result not shown). Blood samples were further collected from 24 relatives to investigate whether the mutation co-segregates with the cancer phenotypes. Two of the seven family members with breast or ovarian cancer were proven mutation carriers, while a third one was an obligate carrier (one of her children had the mutation). Samples from the remaining four breast cancer patients were not available. On the other hand, other cancers in male relatives were not linked to this mutation since none of the 3 patients tested carried the mutation.

a)

```
                                  1739
BRCA1   agaatcactcGAAAAAGAATCTGCTTT [ Alu-polyA > tttGAAAAAGAATCTGCTTTcaaaacgaaa

                                156
BRCA2   gaacctgcagAAGAATCTGAACAT [ Alu-polyA > AAGAATCTGAACATaaaaacaaca
```

b)

```
BRCA1   aatcctaaacccaatagaatcactc/GAAAAAGAATCTGCTTTcaaaacgaaagctgaacctataagc
BRCA2   ccaccctataattctgaacctgcag/AAGAATCTGAACATaaaaacaacaattacgaaccaaacc
First nick consensus site     tt/AAAA
```

c)

```
AluSq    ggccgggcgcggtggctcacgcctgtaatcccagcactttgggaggccgaggcgggTggatcaCCTgag
BRCA1    t...a.....................................................--a..
AluSp    ..................................................c.............
BRCA2    t............c.....................................c.......--...
AluYa5   ..................................................c.......--...

AluSq    gTcAGgagTtcgagaccaGccTgGCCaaCaTGgTgaaaccccgtctctacTaaaaAtacaaaaa----T
BRCA1    ...ga...a.........c.............................c......g......aaaa.
AluSp    ...g..................a.........a..................................
BRCA2    ........a....c....t..c...tg.a.c.................................a....
AluYa5   ........a.........t..c...t..a.c.................................a....

AluSq    tagcCgggcgTGgtggcgGGCgcctgtaAtccCagctactCgggaggctgaggcaggagAatCgcTtga
BRCA1    .....a...a.......a..t.....................t.............................
AluSp    ...................cat.....................................................
BRCA2    ..........a.............g..........t.....................g..g...
AluYa5   ..........a.............g..........t.....................g..g...



AluSq    AcccgggaGgCGgagGTtgcAgtgagccgAgatCGcgccaCtgcaCtccagcctgggcAacaAgagcga
BRCA1    ...................g.......a.....a.....t...................a.....t..
AluSp    ...................g...................t.........................
BRCA2    ..............c..................c.......................g...-......
AluYa5   ..............c..................c.......................g...-......

AluSq    AacTcCgtctca
BRCA1    .....t......
AluSp    ............
BRCA2    g......c....
AluYa5   g...........
```

Figure 2. Description of the two Alu element insertions isolated in the *BRCA1* and *BRCA2* genes. (**A**) Both Alu elements are inserted in the same orientation as their target gene, between positions 1739 and 1740 within exon 11 of *BRCA1* and between cDNA positions 156 and 157 within exon 3 of *BRCA2*. Note the presence of a short sequence duplication (the TSD, represented in uppercase) flanking both Alu elements that is respectively 17 and 14 bp long. (**B**) Representation of the wild type sequences of *BRCA1* and *BRCA2* that are targeted by the Alu retrotransposons. The previously described consensus site for the nicking activity (Jurka 1997) is also indicated, the "/" is at the position were the first nick is made during the retrotransposition process (the real nick occurs on the reverse strand). Note the presence of an identical 9 bp long motif (underlined) located within the TSD and present in both target sites but at variable distance from the nicking site. (**C**) Alignment of the Alu sequences identified in the BRCA genes with Alu consensus sequences from the AluSq, AluSp and AluYa5 sub-families (RepBase at www.girist.org/Repbase_Update.html). Each dot stands for the same nucleotide as the one indicated in the consensus AluSq sequence. The diagnostic nucleotides (the nucleotides that enable to discriminate the different Alu sub-families) are in uppercase.

Alu element insertion in the BRCA1 gene

Index patient from family 279 is of Belgian origin and belongs to a family with four cases of breast cancer. She had breast cancer at age 33, while her deceased mother had bilateral BC and ovarian cancer. When performing the diagnostic mutation screen, the PTT revealed a clear additional band expected to correspond to a truncated mutant protein (Fig. 3a). However, attempts to identify the mutation by sequence analysis using purified PCR fragments as template repeatedly failed. The analysis of a blood sample from an affected aunt of the index patient led to an identical result. When performing a second PTT experiment in which 2 control samples were included from patients harboring known BRCA1 mutations that generate truncated proteins of similar sizes (the aim was to better define the location of the putative mutation), we noticed that the ratio of truncated over wild type protein was significantly lower in the test sample compared to the 2 control samples, suggesting that the allele carrying the unknown mutation was less efficiently amplified than the wild type allele (Fig. 3b). Under-representation of the mutant allele within the template DNA used in the sequencing reaction might thus also be the reason why we at first failed to detect the mutation. Having experienced similar problems when identifying the previously described mutation in BRCA2 (c.156_157insAlu), we hypothesized that the putative mutation could result from the insertion of a relatively large DNA fragment, possibly an Alu repeat. Indeed, the mutant allele could be specifically PCR amplified when using a primer combination including one primer specific for the BRCA1 sequence and the other specifically recognizing Alu sequences (results not shown). The mutation was finally identified as an Alu element insertion belonging to the AluSp/AluSq family (c.1739_1740insAlu, Fig. 2). The same Alu insertion was subsequently identified in another apparently unrelated Belgian family and in both families the mutation co-segregated with the disease since the 4 cancer patients tested carried the mutation (results not shown). The nucleotide sequence of the Alu element present in the BRCA1 gene, as well as the flanking short target site duplications (TSD), were identical in both families. In addition, using a set of polymorphic markers located close to or within the BRCA1 gene (D17S855, D17S1322, D17S1323 and D17S1327) we could show that the allele carrying the mutation shared a common haplotype in both families (results not shown). Together, these data suggest that these families have a common ancestor, although this could not be confirmed by pedigree analysis. The effect of the Alu insertion in the BRCA1 gene was further investigated at the mRNA level but the obtained results were not conclusive.

DISCUSSION

Although linkage analysis suggests that BRCA1/2 mutations must be responsible for breast/ovarian cancer predisposition in more than 80% of the families with high incidence of the disease (Ford et al. 1998), the mutation detection rates obtained in diagnostic settings with commonly used PCR based screening methods are usually much lower. Because of less stringent intake criteria used in diagnostic settings, clustering of sporadic breast cancer cases may frequently occur. In addition, mutations in other cancer predisposing genes may contribute to the cancer phenotype. However, the efficiency of the diagnostic BRCA1/2 screening procedures can also be questioned.

Herein we report on the isolation and characterization of two different de novo Alu insertions in 3 out of 50 families. These mutations were missed with the routinely used PCR based standard screening protocol: one mutation was discovered by Southern blot analysis, while the other was identified using an Alu specific detection method (results not shown). When analysing the PCR products covering the regions wherein the two Alu elements had inserted on agarose gel, we systematically observed that the mutant allele was under-represented or totally absent. Increasing the elongation time could in some cases ameliorate the recovery of mutant amplicons. Since the sizes of the mutant and wild type PCR fragments differ by only 300 bp, one would expect that both alleles can be amplified with the routinely used conditions. However, the two alleles amplify in a competitive manner and small differences in amplification efficiency might result in over-representation of one allele. For instance, if one allele is 10% less efficiently amplified, 40 times less PCR product will be generated after 35 exponential cycles. Increasing the extension time might in turn attenuate the results of this competition. We experienced that especially sequence analysis (in contrast to PTT) is very sensitive to unequal allele representation.

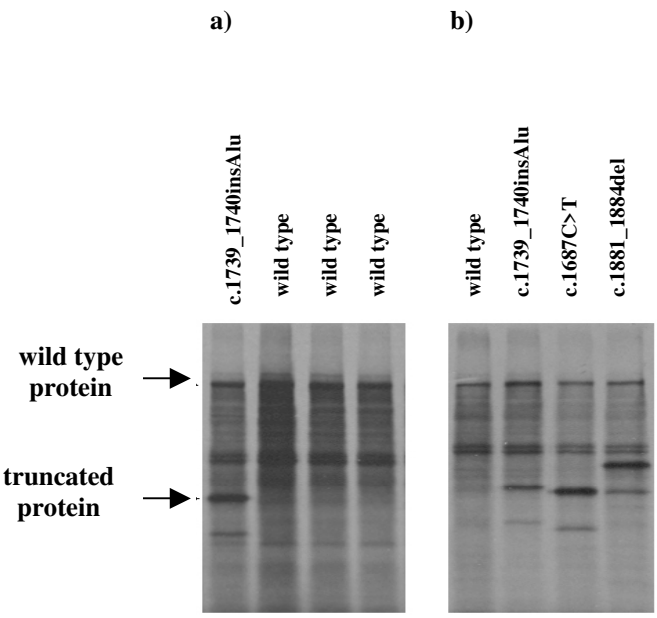

Figure 3. (**a**) Results of a PTT performed on 4 patients and covering the 5' half of exon 11 from *BRCA1*. Three patients show a typical wild type band pattern with a main band corresponding to the in vitro synthesized wild type protein fragment. In the first lane an additional band probably generated by a truncated protein is also clearly observed. (**b**) PTT performed respectively on a wild type control, the patient with the Alu insertion, and two other control patients harboring mutations that generate truncated proteins of similar sizes.

In an attempt to estimate the contribution of de novo Alu/L1 integrations as disease causing mutations, Kazazian Jr (1999) calculated that about 1 mutation out of 600 in humans would result from retrotransposon-mediated insertion. The BIC database (Szabo et al. 2000; http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/) in which the BRCA1 and BRCA2 mutations are registered counts more than 1750 different cancer causing mutations, but only one results from an Alu element insertion (Miki et al. 1996). In contrast, among the 35 different mutations we have identified, 2 resulted from an Alu insertion. This suggests that Alu insertions in the BRCA1/2 genes are likely to be missed with the screening approaches currently used worldwide, and this might by extension be the case for other genes as well. This conclusion is further supported by the fact that this type of mutations is most frequently identified in genes located on the X chromosome of male hemizygotes.

In family 059, the Alu insertion leads to the in-frame deletion of exon 3 in the mutated BRCA2 mRNA. Exon 3 was previously reported to encode a transcriptional activation domain (Milner et al. 1997), and recently this same domain was shown to interact with EMSY, a protein most probably involved in chromatin remodeling that is over expressed in a fraction of sporadic breast cancers (Hughes-Davies et al. 2003). The importance of exon 3 in the tumor suppressor function of BRCA2 is also suggested by Nordling et al. (1998) who described a family harboring a BRCA2 mutation that resulted in the same in-frame exon skipping. In that family as well as in ours, cancer was observed in male family members. There was however no association between mutation carriership and cancer development in males in our family. Using polymorphic markers located on chromosome 10 we could also exclude that a mutation in the PTEN gene (MIM# 601728; GenBank: U93051, NM_000314) is responsible for the occurrence of cancer in male family members (results not shown).

As is typical for Alu and L1 element transposition, the 2 insertions described herein were flanked by a TSD of respectively 17 and 14 bp. The target sequences for the nicking activity showed respectively two and three mismatches with the 6 base pair long consensus nicking signal (5'-TT/AAAA-3') previously reported (Jurka 1997). Most intriguing was the presence, in both Alu target sites and within the TSDs, of a conserved 9 bp long sequence (5'-AAGAATCTG-3'). The orientation of the inserted Alu elements, when compared to this specific sequence, is conserved. Moreover, this specific sequence is located at a short butvariable distance from the nicking site (Fig. 2). Because the probability that this situation would occur by chance equals 1/4855, it is tempting to hypothesize that this 9 bp long sequence includes a specific recognition site for the transposition machinery. However, we could not find this specific sequence in any of the previously reported disease causing de novo Alu insertions. On the other hand, Feng et al. (1996) could purify a L1 encoded endonuclease (L1-EN) with in vitro nicking activity on pBS plasmids and identified a particular motif, present in several copies within the plasmid, that might be involved in the process of nicking site recognition. Interestingly, this sequence (5'-AAAGGATCT-3') presents a good homology with the conserved sequence we have identified. Further investigation of this putative (degenerated?) motif , for instance by monitoring the in vitro nicking activity of the L1-EN enzyme on synthetic DNA templates, might thus contribute to a better understanding of the retrotransposition mechanism in humans.

Alu elements originated in the primate lineage and expanded during the past 65 million years to reach more than one million copies in the actual human genome. However, only a very small fraction of these Alu copies have maintained their ability to retrotranspose, the so-called 'master' or source genes (reviewed in Batzer and Deininger, 2002). Since mutations can accumulate in these master genes and are subsequently transmitted to their transposed copies, primate evolution has led to the generation of several distinct Alu subfamilies that are characterized by a hierarchical series of mutations. The master genes active some 50 million years ago were all of the AluS or AluJ subtypes and are responsible for the large majority (>850.000) of Alu copies currently present in the human genome, while the master genes responsible for the more recently integrated Alu elements belong to one of the AluY subfamilies. The Alu element found in the BRCA2 gene belongs to the AluYa5 subfamily but presents 5 mismatches with the consensus sequence at nondiagnostic positions (including one at position 1). An Alu element located on chromosome 11 presenting only 2 mismatches (at positions 1 and 14) with the element identified in BRCA2 has been reported by Hattori et al. (accession AP003441, position 105369), indicating that these elements must have transposed recently. The Alu element found in the coding sequence of the BRCA1 gene however belongs to the AluSq/Sp subfamily. This was unexpected as these 'old' elements are supposed to be non functional fossil relics that are unable to retrotranspose for some 30 million years. The high number of mismatches when comparing with the AluSq/Sp consensus sequences (about 20) might suggest that this Alu element indeed inserted within the BRCA1 gene long before human speciation. On the other hand, based on the fact that a few intact polymorphic AluS elements (elements with polyA tails and TSDs, and present as a single allelic copy) were found in the human genome, Bennett et al. (2004) concluded that at least some of the AluS copies are likely to have retained the ability to transpose long after the majority of AluS elements became transpositionally inactive. In addition, when screening the gene databases, we found an AluSq element with intact polyA tail and TSDs presenting only one mismatch with the copy we identified in BRCA1 (Muzny et al., accession AC090671, position 26171). Taken together, these data strongly suggest that particular AluS elements are still able to retrotranspose in vivo.

ACKNOWLEDGMENTS

We thank the families for their cooperation. Goele Van Hassel and Kurt De Neef are acknowledged for their technical support. This work was supported by the FWO, the BFTK and the "Wetenschappelijk Fonds Willy Gepts" of the AZ-VUB.

REFERENCES

Batzer MA, Deininger PL. 2002. Alu repeats and human genomic diversity. Nat Rev Genet 3:370-380.

Bennett EA, Coleman LE, Tsui C, Pittard WS, Devine SE. 2004. Natural genetic variation caused by transposable elements in humans. Genetics 168:933-951.

Deininger PL, Batzer MA. 1999. Alu repeats and human disease. Mol Genet Metab 67:183-193.

Dewannieux M, Esnault C, Heidmann T. 2003. LINE-mediated retrotransposition of marked Alu sequences. Nat Genet 35:41-48.

Feng Q, Moran JV, Kazazian HH Jr, Boeke JD. 1996. Human L1 retrotransposon encodes a conserved endonuclease required for retrotransposition. Cell 87:905-916.

Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BA, Gayther SA, Zelada-Hedman M and the Breast Cancer Linkage Consortium. 1998. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 62:676-89.

Ganguly T, Dhulipala R, Godmilow L, Ganguly A. 1998. High throughput fluorescence-based conformation-sensitive gel electrophoresis (F-CSGE) identifies six unique BRCA2 mutations and an overall low incidence of BRCA2 mutations in high-risk BRCA1-negative breast cancer families. Hum Genet 102:549-556.

Goelen G, Teugels E, Bonduelle M, Neyns B, De Grève J. 1999. High frequency of BRCA 1/2 mutations in 42 Belgian families with a small number of symptomatic subjects. J Med Genet 36:304-308.

Hogervorst FB, Cornelis RS, Bout M, van Vliet M, Oosterwijk JC, Olmer R, Bakker B, Klijn JG, Vasen HF, Meijers-Heijboer H, Menko FH, Cornelisse CJ, den Dunnen JT, Devilee P, van Ommen G-JB. 1995. Rapid detection of BRCA1 mutations by the protein truncation test. Nat Genet 10:208-212.

Hughes-Davies L, Huntsman D, Ruas M, Fuks F, Bye J, Chin SF, Milner J, Brown LA, Hsu F, Gilks B, Nielsen T, Schulzer M, Chia S, Ragaz J, Cahn A, Linger L, Ozdag H, Cattaneo E, Jordanova ES, Schuuring E, Yu DS, Venkitaraman A, Ponder B, Doherty A, Aparicio S, Bentley D, Theillet C, Ponting CP, Caldas C, Kouzarides T. 2003. EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer. Cell 115:523-535.

International Human Genome Sequencing Consortiom. 2001. Initial sequencing and analysis of the human genome. Nature 409:860-921.

Jurka J. 1997. Sequence patterns indicate an enzymatic involvement in integration of mammalian retroposons. Proc Natl Acad Sci 94:1872-1877.

Kazazian HH Jr. 1999. An estimated frequency of endogenous insertional mutations in humans. Nat Genet. 22:130.

Luan DD, Korman MH, Jakubczak JL, Eickbush TH. 1993. Reverse transcription of R2Bm RNA is primed by a nick at the chromosomal target site: a mechanism for non-LTR retrotransposition. Cell 72:595-605.

Miki Y, Katagiri T, Kasumi F, Yoshimoto T, Nakamura Y. 1996. Mutation analysis in the BRCA2 gene in primary breast cancers. Nat Genet 13:245-247.

Milner J, Ponder B, Hughes-Davies L, Seltmann M, Kouzarides T. 1997. Transcriptional activation functions in BRCA2. Nature 386:772-773.

Nordling M, Karlsson P, Wahlstrom J, Engwall Y, Wallgren A, Martinsson T. 1998. A large deletion disrupts the exon 3 transcription activation domain of the BRCA2 gene in a breast/ovarian cancer family. Cancer Res 58:1372-1375.

Ostertag EM, Kazazian HH Jr. 2001. Biology of mammalian L1 retrotransposons. Annu Rev Genet 35:501-538.

Ovchinnikov I, Troxel AB, Swergold GD. 2001. Genomic characterization of recent human Line-1 insertions: evidence supporting random integretion. Genome Res 11: 2050-2058.

Petrij-Bosch A, Peelen T, van Vliet M, van Eijk R, Olmer R, Drusedau M, Hogervorst FB, Hageman S, Arts PJ, Ligtenberg MJ Meijers-Heijboer H, Klijn JG, Vasen HF, Cornelisse CJ, van 't Veer LJ, Bakker E, van Ommen GJ, Devilee P. 1997. BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients. Nat Genet 17:341-345.

Prak ET, Kazazian HH Jr. 2000. Mobile elements and the human genome. Nat Rev Genet 1:134-144.

Puget N, Torchard D, Serova-Sinilnikova OM, Lynch HT, Feunteun J, Lenoir GM, Mazoyer S. 1997. A 1-kb Alu-mediated germ-line deletion removing BRCA1 exon 17. Cancer Res 57:828-831.

Scott AF, Schmeckpeper BJ, Abdelrazik M, Comey CT, O'Hara B, Rossiter JP, Cooley T, Heath P, Smith KD, Margolet, L. 1987. Origin of the human L1 element: proposed progenitor genes deduced from a consensus DNA sequence. Genomics 1:113-125.

Smith TM, Lee MK, Szabo CI, Jerome N, McEuen M, Taylor M, Hood L, King MC. 1996. Complete genomic sequence and analysis of 117 kb of human DNA containing the gene BRCA1. Genome Res 6:1029-1049.

Swensen J, Hoffman M, Skolnick MH, Neuhausen SL. 1997. Identification of a 14 kb deletion involving the promoter region of BRCA1 in a breast cancer family. Hum Mol Genet 6:1513-1517.

Szabo C, Masiello A, Ryan JF, The BIC Consortium, Brody LC. 2000. The breast cancer information core: database design, structure and scope. Human Mutation 16:123-131.