The impact of *Alu* elements on human genome and its role in hereditary breast and ovarian cancer (HBOC) syndrome

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Introduction

•Transposable elements (TEs), also called "jumping genes", are pieces of DNA sequence that can move from site to site in (and sometimes between) genomes. The contribution of TEs to the human genome (45%) is remarkable compared with protein-coding regions, which represent about 1.5% of the human genome.

•TEs can be separated in two major classes: DNA transposons and retrotransposons. In the retrotransposons we can find two groups depending on the presence or not of LONG TERMINAL REPEATS (LTRs). However, the most of human TEs are result of non-LTR retrotransposons activity, represented by LINE-1 (or L1 -16,9%-), *Alu* (-10,6%-which belong to SINEs -short interspersed elements) and *SVA* elements (0,2%).

•GOALS of the project:

- **To explain the impact and importance of *Alu* elements on the human genome.**
- **To understand the mechanisms by which they act and their consequences on the integrity of the genome.**
- **To explain the role of *Alu* elements in breast/ovarian familial cancer focusing on *BRCA1* and *BRCA2* genes.**

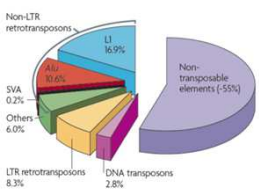


Figure1. Proportion of TEs in human genome[1]

Alu elements

The *Alu* family represents a huge lineage of retrotransposons, whose origin and amplification coincided with the radiation of primates 65 million years ago. They are non-autonomous retrotransposons that mobilize in a "copy and paste" event and only a very small fraction of them are retrotranspositionally competent. Moreover, there are about 1,4 million copy numbers of *Alu*s in human genome (10,6%) and they tend to be in the GC-rich regions (gene-rich regions).

Structure of *Alu* element

Figure2. *Alu* element's structure. Two dimmers separated by a short A-rich region and a long Arich region in the 3' end. In the body element, there is the RNA polymerase III promoter (internal components boxes A and B). The complete *Alu* element is flanked by direct repeats of variable length. Moreover, various distances downstream of the element there is a region (TTTT) where the transcription ends. [2]

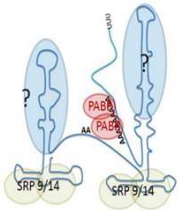


Figure3. The *Alu* RNA product (transcribed by RNA pol III) is folded in two monomer units. Several proteins bind to it:

- 7SL RNA SRP9/14 heterodimer
- PolyA-binding protein (PABP).

They seem to help the *Alu* RNA to associate with a ribosome where ORF2 protein (ORF2p) is being translated from L1 elements.[2]

Mechanism of insertion

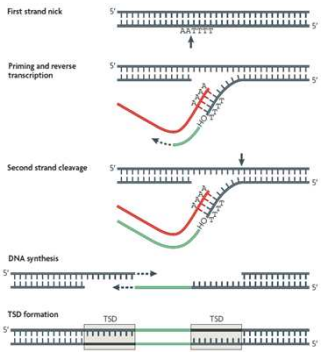


Figure4. Mechanism of amplification. After transcription by RNA pol III, insertion's event needs the ORF2 product of L1 (ORF2p), which has endonuclease and reverse transcriptase activity.[1]

ORF2p cleaves the DNA at a T-rich region → T-rich region primes reverse transcription by ORF2p on the 3' A-tail of *Alu* → - 2nd nick on the second strand - Second-strand synthesis is primed → Short direct repeats flanking *Alu* are formed

Mutagenic effects

There are several processes and mechanisms by which *Alu* elements can alter the correct expression of a gene.

***Alu* retrotransposition**

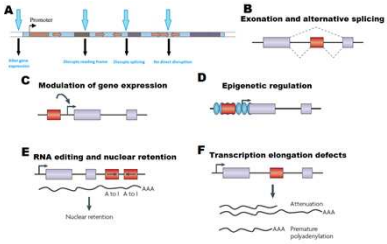
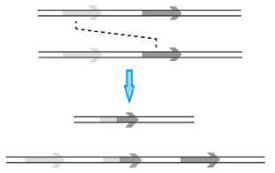


Figure5. (A) Different places where *Alu* can land (blue arrows) in the gene and the consequences in expression of the gene. (B-F) Examples of effects.[1 modified]

However, these mechanisms may be related directly with insertions or can be caused by mutations in silent *Alu* elements

***Alu*-*Alu* Recombination**

Figure6. Unequal homologous recombination event. This event can cause duplication and/or deletion of the sequences between the recombined *Alu*s.



Role of *Alu* elements in hereditary breast and ovarian cancer (HBOC) syndrome: *BRCA1* and *BRCA2* genes

Breast cancer is the most common cancer that affects women worldwide. However, only a small portion of them are caused by hereditary mutations (5-10%). The most mutated genes found in this kind of familial cancer are *BRCA1* and *BRCA2*. The germ-line mutations in the breast cancer susceptibility genes, *BRCA1* and *BRCA2*, are responsible for inherited susceptibility to breast and ovarian cancer.

Cancer Type	General Population Risk	Mutation Risk	
		BRCA1	*BRCA2*
Breast	12%	50%-80%	40%-70%
Second primary breast	3.5% within 5 years Up to 11%	27% within 5 yrs	12% within 5 yrs 40%-50% at 20 yrs
Ovarian	1%-2%	24%-40%	11%-18%
Male breast	0.1%	1%-2%	5%-10%
Prostate	15% (N. European origin) 18% (African Americans)	~30%	<39%
Pancreatic	0.50%	1%-3%	2%-7%

Table 1. Mutation risks in *BRCA1*/*BRCA2*-associated cancers.

BRCA1 gene

- Located in 17q21. It is about 81 kb long and has 24 exons.
- The protein (1863 aa) plays critical **roles in DNA repair, cell cycle checkpoint control, and maintenance of genomic stability** → tumor suppressor gene
- About **41.5% is composed of *Alu* elements → higher rate of rearrangements** than *BRCA2* gene

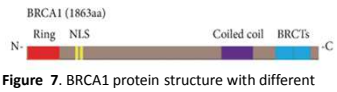


Figure 7. BRCA1 protein structure with different domains.[3]

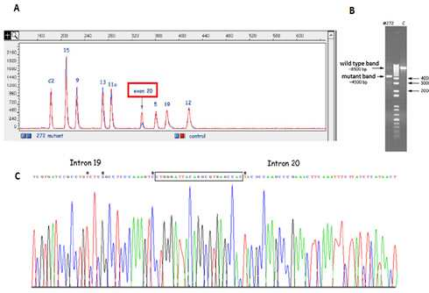


Figure 8. Characterization of exon 20 deletion in Greek families.[4]
A: Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF) → Patient (blue) and control (red).
B: Long-range PCR → comparing wild type band and mutant band
C: Sequence electropherogram→ deletion breakpoint.

Recombination between two *Alu*Y (introns 19 and 20) → 4.2 kb deletion (including exon 20) → In-frame deletion of 20 exon → Loss of BRCT domain

BRCA2 gene

- Located in 13q13. It encodes a 10,4 kb transcript → 27 exons
- The protein (3,418 aa) is a **tumor suppressor gene** and plays an important role in **DNA repair** too.
- About **17% is composed of *Alu* elements**
- **Less rearrangements** than *BRCA1* gene

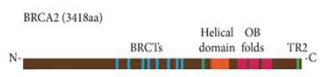


Figure 9. BRCA2 protein structure with different domains.[3]

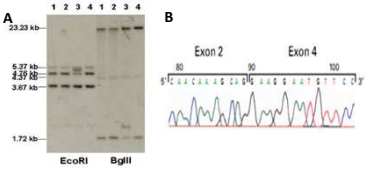


Figure 10. Genomic insertion of an *Alu* in Portuguese origin families (c.156-157insAlu).[5]
A: Southern blot (exons 2-9 of *BRCA2*). In lane 3 (patient) there is an additional band → insertion (350 bp)
B: Sequencing of cDNA revealed exon 3 skipping

Insertion of *Alu*Ya5 subfamily into the exon 3 → It leads to alter the splicing → In-frame exon 3 skipping → Loss of transactivation domain

Conclusions

Alu elements

- ✓***Alu* elements are very abundant in human genome (10,6%) and only a small portion of them have conserved their retrotransposition capacity.**
- ✓***Alu* elements have different ways to alter the integrity of human genome: *Alu* retrotransposition activity (about 0,1% of human diseases) and *Alu*/*Alu* recombination (0,3% of diseases).**
- ✓**Both mechanisms have mutagenic effects: insertions and deletions, duplications, alteration of alternative splicing and changes in gene regulation.**

***Alu* elements in HBOC**

- ✓***BRCA1* gene is rich in *Alu* elements (41,5%) → it is frequent to observe large rearrangements due to *Alu*/*Alu* recombination.**
- ✓***BRCA2* gene is more affected by retrontransposition activity (because it has less density of *Alu*s - 17% -).**
- ✓**Mutations caused by *Alu*s are underestimated because screening techniques have not detected them over last years.**
- ✓**New NGS approaches and bioinformatics are beginning to address the relationships between *Alu* elements and genetic diseases.**

References

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