IDENTIFICATION OF RETROTRANSPOSON INSERTION MUTATIONS IN HEREDITARY CANCER

Yaping Qian, PhD; Debora Mancini-DiNardo, PhD; Thaddeus Judkins, BS; Hannah C. Cox, PhD; Courtney Daniels, BS; Jayson Holladay, BS; Matthew Ryder, BS; Bradford Coffee, PhD; Karla R. Bowles, PhD; Benjamin B. Roa, PhD

Myriad Genetics, Inc., Salt Lake City, UT

BACKGROUND

- Retroelements (RE) such as Alu and L1 elements are located throughout the human genome, with Alu comprising 11% of total genomic sequence, and L1 elements comprising 17% of genomic sequence.[1]
- REs may cause human diseases by either mediating unequal crossovers between adjacent elements that result in deletions or duplications, or by inserting into a gene, disrupting gene function.[2-6]
- Traditional assays or techniques may underestimate the presence of large insertions.
- The aim of this study was to assess RE insertions in patients for whom hereditary cancer genetic testing was performed.

METHODS

- Patients underwent hereditary cancer genetic testing via single-syndrome testing (HBOC, Lynch syndrome) or pan-cancer panel testing (includes *BRCA1, BRCA2, TP53, PTEN, MLH1, MSH2, MSH6, PMS2, EPCAM, APC, BMPR1A, CDH1, CDKN2A, MUTYH, SMAD4, STK11, CHEK2, PALB2, ATM, NBN, BARD1, BRIP1, CDK4, RAD51C* and *RAD51D*).
- Sequencing and large rearrangement (LR) analysis was performed for all genes except *EPCAM*, for which only LR analysis was performed.
- Clinical information was obtained from test request forms completed by ordering healthcare providers.
- Multiple assays were used to detect the large insertions: Next Generation Sequencing (NGS), multiplex quantitative PCR.
- Specific RE insertions were also confirmed by PCR and sequencing analysis.

RESULTS

- We have identified 17 unique large RE insertions in the exonic regions of 7 genes, which are classified as pathogenic variants (Table 1).
  - 15 Alu insertions were identified in *APC, ATM, BRCA1, BRCA2, MSH2,* and *PALB2*.
  - A full-length L1 and a truncated L1 insertion were found in *MLH1*.
- 9 exonic Alu insertions were identified in *BRCA2*, compared to 1 insertion in *BRCA1*.
  - The personal and family cancer histories for individuals with the RE insertions in *BRCA2* include breast and/or ovarian cancer, similar to those carrying other pathogenic truncating variants.

Table 1. Retroelement Insertions Identified by Panel Testing

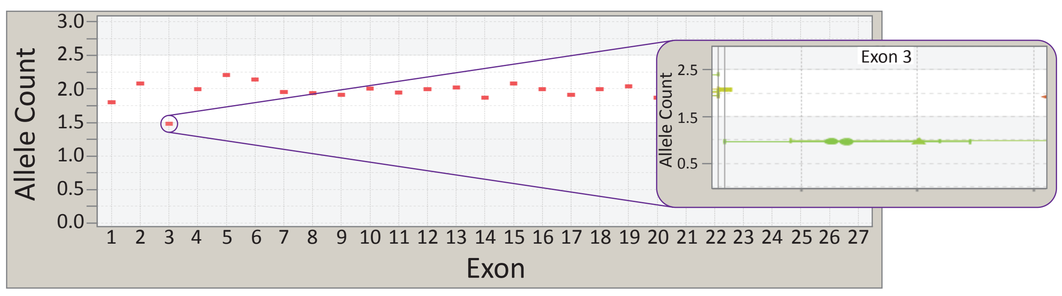
Gene	RE	Number of Observations	Number of Unique Insertions
APC	Alu	2	1
ATM	Alu	1	1
BRCA1	Alu	1	1
*BRCA2**	Alu	101	9
MLH1	L1	4	2
MSH2 **	Alu	2	2
PALB2	Alu	1	1

RE - retroelement; N - number of observations
**Includes 1 previously identified RE in reference (2)*
***Includes 1 previously reported RE in reference (4)*

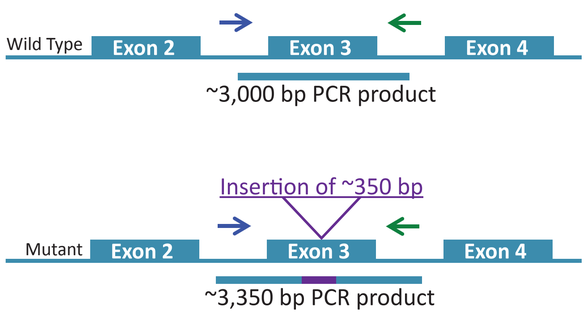
- An Alu insertion in *BRCA2* (c.156_157insAlu) confirmed via long range PCR is shown in Figure 1.
- Haplotype analysis suggests that two Alu insertions in *BRCA2* may be founder mutations in Latin American/Caribbean (c.5007_5007insAlu) and African (c.3407_3408insAlu) populations (Table 2).

Figure 1. *BRCA2* c.156_157insAlu (Portuguese Founder Insertion)

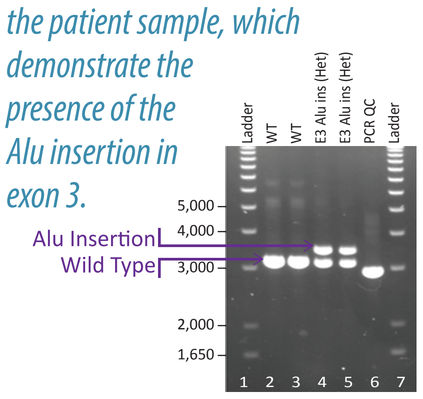
A) *Representative example of Alu insertion detected by NGS. Red squares represent the average dosage of several overlapping amplicons for each exon. Exons at normal dosage align to 2 on the Y-axis, 1 for deleted exons, and 3 for duplicated exons. The enlarged view shows decreased dosage in amplicons covering exon 3, making the insertion appear as a deletion.*



B) *Scheme of long range PCR analysis designed to characterize the large insertion in the suspected region. The insertion adds 350 bases to the affected allele.*



C) *Results of LR-PCR run on an agarose gel. Lanes 4 and 5 contain the patient sample, which demonstrate the presence of the Alu insertion in exon 3.*



D) *Representative sequencing data showing the point of Alu insertion in exon 3 highlighted in blue.*

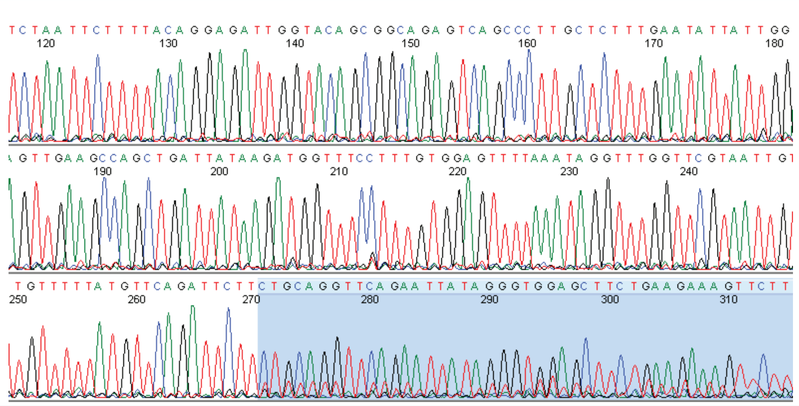


Table 2. Ancestry and Personal/Family Histories of Individuals with Alu Insertions in *BRCA2*

Ancestry	N (%)	iBC	OC	None	NS
c.156_157insAlu (n=35)					
Latin American / Caribbean	19 (54.3%)	16 (45.7%)	1 (2.9%)	14 (40.0%)	4 (11.4%)
Western / Northern European	5 (14.3%)				
Central / Eastern European	1 (2.9%)				
Unspecified	10 (28.6%)				
c.3407_3408insAlu (n=29)					
African	19 (65.5%)	15 (51.7%)	1 (3.4%)	9 (31.0%)	4 (13.8%)
Native American	2 (6.9%)				
Latin American / Caribbean	1 (3.4%)				
Unspecified	7 (24.1%)				
c.5007_5008insAlu (n=4)					
Latin American / Caribbean	4 (100%)	1 (25.0%)	0	3 (75.0%)	0

iBC - invasive breast cancer; OC - ovarian cancer; NS - not specified

CONCLUSIONS

- Identification of RE insertions is technically challenging and highly dependent on the location of the inserted sequence. However, careful analysis and characterization of regions exhibiting evidence of an RE insertion forms an essential part of a comprehensive genetic testing strategy.
- We have identified 17 pathogenic large rearrangements in 112 individuals caused by the insertion of retrotransposons in 7 genes assessed in our hereditary pan cancer panel.
- Two Alu insertions in *BRCA2* suggest possible founder mutations in individuals of Latin American / Caribbean and African ancestry.

REFERENCES

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