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DNA Breakpoint Assay Reveals a Majority of Gross Duplications Occur in Tandem Reducing VUS Classifications in Breast Cancer Predisposition Genes

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BACKGROUND

- Gross duplications are typically detected by aCGH or MLPA which are high-throughput methods that can detect copy number changes but cannot detect whether a duplication is tandem.
- Determination of a tandem finding is essential to inform the classification of gross duplications as only those that are found in tandem can be expected to disrupt gene structure and function, and without this knowledge these variants are of unknown significance (VUS).
- The literature supports repeat-element-mediated gross rearrangements, most notably by Alu elements which are highly enriched in the human genome [1, 2, 3].
- Among the genes that are especially enriched for Alu elements is *BRCA1* which experiences a disproportionate amount of gross duplications[3] relative to other genes tested in this project including *ATM, BRCA2, CDH1, CHEK2,* and *PALB2.*

Project Design

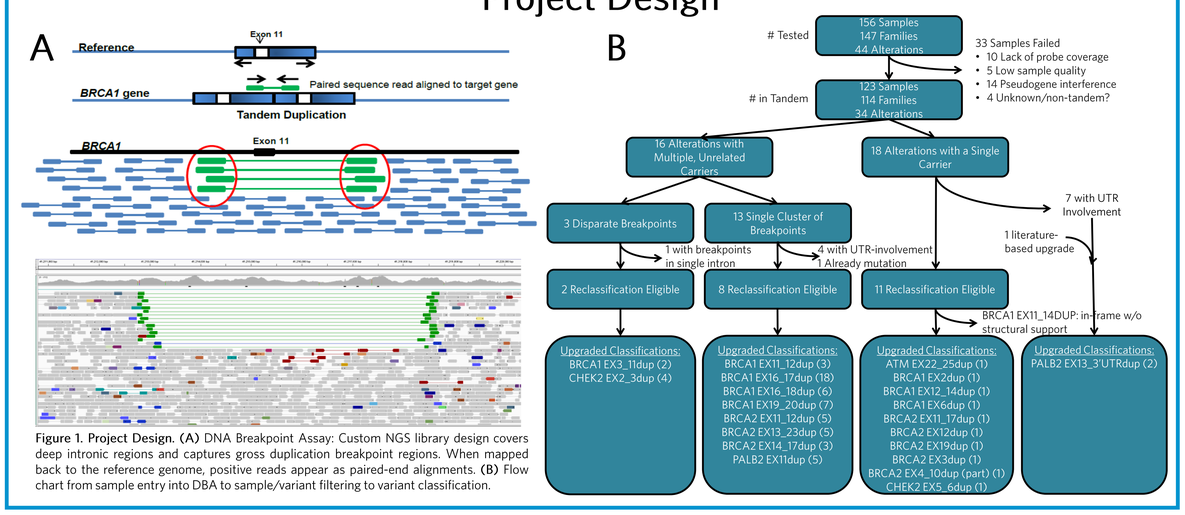


Figure 1. Project Design. (A) DNA Breakpoint Assay: Custom NGS library design covers deep intronic regions and captures gross duplication breakpoint regions. When mapped back to the reference genome, positive reads appear as paired-end alignments. **(B)** Flow chart from sample entry into DBA to sample/variant filtering to variant classification.

METHODS

- DNA Breakpoint Assay (DBA) is a custom, paired-end, NGS library that was designed to capture and detect deep-intronic DNA breakpoints in gross duplications in *BRCA1, BRCA2, ATM, CDH1, PALB2* and *CHEK2* (Figure 1A).

RESULTS

- 156 samples from 147 families carrying 44 unique gross duplications were ascertained for tandem status using the DNA breakpoint assay (Figure 1B). This represents a substantial subset of the entire clinical cohort (Figure 2 & 3).
- Of the 34 gross duplications that were confirmed as tandem, many were shown to overlap with Alu elements (Table 1)
- 12 tandem duplications were considered ineligible for reclassification due to the involvement of a UTR, being contained within a single intron, or classified pathogenic.
- Of the remaining 21 tandem duplications, all-but-one were upgraded to pathogenic leading to a substantial reduction in VUS rates for these gross duplications (Figure 4).

Table 1. Tandem duplications Identified in Breast Cancer Predisposition Genes as Identified by DNA Breakpoint Analysis

Gene	Gross duplication	Effect	Classification Before	Classification After	# of Tandem Findings/ Total Tested	5'Breakpoint Element[d] (Intron)	3'Breakpoint Element[d] (Intron)
ATM	EX22_25dup	Frameshift	VUS	Mutation	1/1	LINE L1P3	AluSx/+ (25)
BRCA1	5'UTRdup	Unknown	VUS	VUS	1/1	None (Intron 1 of AK311131)	None (5'UTR)
	5'UTR_EX1dup	Unknown	VUS	VUS	3/4	None (Intron 3 of AK093551)	None (1)
	5'UTR_EX6dup	Unknown	VUS	VUS	2/2	None (Intron 1 of AK311131)	None (Exon 7)
	5'UTR_EX9dup	Unknown	VUS	VUS	1/2	AluSc8 (Intron 3 of *AK093551*)	None (9)
	5'UTR_EX19dup	Unknown	VUS	VUS	1/1	None (Intron 3 of AK093551)	AluSc8/- (19)
	5'UTR_EX20dup	Unknown	VUS	VUS	2/2	None (Intron 2 of MPP2)	AluSc/+ (20)
	EX2dup	In-Frame	VUS	Mutation	1/1	AluSg/+ (1)	None (2)
	EX3_11dup	Frameshift	VUS	Mutation	2/2	None (2)	None (11)
	EX6dup	Frameshift	VUS	Mutation	1/1	AluSc5/+ (5)	AluSp/+ (6)
	EX11dup	Frameshift	Mutation[a]	Mutation	21/21	AluSx-AluY-AluSx/+ (10)	FLAMC-AluSx1-FLAMC/+ (11)
	EX11_12dup	Frameshift	VUS	Mutation	2/2	AluSx-AluY-AluSx/+ (10)	AluSx1/+ (12)
	EX11_14dup	In-Frame	VUS	VUS[b]	1/1	AluSx-AluY-AluSx/+ (10)	AluSp/+ (14)
	EX12_14dup	Frameshift	VUS	Mutation	1/2	FLAMC-AluSx1-FLAMC/+ (11)	AluSp/+ (14)
	EX16_17dup	Frameshift	VLP	Mutation	5/5	AluSg/+ (15)	AluSz/+ (17)
	EX16_18dup	Frameshift	VUS	Mutation	5/5	AluSg/+ (15)	AluSq2/+ (18)
	EX19_20dup	In-Frame	VLP	Mutation	4/4	AluSg/+ (18)	AluSz/+ (20)
	EX21_3'UTRdup	Unknown	VUS	VUS	1/1	AluSz/+ (20)	MIR1_Amn (3' downstream)
BRCA2	EX3dup	Frameshift	VUS	Mutation	1/1	AluY/- (2)	AluY/- (3)
	EX4_10dup(Partial)	Frameshift	VUS	Mutation	1/1	AluY/- (3)	None (Exon 10)
	EX11_12dup	Frameshift	VUS	Mutation	2/2	LINE L4 (10)	AluSz6/+ (12)
	EX11_17dup	Frameshift	VUS	Mutation	1/1	None (11)	AluSg4/- (17)
	EX12dup	Frameshift	VUS	Mutation	1/1	AluSq10/- (11)	AluSc8/- (12)
	EX13_23dup/trip	Frameshift	VUS	Mutation	2/2	LINE L2a (12)	LINE L2a (23)
	EX14_17dup	Frameshift	VUS	Mutation	2/2	AluSx /+ (13)	AluSz/+ (17)
	EX19dup	Frameshift	VUS	Mutation	1/1	None (Exon 18)	None (19)
CDH1	IN2dup (a)	Unknown	VUS	VUS	2/2	None (2)	None (2)
	IN2dup (b)			VUS	10/10	AluSp/- (2)	AluSz/- (2)
	EX3_3'UTRdup	Unknown	VUS	VUS	1/2	LINE L2a (2)	MIRc (Intron 7 of *TANGO6*)
CHEK2	5'UTR_EX1dup	Unknown	VUS	VUS	1/1	None (Intron 1 of ZNRF3)	MER51A [LTR] (1)
	EX2_3dup (a)	In-Frame	VUS	VLP	2/2	AluSc8/+ (1)	AluSp/+ (3)
	EX2_3dup (b)			VLP	1/1	None (1)	None (3)
	EX2_3'UTRdup	Unknown	VUS	VUS	34/35	AluSx-AluSp-AluSx/+ (1)	AluSx/+ (Intron 1 of *TTC28*)
	EX5_6dup	Frameshift	VUS	Mutation	1/1	AluY/+ (4)	AluY/+ (6)
PALB2	EX11dup (a)	Frameshift	VUS	Mutation	3/3	AluYa5/+ (11)	AluY/+ (12)
	EX11dup (b)			Mutation	1/1	AluSz/+ (11)	AluSx/+ (12)
	EX13_3'UTRdup	Splicing[c]	VUS	Mutation	1/1	AluY/+ (12)-Estimated w/i 300nt	None (Intron 2 of NDUFAB1)

[a]Previously established as a well-known founder mutation
[b]Small in-frame gross duplication in an unstructured region of *BRCA1* leading to uncertain structural impact
[c] Tandem finding in concert with a publication at the time of assay lead to reclassification of this variant due to the activation of a cryptic donor in the last exon leading to frameshift
[d]Element orientation relative to the gene is indicated when possible (elements located in other genes do not have orientation indicated): (+) same orientation as the gene; (-) opposite orientation as the gene

Gross Duplications Detected and Identified as Tandem

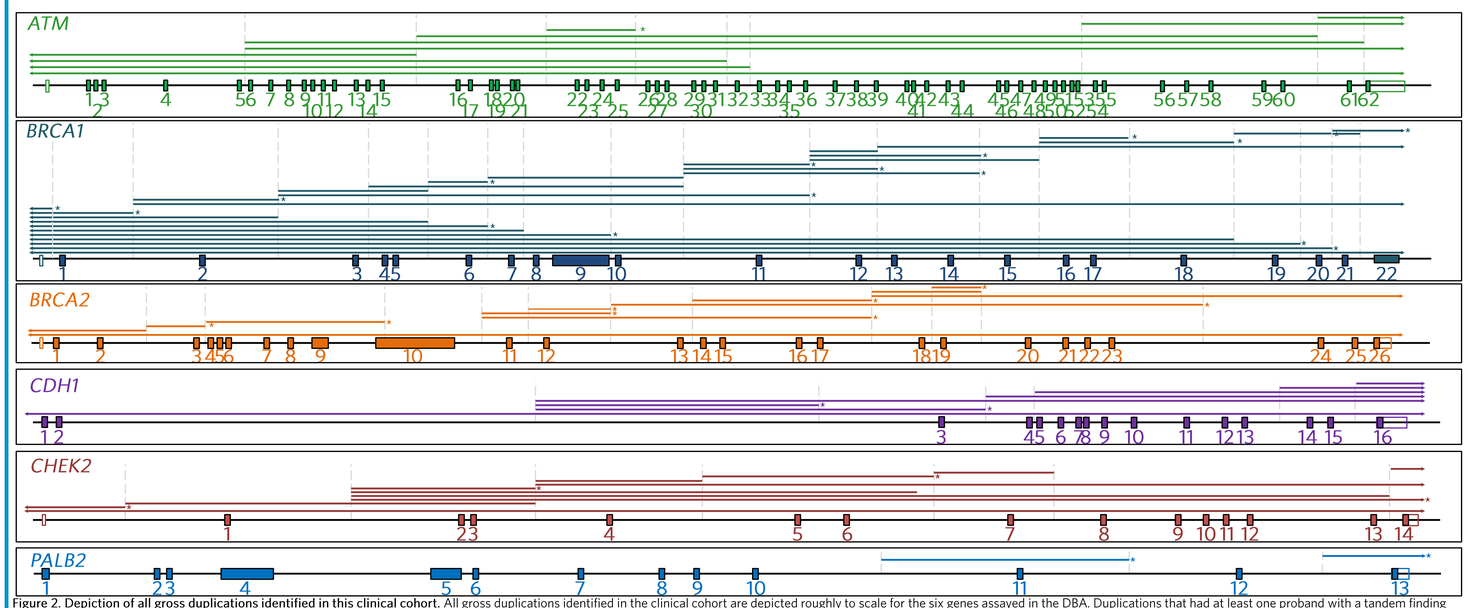
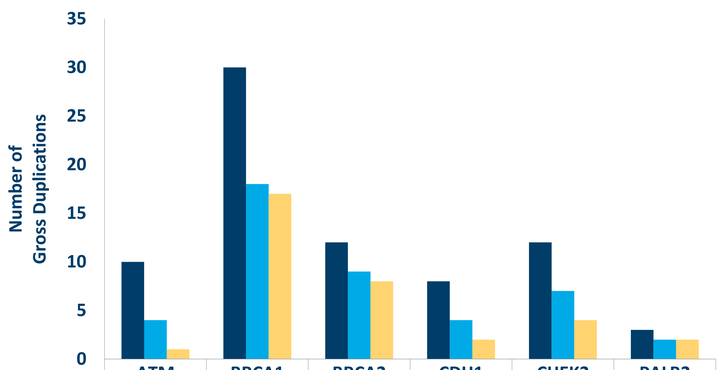


Figure 2. Depiction of all gross duplications identified in this clinical cohort. All gross duplications identified in the clinical cohort are depicted roughly to scale for the six genes assayed in the DBA. Duplications that had at least one proband with a tandem finding are indicated by * after the line representing that duplication. Note that this depiction does not imply to-scale the location of identified breakpoints rather breakpoints are randomly placed near the middle of the involved intron. Dashed vertical lines are placed as a visual aide.

Number of Gross Duplications Detected, Tested and Identified as Tandem in This Clinical Cohort



	ATM	BRCA1	BRCA2	CDH1	CHEK2	PALB2
Detected	10	30	12	8	12	3
Tested	4	18	9	4	7	2
Tandem	1	17	8	2	4	2

Figure 3. Tested cohort of samples and alterations per gene. Number of duplications detected (navy), tested (cyan) and identified as tandem (yellow) in this clinical cohort per gene.

Classification Rates for Gross Duplications Before and After DNA Breakpoint Analysis

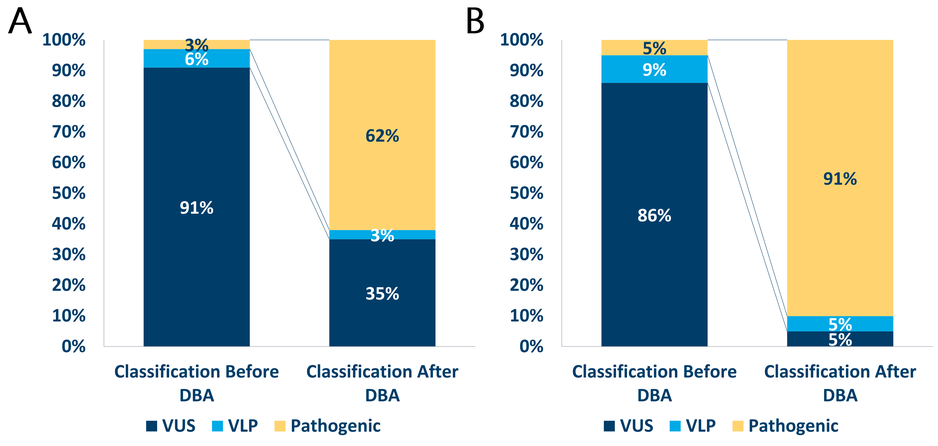


Figure 4: Classification rates for gross duplications before and after DBA . (A) Percent classification ascribed to each tandem gross duplication before and after DBA-tandem-finding. **(B)** Percent classification ascribed to each reclassification-eligible gross duplication before and after DBA-tandem-finding. Eligible gross duplications must have been identified in tandem, must not have involved a 5' or 3' UTR/up- or downstream region, and must have not been contained within a single intron. This does not include PALB2 Ex13_3'UTRdup which was reclassified as pathogenic in light of 3'-UTR-involvement

TAKE-HOME POINTS

- This is, to our knowledge, the largest collection of gross duplication breakpoints evaluated to-date.
- Gross duplications should be considered variants of unknown significance (VUS) until tandem status can be resolved and used to inform the subsequent classification.
- A vast majority of gross duplications in *ATM, BRCA1, BRCA2, CDH1, CHEK2,* and *PALB2* were identified in tandem in this clinical cohort (78-97%).
- Many gross duplications had breakpoints where at least one, if not both, breakpoints fell within an Alu element, which is consistent with foregoing literature reports.
- Gross duplications involving 5' and 3' UTRs cannot generally be considered for reclassification even if identified in tandem because it does not appear alter the basic gene structure and the qualitative impact is not predictable.
- Use of the DBA reduced VUS rates from 91% to 35% for all gross duplications identified in these six genes and when considering only those duplications that are reclassification-eligible, VUS rates dropped to just 5% leading to better clinical management for their carriers.

REFERENCES

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3. Pavlicek A, Noskov VN, Kouprina N, Barrett JC, Jurka J, Larionov V. Evolution of the tumor suppressor BRCA1 locus in primates: implications for cancer predisposition. *Hum Mol Genet* Nov 15 2004;13(22):2737-2751.

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