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

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Purpose: Gross duplications are ambiguous in terms of clinical interpretation due to the limitations of the detection methods which cannot infer the context of the duplications: namely whether the duplication occurs in tandem or is duplicated and inserted elsewhere in the genome. Using a novel, high-throughput, DNA Breakpoint Assay (DBA), we investigated the proportion of gross duplications occurring in tandem in breast cancer predisposition genes with the intent of informing the classification of such mutations.

Methods: A novel DNA Breakpoint Assay (DBA) was employed to detect tandem duplications. The DBA is based on custom, paired-end, NGS probes designed to capture deep-intronic DNA sequences for six breast cancer predisposition genes: *BRCA1*, *BRCA2*, *ATM*, *CDH1*, *PALB2* and *CHEK2*.

Results: DBA allowed us to ascertain breakpoints for 44 unique gross duplications from 156 consenting probands. We determined that the duplications occurred in-tandem in 123 (79%) individuals from this cohort, while the remainder have unknown tandem status. Among the in-tandem gross duplications that were eligible for reclassification, 95% of them were upgraded to pathogenic mutations.

Conclusion: DBA is a novel, high-throughput, NGS-based method that can inform the tandem status and predicted reading frame of gross duplications, thereby contributing to the correct classification of these alterations. This method revealed that most gross duplications in the investigated genes occurred *in tandem* and resulted in a classification of pathogenic mutation which helps to secure the necessary screening, prophylaxis and treatment options for their carriers.

[1] The authors wish it to be known that, in their opinion, the last two authors should be regarded as joint Last Authors.