Prevalence and Characterization of Triplications in Genes Associated with Hereditary Cancers

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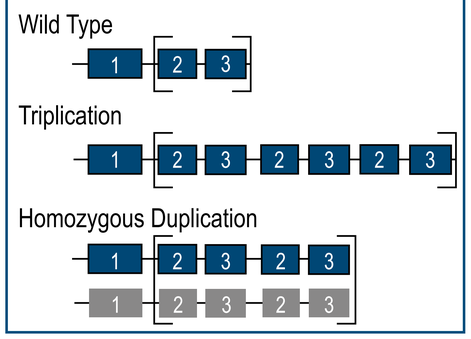
BACKGROUND

- The interpretation of triplications identified using hereditary cancer testing can be challenging.
- For example, an apparent triplication may be a true triplication or a homozygous duplication (Figure 1).
- Follow-up molecular testing is required to provide more accurate information regarding pathogenicity of the triplication as well as potential cancer risks.

Figure 1. *True Triplication versus a Homozygous Duplication*



METHODS

- We assessed individuals who had an apparent triplication identified by hereditary cancer genetic testing at a single laboratory over a 25 year time period.
- The process for the characterization of apparent triplications is shown in Schematic 1.

Schematic 1. *Work Flow for Triplication Characterization*

Primary Identification	• Multiplex quantitative PCR or • Next Generation Sequencing (NGS) dosage analysis
Confirmatory Testing	• Long Range PCR (LR PCR) or • Multiplex ligation dependent probe amplification (MLPA) or • Multiplex quantitative PCR or • Microarray comparative genomic hybridization (microarray CGH)
Possible Additional Analyses	• Dosage NGS • NGS allele frequency analysis • LR PCR followed by Sanger sequencing • Follow-up family testing

RESULTS

Table 1. *Apparent Triplications*

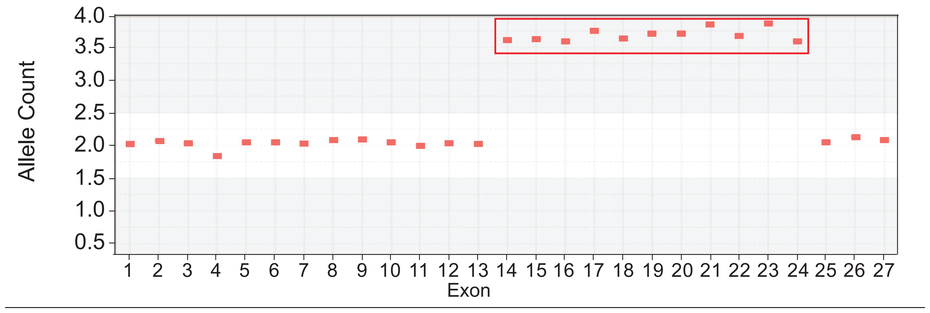
Gene	Unique Variants
BRCA1	3
MSH2	2
RAD51C	2
CDKN2A	1
MUTYH	1
BMPR1A	1
ATM	1
BRCA2	1
PALB2	1
CHEK2	1
Total	14

- 14 unique apparent triplications were identified in 10 different genes (Table 1).
  - 5 whole gene, 5 terminal (span the 5' or 3' end of the gene), and 4 intragenic apparent triplications.
  - Apparent triplications were reported for 125 individuals.
- Triplication of exons 14–24 in *BRCA2* accounted for 84% (105) of all reported triplications.
  - Confirmatory testing is consistent with a true triplication occurring within the DNA-binding domain of *BRCA2* (Figure 2).
  - This triplication has been traced primarily to a large North American kindred.
- An apparent triplication of exons 61–62 in *ATM* (commonly referred to as exons 64 and 65 in the scientific literature) has been identified in 2 individuals: however follow-up testing is consistent with a heterozygous duplication (Figure 3).
  - Analysis of personal and family history is not consistent with an increased breast cancer risk associated with duplication of *ATM* exons 61–62 (n=188).

Figure 2. *Triplication in BRCA2 (exons 14–24)*

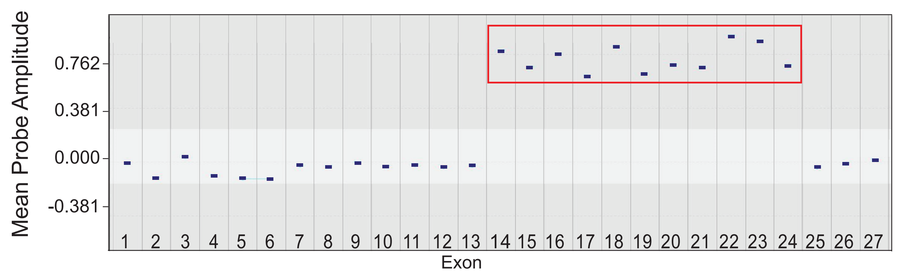
A. NGS dosage analysis

Red rectangles represent the average dosage of several overlapping amplicons for each exon. Exons at normal dosage approximately align to 2 on the Y-axis, 1 for deleted exons, and 3 for duplicated exons.



B. Microarray CGH

Blue rectangles represent probe clusters. Exons at normal dosage approximately align to 0.0 on the Y-axis, -0.5 to -1.0 for deletions, 0.29 to 0.58 for duplications, and 0.75 for triplications.



C. Schematic of *BRCA2* showing the location of the observed triplication

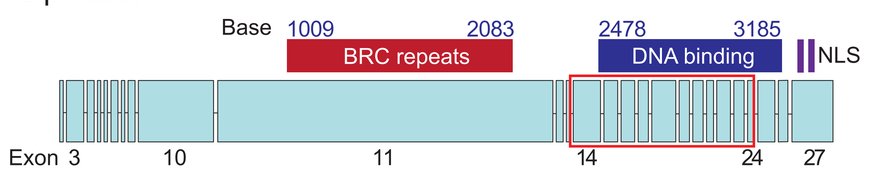
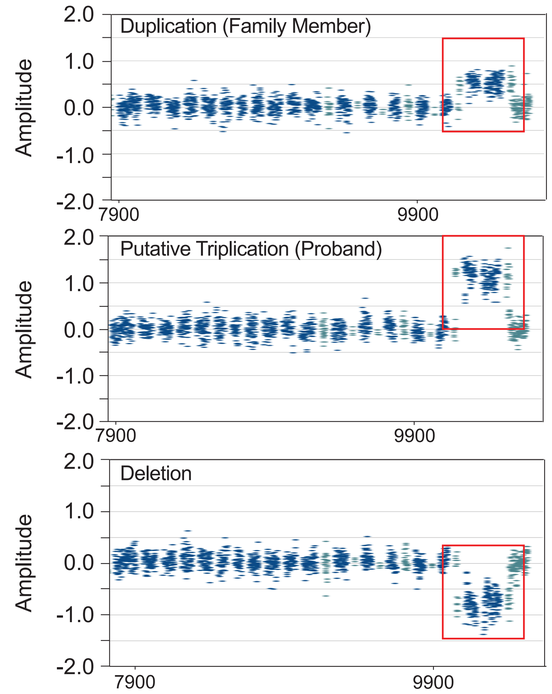


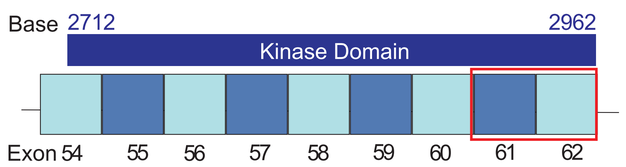
Figure 3. *Apparent triplication in ATM*

A. Microarray CGH Data

Blue symbols represent probe clusters. Examples are shown for a duplication, triplication, and a deletion of ATM exons 61-62.



B. Schematic of a portion of *ATM* showing the location of the observed mutation



CONCLUSIONS

- Establishing the pathogenicity of an apparent triplication requires additional effort because dosage analysis alone is insufficient to distinguish true triplications from homozygous duplications.
- A thorough approach to large rearrangement identification, characterization, and classification of these rare variants is required to provide accurate information regarding hereditary cancer risk to both patients and their families.

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