Apparent Gene Conversion Event Detected in *CHEK2* using Next Generation Sequencing Analysis

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BACKGROUND

- Pathogenic variants (PVs) in *CHEK2* are associated with an increased risk of breast and colon cancer.
- Identification of PVs in *CHEK2* is complicated by the existence of several paralogues in the human genome.
- *CHEK2* pseudogenes overlap with exons 10 through 14 and share 95–98% sequence homology with *CHEK2*.
- Pseudogene-mediated gene conversion is an important mechanism by which PVs can arise and cause human disease.
- Although pseudogene-mediated gene conversion is known to occur in genes such as *PMS2*, it has not yet been documented in *CHEK2*.
- Here, we present the first known case of a pseudogene-mediated gene conversion event in *CHEK2*.

METHODS

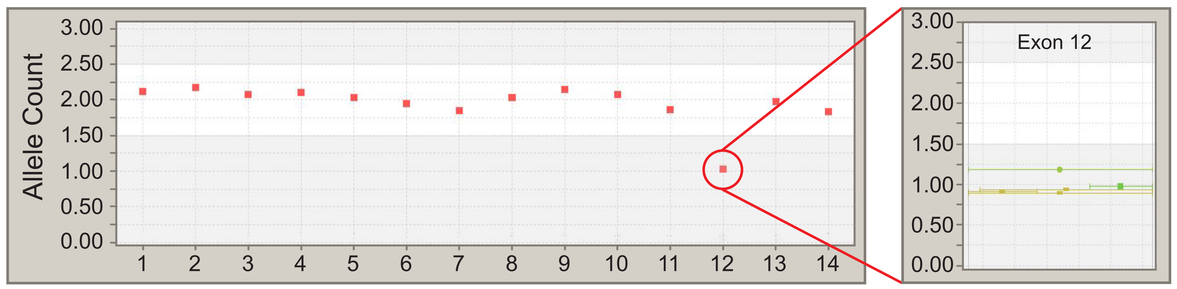
- An apparent exonic deletion in *CHEK2* was observed using amplification-based NGS dosage analysis for hereditary cancer testing with a 25-gene panel.
- Follow-up testing was performed using multiplex ligation-dependent probe amplification (MLPA) and long-range PCR (LR PCR), followed by nested Sanger sequencing.

RESULTS

Figure 1. *CHEK2 variant identified in a 53 year old woman with no personal cancer history and a family history of pancreatic, prostate, ovarian, and breast cancer.*

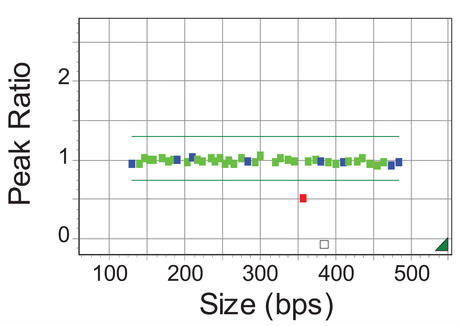
A. NGS dosage analysis shows an apparent deletion of exon 12 in *CHEK2*

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. Enlarged views show decreased dosage in amplicons within exon 12.



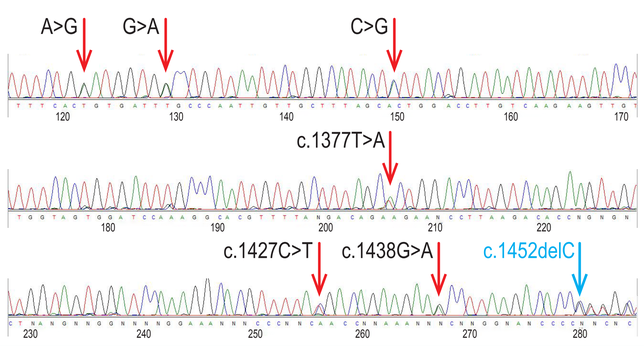
B. Confirmatory MLPA

The red box represents a decrease in dosage at exon 12. Green represents exons present at normal dosage and blue represents control probes.



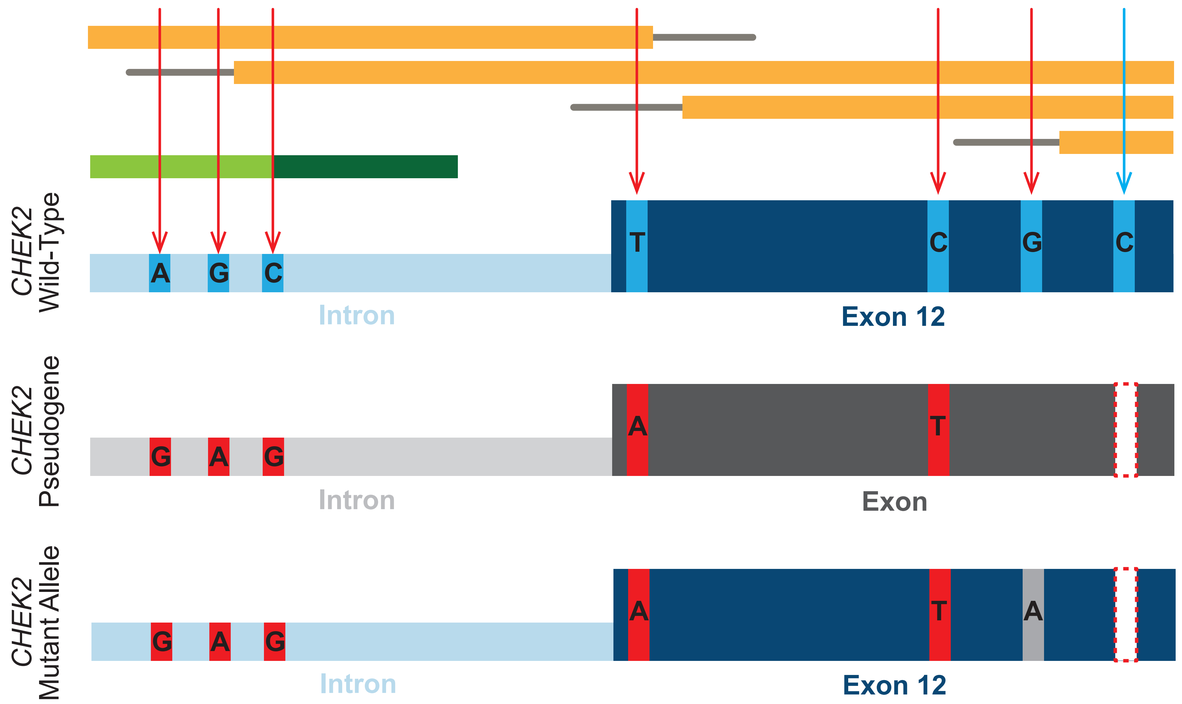
C. Sanger sequencing

Multiple rare heterozygous variants were identified in exon 12, including a pathogenic frameshift mutation (blue), which indicates that exon 12 was not truly deleted.



D. Schematic of NGS and MLPA probes

Several sequence variants (arrows) were located under NGS primers, shown in gray, and one variant was located under the ligation site of the MLPA probe, shown in green. This resulted in the apparent deletion of exon 12 by NGS and MLPA.



Several variants were mapped to a *CHEK2* pseudogene, indicating a gene conversion event. Therefore, the pathogenic frameshift mutation in exon 12 was acquired due to *CHEK2* pseudogene-mediated gene conversion.

CONCLUSIONS

- To our knowledge, this is the first evidence of a pseudogene-mediated gene conversion event in *CHEK2*, which underscores the importance of gene conversion as a mechanism for the acquisition of pathogenic germline variants in this cancer-risk gene.
- Furthermore, the case illustrates the need for comprehensive laboratory programs to clarify and correctly interpret genetic test results, particularly for genes with highly homologous pseudogenes.

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