Titel:

Identification and Categorization of Germline Trio Variants

Abstract:

Introduction

Whole-exome sequencing (WES) variant calling is a non-trivial analysis that offers critical insight into the origin and development of cancer types. For paediatric cancers in particular, analyzing a trio of samples consisting of DNA from both parents and the affected child allows to discriminate between inherited (transmitted) and new (de-novo) mutations. Detected patterns, i.e. concomitant mutations within the same pathway, facilitate understanding of the disease's underlying mechanisms and may indicate future therapeutic targets.

Methods and datasets

We chose a docker-based pipeline approach for the identification of both transmitted and de-novo variants in germline trios. The trio pipeline was designed to combine a standard alignment and variant calling with the state-of-the-art programs bwa-mem [1] and VarScan 2 [2], and included a rigorous filtering and categorization scheme inspired by Zhang et al. [3]. Identified variants were annotated and classified according to their predicted impact. Filters were set to be more stringent for transmitted mutations to increase precision, while weaker cutoffs were applied for de-novo detection to increase sensitivity.
157 WES trios were sequenced in total, including two trios featuring a healthy sibling of a cancer-affected child. The most prevalent disease was AML, followed by Hodgkin's lymphoma and neuroblastoma.

Results and discussion

On average, 433 transmitted and 10 de-novo mutations were identified per child. In addition, a number of family trios revealed evidence for a “two-hit” cancer constellation, with transmitted variants from both healthy parents affecting the same cancer-related pathway in their child.
Given the increasing importance of NGS variant analyses in clinical routine settings, the trio pipeline is currently being updated with the latest annotation databases and functionality to allow for a semi-automated analysis via a web-interface.

References

[1] Li (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997v1 [q-bio.GN]

[2] Koboldt et al. (2012) VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing, Genome Res., 22(3), 568-76.

[3] Zhang et al. (2015) Germline mutations in predisposition genes in pediatric cancer. N Engl J Med., 373:2336-2346