Advancement of point-of-care *BRCA1/2* testing to whole genome sequencing in clinical practice

Kotze MJ,[1] Hector S,[2] Mbuli-Robertson DJ,[1] Oosthuizen J,[3] van der Merwe NC.[3]

1.Division of Chemical Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa.

2.Department of Biomedical Sciences and SAMRC Cardiometabolic Health Research Unit, Cape Peninsula University of Technology, Bellville, South Africa.

3.Division of Human Genetics, Faculty of Health Sciences, University of the Free State and National Health Laboratory Service, Universitas Hospital, Bloemfontein, South Africa

Introduction: The genetic basis of early-onset familial breast cancer remains incompletely characterised, despite the use of targeted multi-gene next-generation sequencing (NGS) panels in both public and private healthcare settings. In South Africa, nine founder/recurrent variants account for over 70% of pathogenic *BRCA1/2* variants in patients with hereditary breast and ovarian cancer, which enables the development of a rapid point-of-care (POC) test kit to complement genetic counselling and guide further testing. Whole genome sequencing (WGS) can address the remaining diagnostic gap, but uptake is hindered by delays from counselling to data analysis and reporting of the results.

Aim: To accelerate clinical implementation of WGS, we developed an open-source bioinformatics pipeline integrating first-tier POC test results into the laboratory workflow for rapid quality checks and streamlined variant prioritisation.

Materials and Methods: The variant prioritisation ordering tool (VPOT) was adapted into a Nextflow-based pipeline, Variant Call Format (VCF)-Prioritise (https://github.com/duncanMR/VCF-prioritise), evaluated in a translational research study (ethics approval ref. S21/07/129_N09/08/224). The tool was applied to WGS data generated at the Genomics Centre of the Medical Research Council (n=6, validation group) and the Centre for Genomics and Proteomics Research (n=7, implementation group) in South Africa. The WGS data sets were filtered and scored across curated cancer, pharmacogenetic, and lifestyle-related gene panels. The initial validation steps informed real-life case studies combining genetic counselling with rapid *BRCA1/2* POC testing using buccal swabs. Global differential methylation analysis based on long-range WGS was performed using Modkit v0.4.1 in three genetically uncharacterised breast cancer patients compared with controls to explore potential epigenetic contributions.

Results: VCF-Prioritise reduced WGS bioinformatics analysis time to under 40 minutes per patient, producing prioritised results in an easily accessible spreadsheet format for clinical interpretation. Comparison with previous first-tier genotype and NGS results validated the clinical usefulness of VCF-prioritise to support multiple sequencing technologies. In a mother-daughter case study, using long-read WGS that enables simultaneous methylation analysis, no pathogenic variants were found. *RPTOR* hypomethylation was present in both these breast cancer patients, while not detected in unrelated patients and controls.

Conclusion: VCF-Prioritise enables rapid prioritisation of WGS-generated gene variants, requiring minimal bioinformatics expertise to implement. Integrating POC genotyping with genetic counselling facilitates the generation of adaptable patient reports at the interface of research and clinical care. This pathology-supported genetic testing framework builds capacity through targeted education and skills transfer, providing a sustainable model for benchmarking emerging sequencing technologies and reanalysing unresolved cases to close the diagnostic gap in familial breast cancer, while advancing pharmacodiagnostic applications in precision oncology.

Acknowledgement: Dr Nicole van der Merwe is thanked for genetic counselling support.