

A rapid and feasible method for detecting pathogenic *BRCA1/2* variants in breast cancer patients

K. Rajan^{1,2}, K. W. Gunawardena³, N. D. Sirisena¹, V. H. W. Dissanayake^{1,4}

¹*Centre for Genetics and Genomics, Faculty of Medicine, University of Colombo, Sri Lanka*

²*Faculty of Life and Medical Sciences, BMS Campus, Colombo, Sri Lanka*

³*Oxford University Hospitals NHS Foundation Trust, United Kingdom*

⁴*Asiri Centre for Genomic and Regenerative Medicine, Colombo, Sri Lanka*

The global and national incidence rate of breast cancer is rising annually. Approximately 5-10% of breast cancers are hereditary and are caused by genetic mutations. Among the cancer-predisposing genes, BRCA1 and BRCA2 are strongly associated with hereditary breast cancers. While several common mutations have been well-characterized, pathogenic single nucleotide variants remain underdiagnosed, particularly in low-resource settings. This study is aimed at developing and optimizing a Tetra Primer-Amplification Refractory Mutation System based PCR (T-ARMS PCR) assay for the accurate and cost-effective screening of four selected pathogenic BRCA1/2 variants. Three pathogenic variants in BRCA1 (c.1575del, c.237del, c.5289del) and one in BRCA2 (c.1296_1297del) were selected for this study. DNA was extracted from the blood samples of a cohort of 44 Sri Lankan hereditary breast cancer patients. Inner and outer primers for each variant were designed manually according to optimum primer parameters published in the literature. The annealing temperature, primer concentration and magnesium chloride concentration were optimized for each variant. Genotyping was performed using the respective optimized protocols, and results were validated using Sanger sequencing for selected samples. Genotyping revealed that one of the samples was positive for the BRCA1:c.237del variant, by producing three bands corresponding to a heterozygous genotype, while the remaining samples were negative. All of the samples were negative for other tested BRCA1/2 variants; c.1575del, c.5289del and c.1296_1297del. It was determined that the generation of only two bands corresponded to the homozygous wild-type genotype. Concordant sequencing results were obtained, which indicated the accurate and reliable use of these T-ARMS PCR assays for genotyping the selected variants. It can be concluded that T-ARMS PCR is a rapid and cost-effective method for targeted screening of rare BRCA1/2 pathogenic variants. It is beneficial in resource-limited settings as an alternative, but can also be used even when sequencing is available.

Keywords: *BRCA1/2, Genotyping, Hereditary breast cancer, Pathogenic variants, T-ARMS PCR*