PP 16 - Design and implementation of a novel pharmacogenetic assay for the identification of CYP2D6*10 genetic variant in a cohort of oestrogen receptor positive breast cancer patients

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Introduction: Tamoxifen is widely used as an adjuvant endocrine therapy in oestrogen receptor (ER) positive premenopausal breast cancer patients. However, in nearly 30% of such patients, pharmacogenomically important variants in the *CYP2D6* gene are known to affect tamoxifen metabolism resulting in reduced drug efficacy. *CYP2D6*10* variant is reported to be the most common variant found in South Asian populations. This study was undertaken to design and implement a novel pharmacogenetic assay for the identification of *CYP2D6*10* gene variant in a Sri Lankan ER positive breast cancer cohort.

Methods: A novel single variant tetra-amplification refractory mutation system (T-ARMS) polymerase chain reaction (PCR) assay was designed for the *CYP2D6*10*:100C>T (rs1065852) variant. The assay was optimized and implemented by genotyping the variant in an existing blood resource obtained from a cohort of breast cancer patients. A total of 70 samples were genotyped. The gel images were interpreted using the following band sizes: Control: 454bp; C allele: 195bp; T allele: 300bp. Genotype frequencies were calculated using the homozygous and heterozygous counts of both alleles. Genotyping results were further validated by Sanger sequencing. Allele frequencies were calculated using the Hardy-Weinberg equation.

Results: The desired specific gel bands for the single variant T-ARMS PCR method were obtained after several steps of optimization. Genotype frequency for *CYP2D6*10 :rs1065852C>T* was: CC - 24.3% (17/70), CT - 75.7% (53/70) and the variant (T) allele frequency was 37.9%.

Conclusion: We successfully designed and implemented a novel genotyping assay for *CYP2D6*10* variant. The minor allele for the variant was identified in heterozygous (C/T) form in the genotyped cohort. This can be introduced as a low-cost, single step optimized pharmacogenomic assay for the breast cancer patients to predict their response to tamoxifen along with assays for the detection of other *CYP2D6* variants that affect tamoxifen metabolism.

Keywords: T-ARMS PCR, Tamoxifen, *CYP2D6*10,* Pharmacogenetics, Genotypes

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