Development of laboratory protocols for detecting epigenetic variants TET2 c.4210C>T (Arg1404Ter) and TET2c.822del (Asn275fs) and study their prevalence in a Sri Lankan Myeloproliferative Neoplasm cohort

W. P. U. S. Weerasinghe[1], S. T. Karunathilake[1], N. N. H. Costha[1], N. Amarasinghe[2], Y. J. Costa[2], H. W. W. Goonasekera[1]

[1]*Center for Genetics and Genomics, Department of Anatomy, Genetics and Biomedical Informatics, Faculty of Medicine, University of Colombo, Sri Lanka*

[2]*Department of Haematology, Colombo North Teaching Hospital, Ragama, Sri Lanka*

Epigenetic variants influence prognosis in myeloproliferative neoplasms (MPN) and their prevalence studies are warranted to enable personalised medicine. Next-generation sequencing platforms limit *TET2* variant profiling due to high cost. A cost-effective, easy to implement genetic testing method would benefit Sri Lankan MPN patients. This study aimed at developing a PCR based protocol and study the prevalence of two Asian specific *TET2* variants; c.4210C>T (Arg1404Ter)[rs974106601] and c.822del (Asn275fs)[rs777145283]. Ethical approval (EC-24-149) was obtained for a descriptive study of patients diagnosed with polycythaemia vera (PV), essential thrombocythaemia (ET), and primary myelofibrosis (PMF) attending the Haematology Clinic, North Colombo Teaching Hospital. Pathogenic variant selection was done using Genome Aggregation and VarSome databases. Genomic DNA was extracted from peripheral blood. Tetra-primer ARMS-PCR, primers were manually designed using the NCBI dbSNP database. Specificity was confirmed through UCSC *in-silico* PCR, to avoid non-specific amplification. Primer optimization included temperature, $MgCl_2$, and primer-ratio gradients to identify ideal annealing and primer concentrations. The finalized PCR protocol was used for variant detection, followed by agarose gel electrophoresis. Results were validated using Sanger sequencing. Statistical analysis was conducted using IBM SPSS Statistics version 19. Samples from 50 long-standing (mean time since diagnosis 10.8 years), treated (100% on treatment), MPN patients were analysed; PV-82% (n=41), ET-10% (n=5), PMF-8% (n=4). The number of patients on remission: 44% (n=22). The optimised Tetra-primer ARMS-PCR results were compatible with Sanger sequencing results. Prevalence for both variants: wild-type 100% (n=50), variant detection rate-0%. A validated, low-cost PCR-based protocol was successfully developed to detect two *TET2* variants, which can be applied for broader *TET2* variant profiling. This first reported study of *TET2* variants in a Sri Lankan MPN cohort showing absence of variants, could reflect low prevalence of these variants in local MPN or clonal deletion following therapy. Larger MPN-population-based studies at diagnosis are recommended, to validate these findings.

Keywords: *Sri Lankan Myeloproliferative neoplasms, TET2c.4210C>T (Arg1404Ter), TET2c.822del (Asn275fs), Tetra-primer ARMS-PCR*