

**Adaptation of Existing Methods of Genotyping
Platelet Polymorphisms Associated with
Cerebrovascular Disease for use within the
Routine Laboratory Setting and Determining the
Relative Frequency in a Cohort of Stroke Patients**

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Declaration

I declare that the dissertation, **Adaptation of Existing Methods of Genotyping Platelet Polymorphisms associated with Cerebrovascular Disease for use within the Routine Laboratory Setting and Determining the relative Frequency in a Cohort of Stroke Patients** is my own unaided work. It is being submitted for a degree of Master of Science in Medicine to the University of Witwatersrand. This work has not been submitted before for any degree or examination at any other University.

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ABSTRACT

Introduction

It is widely recognised that stroke is a multi-factorial disorder in which platelets play a crucial role in thrombus formation resulting in ischaemic stroke. Platelet adhesion and aggregation are initiated by the interaction of various platelet glycoproteins (GP's) such as GPIb α , which binds to von Willebrand Factor and GPIIb/IIIa a fibrinogen receptor. Recent studies have shown that the GP's are polymorphic and the polymorphisms described within GPIb α such as Kozak-5T/C, the variable number of tandem repeats (VNTR) and the Human Platelet antigen 2 (HPA2), have been implicated in the development of stroke, while the P1^A polymorphism of GPIIb/IIIa was found to contribute to "aspirin resistance". Therefore, these polymorphisms may be potentially important for early detection and early intervention and thus setting the need to provide for a high volume genotype testing at health care centres. One of the most used techniques to determine platelet function is platelet aggregometry. However, the major disadvantages of platelet aggregation is that it is influenced by a number of environmental factors and its access is limited to tertiary health centres. Platelet aggregation measures the functional expression of platelets, which is known to deteriorate over time. It is for this reason that new methods at molecular level such as polymerase chain reaction (PCR) are needed to explore the role of genotypic expressions, which are not influenced by environmental factors. Currently, conventional PCR is used to detect platelet polymorphisms in the

research settings and has limitations as a routine diagnostic test. Furthermore, it is time consuming and is prone to contamination. With the recent advances in real-time PCR it is possible to genotype large sample batches rapidly without compromising on the quality, accuracy and precision of results. This study aims to adapt conventional PCR methodology onto a real-time platform for detecting platelet polymorphisms that have been implicated in both stroke and aspirin resistance.

Materials and methods

A total of 60 caucasian patients classified as having ischaemic stroke by virtue of MRI and Doppler analysis from the Stroke Clinic at the Johannesburg Hospital were enrolled for this study. Healthy caucasian individuals (38), age and gender matched were enrolled as controls. DNA samples were extracted from all the subjects and the prevalence of the Kozak –5T/C, HPA-2, VNTR and GPIIIa PI^A polymorphisms were determined first by using conventional PCR and then the real-time LightCycler™ PCR method.

Results

The frequency of the unfavourable alleles (the PI^{A2} allele for the GPIIIa PI^A polymorphism, the T allele for the Kozak –5T/C polymorphism, the B allele for the HPA-2 polymorphism and the C allele for the VNTR polymorphism) of the different GP's were higher in the stroke patients when compared to the control subjects but did not reach statistical significance. There was complete statistical

agreement between the results obtained for the conventional PCR as compared to the results obtained for real-time PCR except for the VNTR polymorphism, due to the difficulty in designing and the unavailability of probes for the real-time PCR assay. However, it is important to note that adapting the real-time PCR as a new methodology would greatly benefit both the patients and the clinicians by providing early detection and the possibility of early therapeutic intervention.

Conclusion

Therefore in conclusion, it is possible to perform not only conventional PCR for platelet polymorphism but also real-time PCR on a large scale without compromising on the quality, accuracy and precision on platelet polymorphisms that play a significant role in stroke and aspirin resistance. However, a larger population based study needs to be performed to confirm the findings.

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LIST OF SYMBOLS AND ABBREVIATIONS

α		alpha
β		beta
bp		base pair
CAD		coronary artery disease
Cox		cyclooxygenase
DNA		deoxyribose nucleic acid
EDTA		ethylenediamine tetra acetic acid
Fig		figure
FRET		fluorescence resonance energy transfer
GP		glycoprotein/s
LED	light emitting diode	
MET	methionine	
μ		micro
OD		optical density
ρ		pica
RFLP		restriction fragment length polymorphism
THR		threonine
UV	ultra violet	
vWF	von Willebrand Factor	

Publication and Presentations

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