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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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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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A dissertation submitted to the faculty of Health Sciences, University of The Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science – Medicine.

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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.

Sadhaseevan Moodly

November 2008

ABSTRACT

Introduction

It is widely recognised that stoke 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 GPIbα, 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 GPIbα 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 PI^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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TABLE OF CONTENTS

TITLE i

DECLARATION ii

ABSTRACT iii

ACKNOWLEDGEMENTS vi

TABLE OF CONTENTS viii

LIST OF TABLES xiv

LIST OF FIGURES xvi

LIST OF SYMBOLS AND ABBREVIATIONS xvii

PUBLICATIONS AND PRESENTATIONS xviii

CHAPTER ONE 1

1. Introduction 2

1.1 Stroke 2

1.2. Platelet Function 11

1.3. Platelet Polymorphisms 13

1.3.1. Glycoprotein Ib/IX/V 14

1.3.1.1 Kozak –5 T/C polymorphism 15

1.3.1.2. Variable Number of Tandem Repeats (VNTR) polymorphism 16

1.3.1.3. Human Platelet Antigen- 2 (HPA-2) Polymorphism 17

1.3.2. GPIIIa PI^A polymorphism 18

1.4. Rationale for this study 20

CHAPTER TWO 21

2. Study Design 22

2.1. The Evolution of Molecular Techniques 22

2.2. Molecular Techniques of Conventional PCR 24

2.2.1. DNA amplification 24

2.2.2. Polymerase chain reaction (PCR) 24

1. Denaturation 25

2. Annealing 25

3. Extension 26

4. Detection Procedure 26

2.3. Real-Time PCR 27

2.3.1. Principle of real time PCR using the Roche LightCycler ™ 27

2.3.2. Detection Formats 28

1. Monitor PCR with SYBR Green I Dye 28

2. Monitor PCR with Hydrolysis probes 29

3. Genotyping and Mutation Detection with SimpleProbe Probes 30

4. Monitor PCR with the LightCycler® HybProbe Format 30

2.4. Experimental Design 32

CHAPTER THREE 33

3. Materials and Methods 34

3.1.1. Patients and Controls 34

3.1.2. Samples 34

3.1.3. Genomic DNA Extraction 35

3.1.3.1. High Pure PCR Template Preparation Kit (Roche)- Method 35

3.2. DNA Quantification 36

3.3. Conventional PCR Methodology 37

3.3.1. Buffers and Solutions 37

3.3.1.2. Tris-Acetic acid-EDTA(TAE),(50xconcentrated stock buffer) 37

3.3.1.3. Tank Buffer (electrophoresis) 37

3.3.1.4. Gel Buffer 37

3.3.1.5. Ethidium Bromide (10mg/ml Stock Solution) 37

3.3.1.6. 2% Agarose gel preparation 38

3.4. Reagents and Materials - conventional PCR 38

3.5. PCR cycling conditions for conventional PCR 43

3.6. Restriction Fragment Length Polymorphisms (RFLP's) 44

3.7. Representation of Kozak -5T/C, HPA-2, VNTR and

GPIIIa PI^A Polymorphisms using Conventional PCR 45

3.7.1. Kozak – 5T/C polymorphism determination – Conventional PCR 45

3.7.2 Human Platelet Antigen (HPA-2) polymorphism – Conventional PCR 45

3.7.3 VNTR polymorphism determination – Conventional PCR 48

3.7.4. GPIIIa $PI^{A1/A2}$ polymorphism determination – Conventional PCR 50

3.8. Real-Time PCR using LightCycler ® 51

3.8.1. LightCycler™ FastStart DNA Master^{PLUS}HybProbe 51

3.8.2. Kit Content 51

3.8.3. Primers and Probes used to detect the target

polymorphisms using real-time PCR 52

2.8.3a.Kozak –5T/C Polymorphism determination- Real-Time PCR 52

2.8.3b. HPA-2 Polymorphism determination -Real-Time PCR 53

2.8.3c. GPIIIa PI^A polymorphism determination- Real-Time PCR 54

3.8.4. Preparation of the PCR Mix for Real-time PCR 55

3.8.5. HPA-2, Kozak and GPIIIa PI^A Cycling Program for Real-Time PCR 57

3.9. Representation of the Melting Curve Analysis of the Platelet Polymorphisms for HPA-2, Kozak and GP IIIa PI^A using Real Time PCR 58

3.9.1. Results for HPA-2, Kozak and GP IIIa PI^A polymorphisms 58

3.9.2. The Application of Real-Time PCR to the VNTR Polymorphism 62

CHAPTER FOUR 63

4. Results and Statistical Analysis of All PCR Results 64

4.1. Calculating gene frequencies as per formula 64

4.2. Hardy Weinberg Equilibrium and Fisher's Exact Test 65

4.3. Kappa statistics 70

CHAPTER FIVE 73

5. DISCUSSION 74

CHAPTER SIX 82

6. CONCLUSION 83

REFERENCES 84

APPENDIX 104

Appendix 1 104

Appendix 2 109

ETHICS APPROVAL 111

Ethics clearance certificate 112

LIST OF TABLES

1. Primer sequences for each platelet polymorphism used in conventional PCR analysis. 39

ii. Reagents and volumes that were used to prepare a master mix 40

solution for both the Kozak and VNTR polymorphism.

iii. Reagents and volumes that were used to prepare a master mix 41

solution for the HPA-2 polymorphism.

iv. Reagents and volumes that were used to prepare a master mix 42

solution for the PI^A polymorphism.

5. The PCR cycling conditions demonstrated in the table are for GPIIIa PI^A, Kozak, VNTR and HPA-2. 43

6. Restriction enzymes and the volumes that were used for each individual reaction in both the PI^A and Kozak polymorphism. 44

7. Primer and Hybridization probe sequence for genotyping Kozak polymorphism 52

8. Primer and Hybridization probe sequence used for genotyping the HPA-2 platelet polymorphism. 53

9. Primer and Hybridization probe sequence used for genotyping the GPIIIa PI^A 54

10. A generic protocol for the preparation of a 20µl standard master mix reaction. 55

11. The PCR cycling conditions for the GPIIIa PI^A, Kozak and HPA-2 polymorphism on the Real-time Lightcycler™. 56

12. The percentage distribution of GPIIIa (PI^A) polymorphism and PI^{A2} Allele frequency in stroke vs controls 66

13. The percentage distribution of Kozak (-5T/C) polymorphism and T Allele frequency in stroke vs controls 67

14a. The percentage distribution of VNTR polymorphism and C Allele frequency in stroke vs controls 68

14b. Breakdown of the calculation for the C allele frequency for the VNTR polymorphism. 69

15. The percentage distribution of HPA-2 polymorphism and BB genotype frequency in stroke vs non stroke individuals. 69

16. Correlation between the lightCycler PCR and the conventional PCR method for GPIIIa PI^A polymorphism. 70

17. Correlation between the Real-Time PCR and the conventional Kozak polymorphism. 71

18. Correlation between the Real-Time PCR and the conventional HPA-2 polymorphism. 72

19. Real-Time PCR - Initial Cost 109

20. Conventional PCR – Initial Cost 110

LIST OF FIGURES

1. Representation of the Kozak genotype that were generated by using gel electrophoresis. 46

2a. Representation of the HPA-2, Thr 234 bp (A) allele using gel electrophoresis. 47

2b. Representation of the HPA-2, Met 234 bp (B) allele using gel electrophoresis. 47

3a. Representation of the VNTR alleles that were generated by using gel electrophoresis. 48

3b. Representation of the VNTR alleles that were generated by using gel electrophoresis (CC genotype). 49

3c. Representation of the VNTR alleles that were generated by using gel electrophoresis (BB genotype). 49

4. Image of the GPIIIa PI^A genotype that were generated using gel electrophoresis. 50

5. Melting curve analysis for HPA-2 polymorphism. 59

6. Melting curve analysis for GPIIIa PI^A polymorphism. 60

7. Melting curve analysis for Kozak polymorphism. 61

LIST OF SYMBOLS AND ABBREVATIONS

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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