UNIVERSITI TEKNOLOGI MARA

OPTIMIZATION OF PCR METHOD FOR THE POLYMORPHIC DETECTION OF BETA-2 ADRENERGIC RECEPTOR GENE

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ABSTRACT

The aim of this study is to perform a one step direct PCR method in identifying polymorphisms of five alleles of interest of the beta-2 adrenoceptor gene (Arg16/ Gly 16, -20T/C, Gln27/ Glu27, -47T/C and Thr164/ Ile164). This one step direct PCR is also expected to produce better results compared to a previous study with the same alleles of interest but using a different method which was the two steps PCR. The method used in the study was multiple allele specific reaction which can allow simultaneous amplification of the five beta-2 adrenoceptor alleles. Specific primers were used to complement the target sequence and produce specific bands of interest. The PCR method was optimized for reproducibility and specificity. Few parameters were frequently adjusted to produce the desired results. The parameters include annealing temperature, cycling conditions, primers and *Taq* polymerase concentration. At the end of the study, the results were unsuccessful due to few problems and possible causes that can be improved in future studies.

CHAPTER 1

INTRODUCTION

1.1 Introduction of ß₂ adrenoceptor gene

The human ß₂ adrenoceptor (ß₂AR) is coded by the ß₂ adrenoceptor gene situated on the long arm (q31-q32) on chromosome 5 and codes for an intronless gene product of approximately 1200 base pairs. It is composed of 413 amino acids.

The ß₂ adrenoceptors are widely distributed in the airway smooth muscle and also in the lung, such as epithelial, endothelial cells, type II cells and mast cells. These receptors are also found in the skeletal muscles and in the liver.

The major response of the airways to stimulation of ß₂ adrenoceptors is relaxation of the airway smooth muscle therefore results in bronchodilation. In the skeletal muscle this receptor acts by promoting potassium uptake and the presence in the liver is to activate glycogenolysis. The ß₂ adrenoceptor agonists have several pharmacologic actions that are important in the treatment of asthma. They relax airway smooth muscle and inhibit release of some bronchoconstricting substances from mast cells.

A number of polymorphisms of the ß₂ adrenoceptor have been explained. Polymorphisms influence the growth of the receptors and it is documented that will lead to the downregualtion of these receptors. Polymorphism of these receptors would also alter receptor function, change in binding affinity towards ß₂ agonists, cause variations among individuals and lead to pathogenesis of some diseases such as bronchial asthma, obesity, hypertension and cystic fibrosis. According to studies, 9 nucleotide polymorphisms were identified but 4 were found to cause amino acid changes at position