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DESIGNING PRIMER FOR EXON 2 OF HLADRB1 GENE IN TYPE 1 DIABETES

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ABSTRACT

Type 1 diabetes is known as a complex disease as several gene mutations are likely to contribute to the disease. The HLA region on chromosome 6p21, which includes the major histocompatibility complex (MHC) class II has been identified as the major genetic determinant for the development of Type 1 diabetes. It has been identified that MHC class II genes DRB1, DQA1, and DQB1 are the loci of greatest genetic risk for this disease. The strongest association of these genes with Type 1 diabetes were observed with DRB1 and DQB1 genes. DRB1 gene was found to influence the risk of DQB1 allele susceptibility. Therefore, HLADRB1 was chosen in this research. The objective of this research is to design a set of primers to amplify exon 2 of HLADRB1 gene using Polymerase Chain Reaction (PCR) technique. A set of primer was designed for the amplification of exon 2 of HLADRB1 gene (NG_029921) by using NCBI Primer BLAST. The primers together with the PCR components were assembled for PCR amplification and optimization which then subjected to gel electrophoresis. The desired amplicons were sent for DNA sequencing and analysis of SNPs were conducted. In the results, seventeen SNP were found for this exon; 9 synonymous and 8 non-synonymous. Deletion of GC at position rs13383 and rs13384 result in the conversion of Serine to Lysine. Insertion of C at 350nt causes a conversion of amino acid from Serine to Threonine whereas at 361nt, insertion of C causes conversion of Glycine into Arginine. In conclusion, the newly designed primer set was able to amplify exon 2 of HLADRB1 gene successfully. However, all SNPs were not reported as clinically significant in the previous reports. Insertion and deletion mutations observed may or may not have altered the function of the resulting

CHAPTER ONE: INTRODUCTION

1.1 Background of Study

About 382 million of people have diabetes worldwide. More than 138.2 million people from the total number came from the West Pacific region and this number will possibly arise to 201.8 million by the year of 2035. In Malaysia, there were 1.9 million of people diagnosed with diabetes in 2013 (International Diabetes Federation, 2014).

According to Jong Koi Chong, the president of Obesity Prevention Council, 3.6 million of adults are assumed to be diagnosed with diabetes in Malaysia. This alarming situation has put the country to be the first country in Asean having the highest number of patients with diabetes. In the western pacific region, this high number of diabetics put Malaysia as the sixth country with diabetes. According to Professor Datuk Ikram Shah Ismail, Malaysia had exceeded the estimation done by the World Health Organization (WHO) with additional 807,000 diabetics in 2025 ("Number of diabetics in Malaysia alarming - Nation | The Star Online," 2013). The prevalence of diabetics in Malaysia are shown in Figure 1.1.