UNIVERSITI TEKNOLOGI MARA

DESIGNING PRIMER FOR EXON 6 OF LEPTIN RECEPTOR GENE

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ABSTRACT

Introduction. Prevalence of obesity is increasing in the world population. Obesity can be defined as excessive fat accumulation due to imbalance between energy intake and energy consumption. The etiology of obesity includes sedentary lifestyles and genetic factors such as leptin deficiency or leptin receptor deficiency. Leptin is a hormone that regulates energy consumption and intake as it affects the satiety center in brain. A Proline316Threonine (P316T) SNP in exon 6 of leptin receptor causes early onset of obesity as there is conversion of nucleotides C156868A. This SNP causes the dysfunction of Fibronectin III domain which involves in activation of leptin receptor. If the receptor cannot be activated, leptin cannot bind to it and send necessary signal to the satiety center. There is limited study on exon 6 of leptin receptor in Malaysia, thus leading to this study which was to design primer to amplify exon 6.

Methodology. The study requires ethic approval as it involved sampling of the human blood. The primer was designed using NCBI Primer BLAST and DNA extraction was done using NucleoSpin® Blood kit. Next, the DNA was amplified and optimized using gradient PCR to obtain the best annealing temperature. The amplicon was subjected for agarose gel electrophoresis to determine the best annealing temperature. The amplicon was sent for purification and DNA sequencing. The sequencing result obtained and SNP was then analyzed.

Results. The DNA yield was 43.4μg/ml and the A260/280 nm was 1.87. The best annealing temperature is 62.3°C. The SNP was absent in the exon 6 of the subject.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Obesity has become pandemic (Prentice, 2006). From 1980 to 2013, obesity and overweight cases have increased 27.5 % in adults and 47.1 % in children as shown in Figure 1.1 (Ng et al., 2014). Obesity can be defined as an abnormal or excessive fat accumulation. It is an important risk factor for several chronic diseases such as cardiovascular disease, diabetes, cancer and liver and gall bladder disease (Maggi, Busetto, Noale, Limongi, & Crepaldi, 2015). The etiology of obesity includes rapid urbanization, lifestyle transition, nutrition and some genetic factors (Chong, Teh, Poh, & Noor, 2014). From the genetic perspective, leptin receptor deficiency can contribute to obesity (I. S. Farooqi & O'Rahilly, 2014).

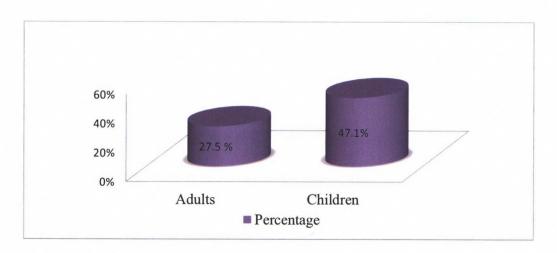


Figure 1.1: Obesity and overweight cases in adults and children.