DETECTION OF HOMOZYGOUS DELETION OF SURVIVAL MOTOR NEURON 1 (SMN1) GENE USING ALLELE-SPECIFIC PRIMER IN SMA PATIENTS

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BIOTECHNOLOGY PROGRAMME
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HOO KIT MEI

THIS DISSERTATION IS PRESENTED TO FULFILL THE PARTIAL REQUIRMENT TO OBTAIN A BACHELOR DEGREE OF SCIENCE WITH HONOURS

BIOTECHNOLOGY PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
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DECLARATION

I hereby declare that the work in this project is of my own except quotations and summaries from the references which have been acknowledged.

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VERIFICATION

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ABSTRACT

This research was carried out to amplify the exon 7 of SMN1 gene by using allele-specific primer and optimize the PCR reagents and parameters for the detection of homozygous deletion of SMN1 gene in SMA patients. It had been noticed that 58.4℃ of annealing temperature gave the optimum condition in PCR amplification for both tel-SMN and β-globin primers. Based on the resulted PCR amplification profiles, both SMN1 and β-globin genes were performed in the normal healthy sample. Meanwhile, the identification of SMN1 gene deletion in SMA patients was verified by the absence of targeted band (SMN1 gene) together with the presence of β-globin gene band. This phenomenon could only be detected in the experiment with higher DNA concentration loaded, that was 68 ng. With the β-globin gene served as an internal control, the result was considered valid for any sample that showed β-globin gene band. In order to confirm the actual size of the PCR products, DNA sequencing was performed for both SMN1 and β-globin genes. However, the resulted sequencing electropherograms were failed to provide the complete sequence for all samples. Hence, PCR-allele specific method is specifically useful in the analysis of homozygous deletion of SMN1 gene in SMA disease but more optimizations are needed for DNA sequencing in order to make this study more reliable and success.

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ABSTRAK

Penyelidikan ini telah dilakukan untuk mengamplikasikan ekson 7 dalam gen SMN1 dengan menggunakan primer alel-khusus dan mengoptimumkan reagen-reagen dan parameter-parameter bagi pengesanan pemotongan homozigus gen SMN1 dalam pesakit-pesakit SMA. Didapati bahawa suhu perlekatan primer pada 58.4 ℃ memberikan keadaan yang optimum dalam amplifikasi PCR bagi kedua-dua primer tel-SMN dan β-globin. Berdasarkan profil-profil amplifikasi PCR yang terhasil, kedua-dua gen SMN1 dan β-globin hadir dalam sampel yang normal dan sihat. Sementara itu, pengecaman pemotongan gen SMN1 di pesakit-pesakit SMA pula dapat disahkan dengan ketiadaan jalur sasaran (gen SMN1) bersama dengan kehadiran jalur gen β-globin. Fenomena ini hanya dapat dikesan dalam eksperimen yang dimuatkan dengan kepekatan DNA yang lebih tinggi, iaitu 68 ng. Dengan adanya gen β-globin sebagai kawalan dalaman, keputusan boleh dikatakan sahih bagi sebarang sampel yang menunjukkan jalur gen β-globin. Untuk memastikan saiz sebenar produk PCR, penjujukan DNA telah dijalankan bagi kedua-dua gen SMN1 dan β-globin. Walau bagaimanapun, elektroferogram-elektroferogram penjujukan yang terhasil adalah gagal untuk membekal jujukan yang lengkap bagi semua sampel. Maka, kaedah PCR alel-khusus ini adalah sangat berguna khususnya dalam analisis mengenai pemotongan homozigus gen SMN1 dalam penyakit SMA tetapi lebih optimasi adalah diperlukan untuk penjujukan DNA supaya kajian ini menjadi lebih menyakinkan dan berjaya.

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LIST OF SYMBOLS

α	Alpha
β	Beta
D	The nonsuperimposable stereoisomer of (L)
L	The nonsuperimposable stereoisomer of (D)
F_1	The first filial generation
F_2	The second filial generation
S	Smooth seed
s	Wrinkled seed
p	Short arm of chromosome
q	Long arm of chromosome
3'	Three prime end of a DNA strand
5'	Five prime end of a DNA strand
+	Position of a nucleotide located in a positive direction within the sequence of a gene
-	Negative/Minus
±	Plus and minus
∞	Infinity
/	Per
~	Approximate
&	And
x	Multiply
∴	As a result

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LIST OF UNITS

%	Percent
˚C	Degree Celsius
ft	Feet
g	Gram
in	Inch
kb	Kilobase
min	Minute
ml	Milliliter
mM`	Millimolar
ng	Nanogram
ng/µl	Nanogram Per Microliter
pH	A standard measure of the acidity of a solution
pmol/µl	Picomole Per Microliter
rpm	Rotation Per Minute
U	Unit
U/µl	Unit Per Microliter
µl	Microliter
V	Volt
X	Times in concentration

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LIST OF ABBREVIATIONS

A	Adenine
ABI	Applied Biosystem
bp	Base pairs
C	Cytosine
ddH_2O	Deionized Distilled Water
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates (dATP, dCTP, dGTP, dTTP)
ddNTPs	Dideoxyribonucleoside Triphosphates
dsDNA	Double-Stranded Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
EtOH	Ethanol
ssDNA	Single-Stranded Deoxyribonucleic Acid
fwd	Forward
G	Guanine
HBB	Beta-hemoglobin
KCl	Potassium Chloride
Mg^{2+}	Magnesium Ion
$MgCl_2$	Magnesium Chloride
mRNA	Mesenger Ribonucleic Acid
^{32}P	Radioactive Phosphorus Group
PCR	Polymerase Chain Reaction
RE	Restriction Enzyme
rvs	Reverse

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SMA	Spinal Muscular Atrophy
SMN	Survival of Motor Neuron
SMN*cen*	Centromeric Survival of Motor Neuron 2 Gene
SMN*tel*	Telomeric Survival of Motor Neuron 1 Gene
ssDNA	Single Stranded Deoxyribonucleic Acid
T	Thymine
TBE	Tris-borate EDTA
tel-SMN	Telomeric Survival of Motor Neuron Primer
tel-SMNex7	Telomeric Survival of Motor Neuron Forward Primer (exon 7)
tel-SMNint7	Telomeric Survival of Motor Neuron Reverse Primer (intron 7)
T_m	Annealing Temperature
Tris-Cl	Tris-Chloride
Tris-HCl	Tris-Hydrogen Chloride
UV	Ultraviolet

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CHAPTER 1

INTRODUCTION

Spinal Muscular Atrophy (SMA) is a common autosomal recessive neuromuscular disease of early life. Childhood-onset proximal SMA is characterized by degeneration of anterior horn cells of the spinal cord and, in some cases, of motor nuclei in brain stem. This degeneration causes the deficiency in the production of SMN protein which is essential for the health of motor neurons. Motor neurons are nerve cells that control muscles used for normal activities such as walking, sitting and swallowing. Consequently, the loss of motor neurons will lead to symmetrical muscles weakness and atrophy, limb and trunk paralysis, respiratory failure, and even infant death among the SMA patients.

SMA affects the voluntary muscles used for crawling, walking, head and neck control, and swallowing. The proximal muscles such as shoulders, hips, and back are often the most severely affected part. However, weakness in the legs is usually more severe than in the arms. As the child with SMA grows, the decrease in motor neurons and the increase demands on the nerve cells make his body doubly stressed. The resulting muscle weakness may lead to further loss of function especially the respiratory system which can lead to an increased tendency for pneumonia and other lung problems.

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Clinically, SMA is divided into four groups based on certain key motor function milestones such as the age of onset, the maximum muscular activity achieved, and survivorship. Type I is the most severe form of SMA with onset at birth or before six months and death of respiratory distress usually within two years. This type of patients will never be able to sit or walk and have difficulties in breathing and swallowing. Type II is the intermediate form SMA which strikes infants between seven and 18 months old. These patients may able to sit unaided or even stand with support. Type III SMA affects children after the age of 18 months and patients are able to stand and walk but may unable to run. Type IV or the adult-onset SMA usually shows symptoms after age 35. The affected patients have muscle weakness, tremor, and twitching from mild to moderate (Families of SMA, 2005).

The survival of motor neuron (SMN) gene is the causing gene for SMA disease. It is present on chromosome 5 at position 5q13 in two inverted copies, telomeric SMN1 and centromeric SMN2. Both genes are nearly similar and encode for the same SMN protein (Lefebvre *et al.*, 1995). However, only SMN1 gene produces the full-length proteins while most SMN2 proteins are shortened due to the lacking of exon 7 in its mRNA. Therefore, SMA disease is caused by the deletions or mutations in SMN1 but not SMN2. The number of copies of SMN2 is only related to the severity of SMA disease. SMA patients are people who have both SMN1 copies deleted while the carriers are missing only one copy (Families of SMA, 2005). Approximately 95% of SMA patients are having homozygous deletion of SMN1 gene whereas the subsequent 5% are having other mutations (Lefebvre *et al.*, 1995).

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Polymerase Chain Reaction (PCR)-based assays are always used for molecular diagnosis. A method called PCR-Allele Specific is one of the tests that developed on the basis of conventional PCR. The speciality of this method is the allele-specific primer that used for amplification because it is designed to only amplify particular desired allele. Since the primer has a high level of specificity in amplifying desired DNA fragment, thus, the resulted PCR products are always highly reliable (Cantafora *et al.*, 1998).

In this study, the PCR-Allele Specific method was applied to detect the homozygous deletion of SMN1 gene in three SMA patients from three to 14 years old. All these patients are reffered to SMA molecular genetic test conducted at Human Genome Centre, Universiti Sains Malaysia Health Campus, Kubang Kerian, Kelantan. The first objective of this study is to amplify the specific exon 7 of SMN1 gene by using allele-specific tel-SMN primer and the second objective is to optimize the PCR reagents and parameters for the detection of homozygous deletion of SMN1 gene in SMA patients. The third objective is to confirm the actual size of both SMN1 and β-globin genes by using DNA sequencing.

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