MOLECULAR ANALYSIS OF SURVIVAL MOTOR NEURON (SMN) AND NEURONAL APOPTOSIS INHIBITORY PROTEIN (NAIP) GENES IN SPINAL MUSCULAR ATROPHY (SMA) PATIENTS IN MALAYSIA

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MOLECULAR ANALYSIS OF SURVIVAL MOTOR NEURON (SMN) AND NEURONAL APOPTOSIS INHIBITORY PROTEIN (NAIP) GENES IN SPINAL MUSCULAR ATROPHY (SMA) PATIENTS IN MALAYSIA

By

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Thesis submitted in fulfillment of the requirements for the Degree of Master of Science

OCTOBER 2007
DEDICATION

To my family, who have always showered their love and support, especially to My mother, Puan Faridah Muhammad, my late father Allahyarham Mohd Shamshudin Daud, my sisters and brothers Kak Anis, Nazila, Fadhli, Sabri, Saffuan, Eizhan, Eqba and Suhaila and also C’Ngah, Mama and families...
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LIST OF ABBREVIATIONS

°C : degree celcius
µl : microliter
$A_{260}/A_{280}$ : ratio of 260 absorbance over 280 absorbance
AFLVs : amplification fragment length variations
ANS : autonomic nervous system
bp : base pair
BSA : bovine serum albumin
Buffer AE : Elution Buffer
Buffer BL : Blood Lysis Solution
Buffer BW : Column Wash Solution B
Buffer TW : Column Wash Solution T
CBs : Cajal bodies
CFTR : Cystic Fibrosis Transmembrane Regulatory
CNS : central nervous system
ddH$_2$O : deionized distilled water
dHPLC : denaturing High Performance Liquid Chromatography
dNTPs : dinucleotide triphosphatase
EDTA : ethylenediamine tetraacetic acid
EMG : electromyography
Gems : Gemini of Cajal bodies
HDAC : histone deacetylase
kb : kilobase
kDa : kilo Dalton
LB : Lithium Boric Acid buffer
mg/ml : milligram per milliliter
MgCl$_2$ : magnesium chloride
min : minute
ml : milliliter
mM : millimolar
NAIP : Neuronal Apoptosis Inhibitory Protein
NCS : nerve conduction studies
ng/µl : nanogram per microliter
nm : nanometer
PBS : phosphate buffer saline
PCR : Polymerase Chain Reaction
PCR-RE : Polymerase Chain Reaction-Restriction Enzyme
PNS : peripheral nervous system
pre-mRNA : precursor messenger RNA
RNA : ribonucleic acid
rpm : round per minute
RT-PCR : reverse transcriptase PCR
SMA : Spinal Muscular Atrophy
SMN : Survival Motor Neuron
SMN1 : Survival Motor Neuron 1
SMN2 : Survival Motor Neuron 2
snRNA : small nuclear RNA
snRNPs : small nuclear ribonucleoprotein
SYBR® Green I : SYBR® Green I Nucleic Acid gel stain
Taq : *Thermophilus aquaticus*
U : unit
UV : ultra-violet
V : voltage
VPA : valproic acid

Objektif kajian ini adalah untuk menentukan frekuensi mutasi delesi gen SMN1 dan NAIP di kalangan pesakit SMA di Malaysia. Selain itu, hubungan antara bilangan salinan gen SMN2 dan tahap keterukan penyakit ini juga dikaui. Sejumlah 69 sampel darah individu normal dan 69 sampel darah pesakit yang disyaki secara klinikal mengalami SMA, diperolehi dari hospital kerajaan dan juga institusi akademik di seluruh Malaysia. Delesi homozigus bagi gen SMN1 ditentukan melalui kaedah PCR diikuti oleh pemotongan menggunakan enzim restriksi. Bagi mengenalpasti delesi homozigus gen NAIP, asai PCR multiplex menggunakan gen β-globin sebagai kawalan dalaman telah dilakukan. Bagi sampel pesakit yang menunjukkan keputusan delesi homozigus SMN1 yang positif, sampel tersebut...
digunakan untuk kajian seterusnya bagi menentukan bilangan salinan gen SMN2 dengan menggunakan Real-time PCR. Sebanyak 81 peratus daripada pesakit yang secara klinikal disyaki menghidapi SMA telah menunjukkan keputusan positif delesi homozigus pada sekurang kurangnya exon 7 gen SMN1. Delesi homozigus NAIP dikenalpasti berlaku pada 9 daripada 42 pesakit yang positif delesi gen SMN1. Tujuh puluh lapan peratus daripada sampel tersebut adalah pesakit SMA jenis I. Analisis kuantifikasi pula menunjukkan bilangan salinan gen SMN2 yang tinggi didapati pada pesakit yang mempunyai fenotip yang tahap keterukan penyakit yang lebih rendah. Keputusan ini telah memberikan penunjuk penting bagi prognosis pesakit. Hasil kajian ini telah menunjukkan bahawa delesi gen SMN1 adalah penyebab utama bagi penyakit SMA di Malaysia. Kaedah pengambilan sampel darah yang tidak invasif berbanding kaedah konvensional ini sangat sesuai bagi tujuan diagnosa terutama bagi bayi yang baru dilahirkan. Delesi bagi gen NAIP lebih banyak didapati pada pesakit yang lebih teruk fenotipnya.
MOLECULAR ANALYSIS OF SURVIVAL MOTOR NEURON (SMN) AND NEURONAL APOPTOSIS INHIBITORY PROTEIN (NAIP) GENES IN SPINAL MUSCULAR ATROPHY (SMA) PATIENTS IN MALAYSIA

ABSTRACT

Spinal Muscular Atrophy (SMA) is a neuromuscular disease which is clinically characterized by progressive muscular weakness and atrophy of the skeletal muscles. This degenerative disease is caused by mutation of the Survival Motor Neuron 1 (SMN1) gene. SMA is classified into 3 subtypes; type I, type II and type III based on age at onset and clinical severity. The variations of severity might be related with mutation or alteration in other associated genes such as Survival Motor Neuron 2 (SMN2) and/or Neuronal Apoptosis Inhibitory Protein (NAIP). The objectives of this study are to determine the deletion frequency of SMN1 and NAIP genes and study the relationship between the copies of SMN2 gene with severity in patients who have SMN1 gene deletion. A total of 69 normal blood samples and 69 blood samples of clinically suspected SMA patients from various hospitals in Malaysia were recruited into this study. Homozygous deletion of the SMN1 gene was determined by PCR method followed by restriction enzyme digestion. NAIP gene deletion was determined by multiplex PCR assay whereby β-globin gene was used as an internal control. Samples found to have deletion of the SMN1 gene were then subjected to real-time PCR for the quantification of the SMN2 gene. Eighty-one percent of patients highly suspected to have SMA showed homozygous deletion of at least exon 7 of SMN1 gene. The NAIP gene deletion was detected in
9 out of 42 patients and 78% of them were patients with type I SMA. Quantification analysis showed a higher copy number of the SMN2 gene in patients with milder phenotype and could be an important indication for prognosis. From this study, deletion of the SMN1 gene was a major cause of SMA in these patients. This non-invasive molecular genetic testing could be a useful tool for the diagnosis of SMA especially in newborn babies. NAIP gene deletion found in this study was mostly seen in severe type of SMA.
CHAPTER 1

LITERATURE REVIEW

1.1 The human body system

The human body is made up of atoms, molecules, cells, tissues and organs. The organization of these organs is called system. The human body systems are the complex units that make up the body and are composed of 11 major systems including integumentary, nervous, skeletal, muscular, cardiovascular, endocrine, respiratory, digestive, reproductive, lymphatic, and urinary system.

1.1.1 Nervous system

The nervous system is the most complex of all human body systems. It is classified into two major divisions; the central nervous system (CNS) and peripheral nervous system (PNS). The central nervous system consists of the brain and spinal cord. The peripheral nervous system consists of all nervous tissues outside the brain and spinal cord (Figure 1.1). Functionally, the nervous system can be divided into the somatic nervous system which controls skeletal muscles, and autonomic nervous system (ANS) that controls smooth muscle, cardiac muscle and glands.
Figure 1.1: Brain and spinal cord build up central nervous system while peripheral nervous systems consist of peripheral nerves and sensory receptors
1.1.2 Development of central nervous system

During embryogenesis, three germ layers, namely endoderm, mesoderm and ectoderm are formed (Table 1.1). Endoderm gives rise to guts while the mesoderm to the rest of the organ. Ectoderm develops into skin and nervous systems. The development of nervous systems starts with the formation of the neural plate. In the third week of human development, neurulation occurs where the surface of ectoderms thickens and begins to sink and fold in on itself. By the end of this process, the neural tube is formed. Finally this neural tube forms the brain and spinal cord which constitutes the central nervous system.

1.1.3 Neuronal development

Neuron is the basic functional unit of the nervous system. Each neuron has two types of fibers extending from the cell body; dendrite and axon (Figure 1.2). The dendrite carries impulses toward the cell body while the axon carries impulses away from the cell body. Nerve cell bodies are derived from the neural tube or neural crest. This nerve cell processes the axons and dendrites which sprout from the cell bodies to the tissues and structures they innervate.

Each neuron is part of a relay system that carries information through the nervous system. A neuron that transmits impulses toward CNS is a sensory neuron while a neuron that transmits impulses away from CNS is a motor neuron. There are connecting neurons within the CNS called synapses. At the synapse, energy is passed from one cell to another by means of a chemical neurotransmitter.
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Figure 1.2: Neuron, the core components of brain, spinal cord and peripheral nerves
1.1.4 Spinal cord

The spinal cord is the connection center for the reflexes as well as the afferent (sensory) and efferent (motor) pathways for most of the body below the head and neck. The spinal cord begins at the brainstem and ends at about the second lumbar vertebra. The spinal cord carries all the nerves to and from the limbs and lower part of the body. It is the pathway for impulses going to and from the brain. A cross-section of the spinal cord reveals an inner section of gray matter containing cell bodies and dendrites of peripheral nerves. The gray matter appears as a thickened and distorted letter ‘H’. The upper arms of the H are referred to as the dorsal horns (posterior horns) and the parts below are referred as the ventral horns (anterior horns). The outer region of white matter contains nerve fiber tracts and myelin sheath and conducts impulses to and from the brain.

1.1.5 Transmission of signal

The axons of ventral horn motor neurons exit via ventral roots. There are two types of motor neurons. Large alpha-motor neurons (skeletomotor neurons) innervate the ordinary skeletal muscle fibers, while gamma-motor neurons (fusimotor neurons) innervate the intrafusal muscle fibers of muscle spindles exclusively. The contractions of skeletal muscles are produced via the activation of the alpha-motor neurons. Damage or degeneration of the alpha-motor neurons causes failure of the impulse to be transferred to a motor unit and will finally affect the stretch reflex action (Figure 1.3).
Figure 1.3: The diagram showing a signal transmission (arrow) from the motor neuron to the muscle.
1.2 Spinal Muscular Atrophy as a neurodegenerative disease

Spinal Muscular Atrophy (SMA) is one of neuromuscular disorders. SMA was first described in the 1890s by Guido Werdnig of the University of Vienna and Johann Hoffmann of Heidelberg University (Markowitz et al., 2004). The term ‘spinal’ was used because the main cause of the disease is degeneration of alpha-motor neuron, located in the anterior horn of the spinal cord. The disruption of the specific neuron causes failure of the impulse to be transferred from the brain to muscle for a response. The effect from transmission failure involves the muscular systems. The muscles that do not function will eventually shrink or undergo wasting (atrophy). This condition mainly affects the proximal voluntary muscles or the muscles closest to the spinal cord, thus affecting activities such as crawling, swallowing, walking, and neck control, eventually leading to death.

1.2.1 Classification and clinical description of SMA

SMA is classified into 3 clinically subtypes; type I, type II and type III based on clinical features, age of onset and development of motor milestone (Munsat, 1992). The diagnostic criteria for SMA were categorized and reported in the International SMA Consortium Meeting (26th -28th June 1992) in Bonn, Germany, published by European Neuro Muscular Center (ENMC). In 1998, the diagnostic criteria was revised and detailed in 59th ENMC International Workshop (Zerres and Davies, 1999) as shown in Appendix 1. The updating of the diagnostic criteria for SMA has been part of an agreement done by groups
of clinicians and researchers of neuromuscular disorders. Later, the diagnostic criteria becomes a guideline for clinicians to diagnose SMA.

### 1.2.1 (a) Type I SMA

Type I SMA is an acute type, also known as Werdnig-Hoffmann Disease. This is the most severe type of SMA. Majority of cases present before the age of 3 months with lack of fetal movements in the final months of pregnancy and weakness at birth. The onset ranges from prenatal period to the age of 6 months. Patients typically present with generalized muscle weakness, poor muscle tone and absence of tendon reflexes. They are hypotonic and never able to sit without support. Fasciculation of the tongue are seen in most but not all patients with type I. Normal reaction to sensory stimuli shows no sensory loss in patients. Mild contractures are often at knees and rarely seen at the elbows. The patients may also present with some ingestion, feeding and secretion problems as a result of the muscle weakness of respiratory and digestive systems. Almost all patients have a life expectancy of less than 2 years. The mortality is typically due to respiratory failure or infection which is caused by weakness in the intercostal and accessory respiratory muscles. A typical clinical appearance of the patient is shown in Figure 1.4.
Figure 1.4: Clinical feature of type I SMA patient. The baby presents with hypotonia
1.2.1 (b) Type II SMA

Type II SMA is the intermediate form with onset after 6 months of age, but less than 18 months. Patients with this type are able to sit independently but could not stand or walk. There is absence of tendon reflexes in about 70 per cents of individuals (Iannaccone et al., 1993). Tongue fasciculation is one of the features that is present in type II. The life expectancy could be until adulthood and the intellectual skills of this group of patients are in the average range. Figure 1.5 shows one of our patients with type II SMA.

1.2.1 (c) Type III SMA

Type III SMA is also known as Kugelberg-Welander Disease. It is the mildest form with the onset after the age of 18 months. Patients are able to stand and walk without aid. The lower limbs are usually more affected than the upper limbs. Affected limbs shows proximal muscle weakness. Patients with type III SMA usually have frequent falls or trouble walking up and down stairs at the age of two to three years. They have normal IQ and usually go to school and learn as other children. Figure 1.6 shows a child with SMA type III.
Figure 1.5: Type II SMA patient. Patient can sit but cannot stand or walk independently
Figure 1.6: Patient with type III SMA is able to stand without support
1.3 Genetics of SMA

1.3.1 Genetic bases of different types of SMA

SMA is an inherited disorder. This autosomal recessive disease is caused by mutation of Survival Motor Neuron (SMN) gene that encodes a multifunctional protein. SMN gene has been characterized as having a duplicated form; SMN1 and SMN2 gene. This gene lies within a large region (about 20kb) containing several genes (Lefebvre et al., 1995). The presence of deletion (90%) and other intragenic mutations (10%) in the telomeric copies known as SMN1 gene in SMA patients confirmed that the SMN1 gene is responsible for this disease. There has been no reported cases of patients losing both the SMN1 and SMN2 genes (Schwartz et al., 1997). The neighboring genes such Survival Motor Neuron 2 (SMN2) and Neuronal Apoptosis Inhibitory Protein (NAIP) are thought to be the modifying genes as the disease varies from mild (type III) to very severe (type I) cases (Wirth et al., 1999, Harada et al., 2002).

1.3.2 Inheritance of the disease

SMA is an autosomal recessive disease which affects 1 in 10000 live births. The overall frequency for a carrier is 1 in 40 (Pearn, 1980). Mutation in either of the alleles causes an individual to be a carrier. If a carrier is married to another carrier, there will be a twenty five percent possibility of having a child with SMA. If both of the mutated allele are transferred from parents, the child will have this fatal disease. The child who received both of the normal alleles will be unaffected.
The possibility for a carrier parents to have an unaffected child with carrier status is 50 percent. When one of the mutated allele is transferred from either mother or father, the child will be a carrier. The explanation of this mode of inheritance is described in Figure 1.7.

1.3.3 The discovery of SMA candidate genes

All three types of SMA; severe, intermediate and mild, have been reported to be due to different mutations at a single locus on the long arm of chromosome 5 (Melki, 1990). Brzustowicz et al., (1990) later mapped the candidate gene at a specific region of 5q12.2-13.3 by linkage analysis.

In the beginning, the severity of this disease was associated with Ag1-CA alleles which are complex marker (DiDonato et al., 1994). This marker is a short tandem repeat of nucleotide CA which is also known as microsatellite. The Ag1-CA alleles are located in each of the promoter region of SMN gene. The numbers of repeats differed in each of the promoter region for each allele. Usually, normal individuals have 2 copies of SMN1 gene and 2 copies of SMN2 gene. Thus, the amplification of Ag1-CA in a normal individual shows 4 different sizes of the marker. DiDonato et al., (1994) found patients with type I SMA predominantly produce a single AFLV allele whereas majority of type II patients amplified an allele with two or three amplification fragment length variants (AFLVs). They suggested that this marker clearly identifies the critical region that should be searched for SMA candidate genes.
Figure 1.7: The possibility of a carrier parents to transfer the genetic information to the offspring. Blue color indicates a normal allele while the white color indicates a mutated allele.
In 1995, Lefebvre et al characterized the SMA-determining gene and found the evidence of a large inverted duplication of an element of approximately 500kb, termed $E^{Tel}$ for the telomeric and $E^{Cen}$ for the centromeric elements. The $E^{Tel}$ ($SMN1$, *Survival Motor Neuron 1*) and $E^{Cen}$ ($SMN2$, *Survival Motor Neuron 2*) were later successfully distinguished by southern blotting analysis (Roy et al., 1995b). Survival Motor Neuron (*SMN*) gene was later found to be the responsible gene for this disease (Lefebvre et al., 1995) while Neuronal Apoptosis Inhibitory Protein (*NAIP*) gene was reported to be deleted in most of the patients with severe type (Roy et al., 1995a).

1.3.3 (a) **Survival Motor Neuron gene**

The *SMN* gene spans about 20kb with 9 exons (Burglen et al., 1996). *SMN* gene is characterized by an inverted duplication which exists in two highly homologous copies known as *SMN1* and *SMN2* gene. Analysis of the genomic sequence of these genes revealed 5 nucleotide differences between *SMN1* and *SMN2*. The differences are one nucleotide in intron 6, one in exon 7, two in intron 7, and one in exon 8 respectively (Figure 1.8).

All the 5 differences between *SMN1* and *SMN2* did not result in any change in the amino acid coded. Both of the genes expressed the same peptide sequence for the SMN protein. However, the alteration in the nucleotide sequence (C to T) in exon 7 of *SMN2* causes splicing of this exon 7 during the transcriptional process. This results in the formation of truncated SMN2 protein (Lorson et al., 1999). Exon 7 of *SMN1* gene encodes a protein with a last terminal-C 16 residues while the transcript of *SMN2* gene is lacking of this vital protein which
Figure 1.8: A) p arm and q arm of the human chromosome 5, B) the inverted duplication region contains SMA-causing gene, C) five differences in nucleotide changes between \textit{SMN1} and \textit{SMN2} genes.
causes the produced protein to be not self-oligomerized (Hofmann et al., 2000) and unstable both in vivo and in vitro.

The SMN gene encodes a 294 amino acid with 38kDa of SMN protein. The SMN protein is ubiquitously expressed and localized in both nucleus and cytoplasm (Coover et al., 1997).

In nucleoplasm, SMN protein is found in a concentrated form in subnuclear structure known as Gems. Gems is also known as ‘Gemini of Cajal bodies’ because of the similarities in number and size with Cajal bodies (Liu and Dreyfuss, 1996). The ultrasructural study showed Gems represent a distinct category of nuclear body (Navascues et al., 2004). This subnuclear structure also gives the same response to metabolic condition as Cajal bodies (CBs). CBs is a nuclear accessory bodies described as a roughly spherical, typically 0.1-1.0 µm in size (Lamond and Carmo-Fonseca, 1993) and exist in about 1-5 per nucleus. This structure is mainly derived from metabolically active cells such as neuron or cells that are highly propagated like cancer cells (Matera, 2003).

The difference between CBs and Gems is the presence of small nuclear ribonucleoprotein (snRNPs) in the CBs. snRNPs is a complex of snRNA protein which consist of four different snRNP (U1, U2, U4/U6, and U5), essential mediators of RNA processing events. Two proteins were identified by Liu and Dreyfuss in 1996 that are essential in the biogenesis and recycling of snRNPs which are SMN and its associated protein, SIP1. This SMN-SIP1 protein complex associate with snRNPs and formed a complex of multiprotein (Fischer
et al., 1997) called spliceosomal snRNPs which is involved in pre-mRNA splicing.

The SMN protein was shown to interact with itself before associating with other protein (Liu and Dreyfuss, 1996). Full-length SMN protein produced by SMN1 is needed for the self-oligomerization before the interaction with other protein to form a large complex of multiprotein.

The expression of SMN protein is normally very high in the spinal cord of normal individuals and was shown to be reduced by 100-fold in samples of type I SMA. In type I SMA fibroblast, the number of gems is greatly reduced compared to type II, type III, carrier and normal individual (Coovert et al., 1997).

To date, there are enough findings to prove that deletion of SMN1 gene being the major cause for the SMA disease. However, the mechanism on how deficiency of SMN protein causes a specific defect in degeneration of motor neuron is still unclear.

1.3.3 (b) Neuronal Apoptosis Inhibitory Protein gene

The Neuronal Apoptosis Inhibitory Protein (NAIP) gene is a part of 500kb inverted duplication on chromosome 5q13. It lies adjacent to the SMN1 gene and close to each other with the ends probably less than 20kb apart. NAIP gene contains at least 16 exons and encodes for 1232 amino acids of 140kDa protein (Roy et al., 1995a).
The protein sequence encoded by *NAIP* gene exons 6-12 contains a region which is homology to baculovirus protein (Birnbaum *et al.*, 1994). This protein was found to inhibit cell apoptosis in insects induced by virus (Clem and Miller, 1993). Expression of *NAIP* in mammalian cells was also shown to inhibit apoptosis induced by a variety of signals (Liston *et al.*, 1996).

The *NAIP* was identified as one of the SMA-related gene after it was found to be deleted in the most severe type of SMA patients. The RT-PCR amplification of RNA from SMA and non-SMA tissue revealed that at least some of the internally deleted and truncated *NAIP* versions are transcribed in SMA patients. Based on the data obtained, deletion of *NAIP* gene was suggested to be consistent with defects in SMA either resulting in or contributing to the SMA phenotype (Roy *et al.*, 1995a). However, until today the role of *NAIP* in SMA has not been fully clarified.

### 1.4 Diagnosis of SMA

Common diagnostic methods for SMA include observing the degeneration of cells from muscle biopsy, electromyography (EMG) and/or Nerve Conduction Studies (NCS).

#### 1.4.1 Muscle Biopsy

Muscle biopsy is performed to examine small muscle tissues, usually taken from the thigh. The tissue is stained and observed under microscope to investigate the degeneration of muscle fibers. Typically, muscle biopsy shows
degeneration of muscle fibers without inflammation, fibrosis or histochemical abnormality. Patients with severe weakness have many small fibers which show features of denervation. However, the set back of this procedure is it is invasive and sometimes may give inconclusive results especially in newborn babies.

1.4.2 Electromyography (EMG) and Nerve Conduction Study (NCS)

EMG is a procedure used to assess motor units in various portions of the body such as cells located in the anterior horn, brain stem, axons, and the muscle fibers they innervate via neuromuscular junctions. An electrical current that passes across the nerve membrane shows up as an electrical activity on the EMG monitor. This procedure is done to exclude the abnormalities of the peripheral neuromuscular system. However, EMG could not be used as a screening procedure for neuromuscular disease because there are too many nerves and muscles that can be assessed by this procedure. Nerve conduction study is done to record the motor and sensory amplitude. In SMA patients, sensory amplitude is usually normal while the motor amplitude is decreased.

1.4.3 Molecular genetic testing

The Polymerase Chain Reaction-Restriction Enzyme (PCR-RE) method established in 1995 by van der Steege et al. has become the most accurate and non-invasive method of diagnosis of SMA compared to muscle biopsy and EMG. A small amount of blood from patient is needed to extract the DNA. This method is simple and suitable to be applied for diagnostic testing.
In 2001, allele-specific PCR was studied as a simple method compared with PCR-RE method (Moutou et al., 2001). This method took a shorter time but needs more evaluation to be applied for genetic diagnosis because of the unique features of this gene. The highly homologous copy of the \textit{SMN1} and \textit{SMN2} genes causes a possibility of mismatch to occur and may result in false-positive.

Another technique of molecular genetic screening and diagnosis is by using denaturing high performance liquid chromatography (dHPLC). This technique has proven to be rapid, accurate and sensitive for the genetic and prenatal diagnosis of SMA (Zhu et al., 2006). However, the cost for the maintenance of the equipment may not be affordable by each hospital or government institute. This equipment is normally available at the referral center and the application of dHPLC for the services of routine screening may not be suitable to be applied in each government hospital.

1.5 Prenatal diagnosis of SMA

The knowledge of genetic information and methods of molecular diagnosis has made it possible for prenatal diagnosis of SMA to be carried out. The source of genetic material is usually the chorionic villus sample.

A more non-invasive procedure has been studied using the circulating fetal cells in maternal blood (Beroud et al., 2003) and fetal normoblasts in maternal blood (Chan et al., 1998). This method is based on separation of fetal cells from maternal cells depending on the size of the cells. Epithelial cells which originate
from the fetus are easily found in maternal blood. The cells are usually larger than red blood cells and other cells and could be separated in accordance with size. After obtaining the fetal DNA sources, the molecular analysis for detecting homozygous deletion can be done by the PCR-RE approach or other molecular methods.

1.6 Therapeutic trials in SMA

Since SMA phenotype is proportional to the amount of full-length protein produced, most of the attempts in therapeutic trials are targeted towards elevating the full-length SMN protein. Aclarubicin is a compound which is able to restore SMN2 splicing pattern in vitro by promoting exon 7 inclusions. However, the side effects and toxicity of this compound makes it unsuitable for the treatment of young SMA patients (Andreassi et al., 2001).

The mouse model on mutant mice carrying the homozygous mutation of SMN1 exon 7 has been studied to determine the neuroprotective activity by riluzole. However, no significant improvements were shown to improve the loss of proximal axons. Furthermore, severe side effects of riluzole in young animals also raised concerns on the potential toxicity in infants (Haddad et al., 2003).

Histone deacetylase (HDAC) inhibitors are also being studied for the therapy of SMA. HDAC inhibitor has been used for the treatment of cancer and neurodegenerative diseases. Valproic acid (VPA), an HDAC inhibitor was tested on fibroblast cultures derived from SMA patients. This well-known drug was able to increase the SMN protein levels by restoring the correct splicing of the