MOLECULAR ANALYSIS OF DYSTROPHIN GENE IN PATIENTS WITH DUCHENNE MUSCULAR DYSTROPHY

By

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CERTIFICATE

This is to certify that the dissertation entitled

" Molecular analysis of Dystrophin gene in patients with Duchenne muscular dystrophy"

is a bonafide record of research work done by

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ABSTRACT

Duchenne muscular dystrophy (DMD) is an inherited disorder characterized by rapidly progressive muscle weakness, which start in the legs and pelvis and later affects the whole body. The responsible gene for DMD has been mapped on the Xp21, namely dystrophin gene. This gene is the largest gene in human being, comprises 79 exons. The function of the gene is to enable muscle fibers to make a protein called dystrophin protein. Muscle fibers in people affected with DMD are extremely deficient of dystrophin protein. Seven exons were found to be the most commonly deleted in patients from South East Asia populations and we choose these seven exons for this study. The seven exons are exon 44, 43, 45, 46, 49, 50, and 51. Seven DMD patients from Malaysia were recruited as our patients of study. 5cc of blood were taken from these patients, DNA were extracted using kit (QiaAMP mini kit). Polymerase Chain Reaction (PCR) was performed to analyze the mutation of dystrophin gene in DMD patients. The objective is to detect the deletion of the seven hotspot exons of the dystrophin gene. We found that the two patients with deletion of all the tested exons. Three patients had at least two deleted while two did not show deletion of any of the seven exons tested. The two patients who did not show any deletion could not be excluded from DMD, as there could be other types of mutation in the Dystrophin gene, which is not tested in this study.

ABSTRAK

Penyakit distrofi otot Duchenne (DMD) adalah penyakit keturunan yang dikenal pasti apabila berlakunya kelemahan fungsi otot dengan cepat terutama pada bahagian kaki dan pelvis. Gen yang terlibat ialah gen distrofin yang dipetakan pada kromosom X, Xp21. Gen ini adalah gen yang terbesar dalam badan manusia, mengadungi 79 exon. Tujuh daripada 79 exon ini telah dikenal pasti sebagai exon yang sering terhapus dan menyebabkan DMD bagi populasi pesakit DMD di bahagian Asia Tenggara iaitu exon 43, 44, 45, 46, 49, 50, dan 51. Tujuh exon ini digunakan bagi menjayakan kajian ini. Gen distrofin diperlukan untuk menghasilkan protein distrofin yang berfungsi dalam menguatkan otot- otot. Gentian otot bagi pesakit DMD didapati mengalami kekurangan protein distrofin yang sangat teruk. Subjek bagi kajian ini ialah pesakit DMD dari seluruh Malaysia. 5cc darah diambil dari pesakit. Pengekstratan DNA dilakukan menggunakan kit. 'Polymerase Chain Reaction (PCR) digunakan untuk menggadakan gen tersebut dan sekaligus melakukan analisa. Objektif bagi kajian ini ialah mengenal pasti exon yang terhapus di antara tujuh 'hotspot' exon berdasarkan kajian- kajian yang lepas. Kami dapati dua pesakit yang diuji mempunyai exon yang terhapus bagi semua exon yang diuji. Tiga pesakit lagi mempunyai sekurang- kurangnya 2 exon yang terhapus manakala 2 pesakit lagi tidak mempunyai sebarang exon yang terhapus. Walaubagaimanapun, dua pesakit yang tidak mempunyai sebarang exon yang terhapus tidak sepatutnya dikecualikan dari DMD kerana mungkin terdapat mutasi lain yang tidak diuji dalam kajian ini.

1.0 INTRODUCTION

Muscular Dystrophy is a group of muscle diseases, which have three features in common. The features of muscular dystrophies are hereditary, progressive and each causes selective pattern of weakness. There are more than 20 types of muscular dystrophy such as Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), Myotonic Dystrophy, Limb-girdle Muscular Dystrophy, Facioscapulohumeral Muscular Dystrophy, Spinal Muscular Atrophy (SMA) and others. All types of muscular dystrophy will gradually weaken the body's muscles. The different types of muscular dystrophy caused by different sets of muscle result in different degree of muscle weakness. More severe damage sets of muscle will produce more severe types of muscular dystrophy. To date, there is no cure for muscular dystrophy but to prevent and treat the conditions. There are so many researches done in diagnosis of muscular dystrophy as well as the classification and to prevent the condition.

Muscular dystrophy caused by incorrect or missing genetic information that prevents the body from making the proteins it needs to build and maintain healthy muscle. Incorrect or missing genetic information could be caused by inheritance. For example, gene of muscular dystrophy such as Spinal Muscular Atrophy is an autosomal recessive. However, in some cases, children affected with muscular dystrophy disease have no family history of muscular dystrophy. They are born with abnormal genes that produce abnormal proteins. Several types of muscular dystrophy are listed in Table 1.

Table 1: Types of muscular dystrophy

Types	Summary
1) Duchenne Muscular Dystrophy	- Occurs because of absent of dystrophin protein due to abnormal dystrophin gene. - Affect mainly boys with 1:3500 boys worldwide. - X-liked recessive disease. - Showed Gower's sign. - Symptoms usually appear around age 5, as the pelvic muscles begin to weaken. - The most common and severe form of muscular dystrophy.

2) Becker Muscular Dystrophy	- Similar types with Duchenne Muscular Dystrophy. - Caused insufficient production of dystrophin protein due to abnormal dystrophin gene. - Affect mainly boys with 1:30,000 boys worldwide. - X-linked recessive disease. - Muscle weakness first begins in the pelvic muscles, and then moves into the shouldes and back. - Less severe than DMD.
3) Myotonic Dystrophy	- Most common adult form of muscular dystrophy. - Caused by a portion of particular gene that is larger than it should be. - Symptoms: Myotonia (muscles have trouble relaxing once contract), muscle weakness, muscle wasting.

4) Limb-girdle Muscular Dystrophy	- Affects boys and girls equally. - Symptoms begin when kids are between 8 and 15 years old. - Muscle dystrophy progresses slowly. - Affecting the pelvic, shoulder and back muscles.
5) Facioscapulohumeral Muscular	- Can affect both boys and girls. - Symptoms usually first appear during the teen ages. - Muscle weakness first develops in the face. - Shoulders and back muscles gradually become weak, affected kids usually have difficulty lifting objects or raising their hands overhead.

Among all muscular dystrophy, Duchenne muscular dystrophy is the most common and most severe type of muscular dystrophy (Chandrasoma and Taylor, 1995). Duchenne Muscular Dystrophy is an inherited disorder characterized by rapidly progressive muscle weakness, which start in the legs and pelvis and later affects the whole body. Duchenne muscular dystrophy and Becker muscular dystrophy is one of the most common X-linked

lethal genetic diseases. These two types of muscular dystrophy are cause by the mutation of same gene, which is dystrophin gene.

Duchenne Muscular Dystrophy is first described by the French neurologist Guillaume Benjamin Amand Duchenne (1806-1875). He also described the nervous and muscular disorder. Although Edward Meryon had recognized similar cases with Duchenne muscular dystrophy 10 years early, Duchenne got the popularity based on his articles and illustrations on the same types of muscular dystrophy. Duchenne Muscular Dystrophy (DMD) is one of the muscular dystrophies characterized by the enlargement of muscle and rapid progression of muscle degradation that occurs early in life, as early as age three. DMD affects only males with rare exception in ratio 1:3500 boys worldwide (Harper, 1989). In 1950's, German doctor, Peter Emil Becker has studied the variant of Duchenne muscular dystrophy and described Becker muscular dystrophy, which is much milder version of DMD. Becker muscular dystrophy (BMD) is one of the muscular dystrophy, which has similar clinical features with Duchenne muscular dystrophy. BMD affects mainly males in ratio 1:222 (Emery *et al.*, 1991). Both Duchenne and Becker muscular dystrophy are the most severe muscular diseases.

In DMD, boys begin to show signs of muscle weakness as early as age 3. Usually kids with DMD will begin to show the symptoms at 5, as the pelvic muscles begin to weaken. By the 1880s, the English neurologist Sir William Gowers was able to identify 81 clinical case reports confirming the clinical features established by Duchenne. Later, the sign that showed only by DMD patient was called Gower's sign (Sinha *et al.*, 1992). Gower's sign is the sign that showed by DMD patients when they want to stand up from flat position. Other signs are fall often, late in learning to walk, enlarge calf muscle, poorly developed of other muscles, typical style of walk called waddling (Pradhan and Mittal,

1995). Children also showed toe walk, which is walk on the toes without the heels hitting the floor. The diseases usually weaken the skeletal or voluntary muscles, those in the arms, legs and trunk. A child may start to struggle to get up from the sitting positions or have a hard time to pushing things, like a tricycle or a wagon. Most of the children will need to use a wheelchair by the age of twelve. By the early teens or even earlier, the boy's heart and respiratory muscles may also affected. By age late teens or twenties, the condition of DMD is severe enough to shorten life expectancy. Kids with DMD also experienced less intelligence and mental retardation, however one third of them may have an average intelligence with others children. Before development of molecular analysis, Duchenne muscular dystrophy was diagnosis by the clinical features that showed by the patients. Sometimes, clinicians misdiagnose the similar types of muscular dystrophy such as Duchenne and Becker muscular dystrophy. However, clinical features also important to proceed any diagnosis especially molecular analysis and any others lab test such as muscle biopsy and serum creatine kinase level (CK). Figure 1 shows the Gower's sign.

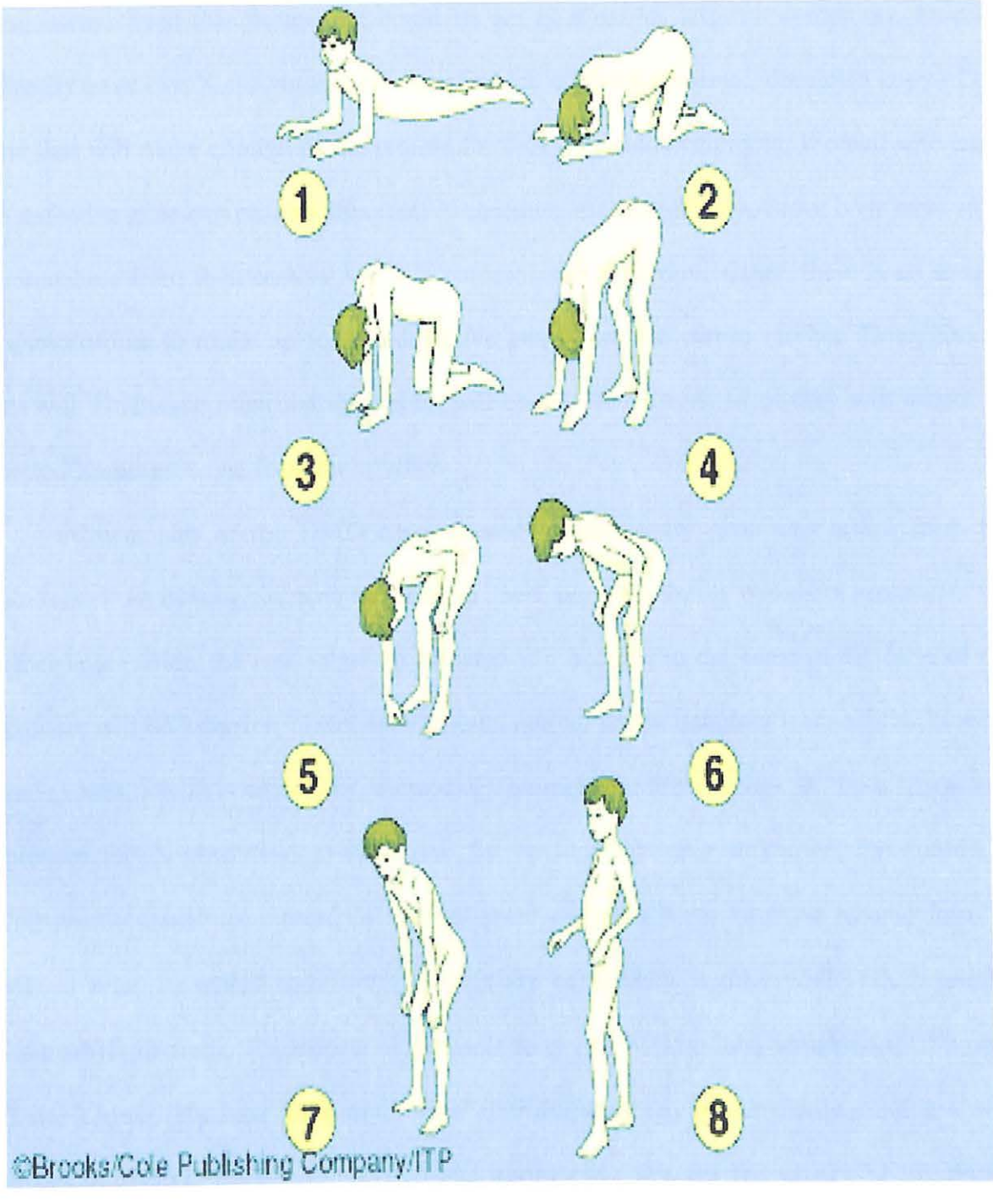

Figure 1: Gower's sign

DMD affect mainly on male because female have two X-chromosome, which make them secure from the disease but could be act as a carrier with no symptoms. Females normally have two X chromosomes, one of which contains a normal, dominant copy of the gene that will make enough of the protein for them to avoid symptoms. Women who carry the defective gene can pass an abnormal X chromosome to their sons. Since boys have an X chromosome from their mother and a Y chromosome from their father, there is no second X chromosome to make up for the defective gene from the carrier mother. Daughters of men with Duchenne muscular dystrophy will always be carriers, since they will inherit an affected X chromosome from their father

Almost half of the DMD cases nowadays, the faulty gene was arisen from the mutation of the dystrophin gene in the boys itself, no other family members carriers. If the mother is a carrier, the risk to get an effected son is 50%. In the same cases, 50% of the daughters will be a carrier. The chance to get a normal son or daughter is also 50%. In some female cases, DMD is caused by skewed X chromosome inactivation. In these cases, two copies of the X chromosome exist, but for reasons currently unknown, the flawed X chromosome manifests instead of the unflawed copy. In these cases, a mosaic form of DMD is seen, in which some muscle cells are completely normal while others exhibit classic DMD findings. The effects of a mosaic form of DMD on long-term period of time is still not known. The best thing to do soon after knowing any of the family members were affected is to seek for genetic advice and appropriate test for the carrier in the family members. Figure 2 shows the X- linked inheritance of the DMD.

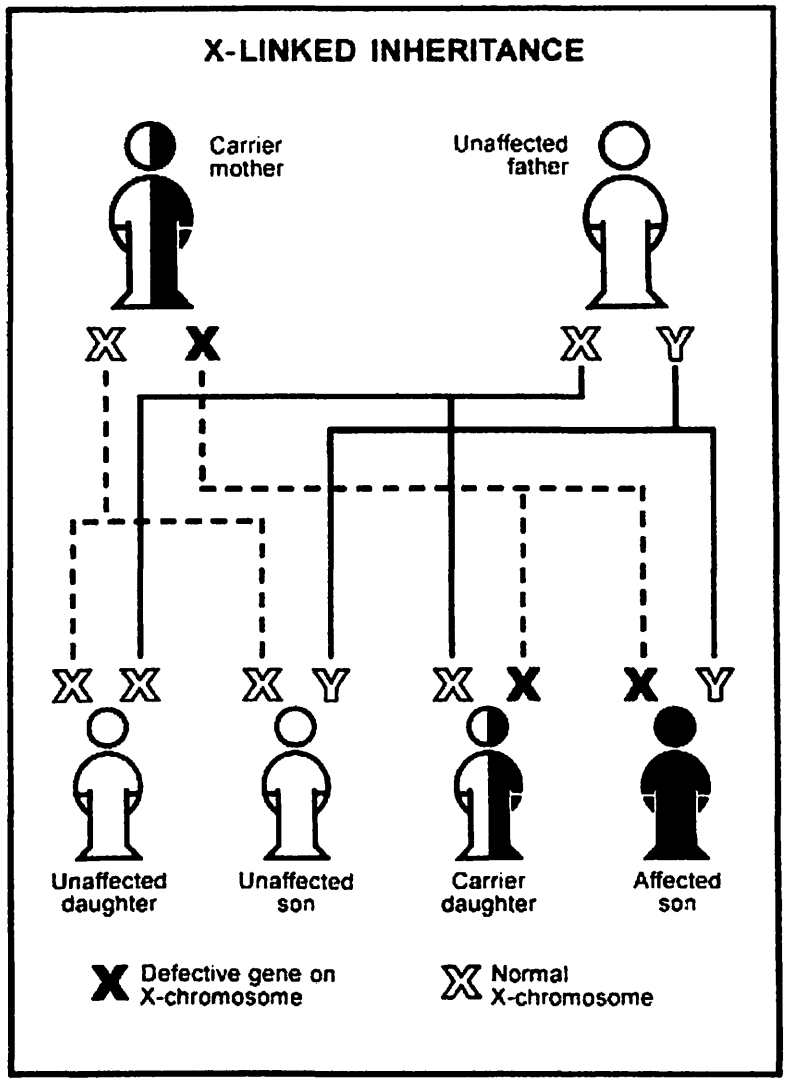

Figure 2: X-linked inheritance

DMD occurs when there are mutations of the dystrophin gene. Dystrophin gene located on the X chromosome with specific locus Xp21.1. Dystrophin gene is the largest gene in human being with 2.3 million base pair. DMD caused by several types of mutations, namely deletions, duplications and point mutations. Deletions accounted for the 50-60% of mutation within the 79 exons of dystrophin gene (Scriver *et al.*, 1995). Multiplex polymerase chain reaction (PCR) can be used to detect 98% of the deletion

(Beggs *et al.*, 1990; Chamberlain *et al.*, 1990). Large duplication and point mutation was the other causes of DMD instead deletion (Read *et al.*, 1988; Tennyson *et al.*, 1995).

Dystrophin gene encoded dystrophin protein, which important to keep muscle cells intact and work properly. Dystrophin protein links actin filaments to a complex of a transmembrane protein hence to the extracellular matrix. Mutations that affected the C-terminus or actin-binding region cause Duchenne muscular dystrophy while mutations that shorten α helix cause Becker muscular dystrophy. Absent of dystrophin protein will lead to muscle damage due to produce of permeable membrane. Figure 3 shows the function of dystrophin protein in the muscles.

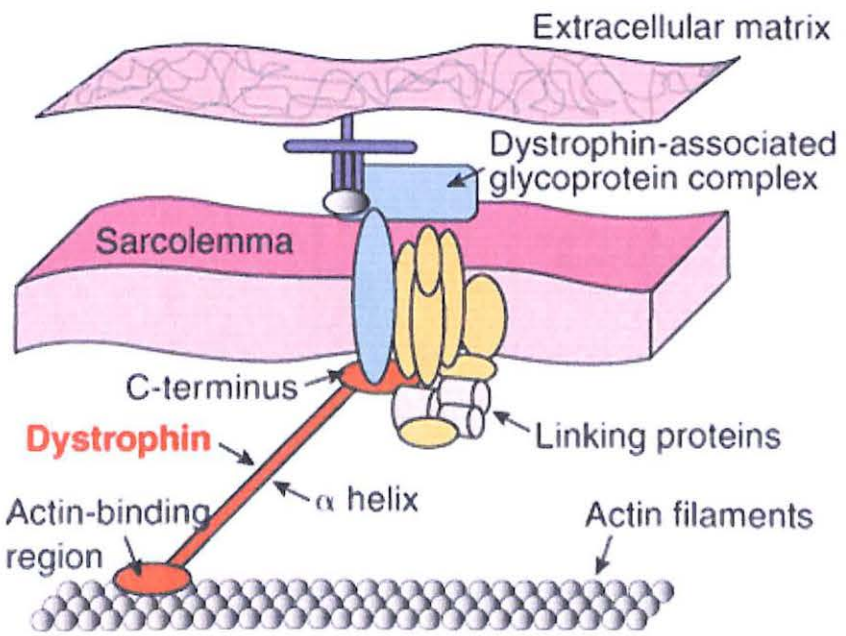

Figure 3: Function of dystrophin gene in the muscles.

DMD can be diagnosed by measuring the serum creatine kinase level (SCK), muscle biopsy, clinical examination with taking a patient and family history and the most accurate test is molecular analysis or DNA analysis. In Malaysia, DMD can be diagnosed

by measuring the serum creatine kinase level (CK), muscle biopsy, and clinical examination.

Molecular analysis of the dystrophin gene has only recently been introduced. Human Genome Center, School of Medical Sciences, Science University of Malaysia was the first center that does the molecular analysis of the dystrophin gene through this research. Molecular analysis of DMD could help us to confirm this disease although there is no cure for DMD till the time of writing. Figure 4 shows X chromosome with the locus of dysrtrophin gene.

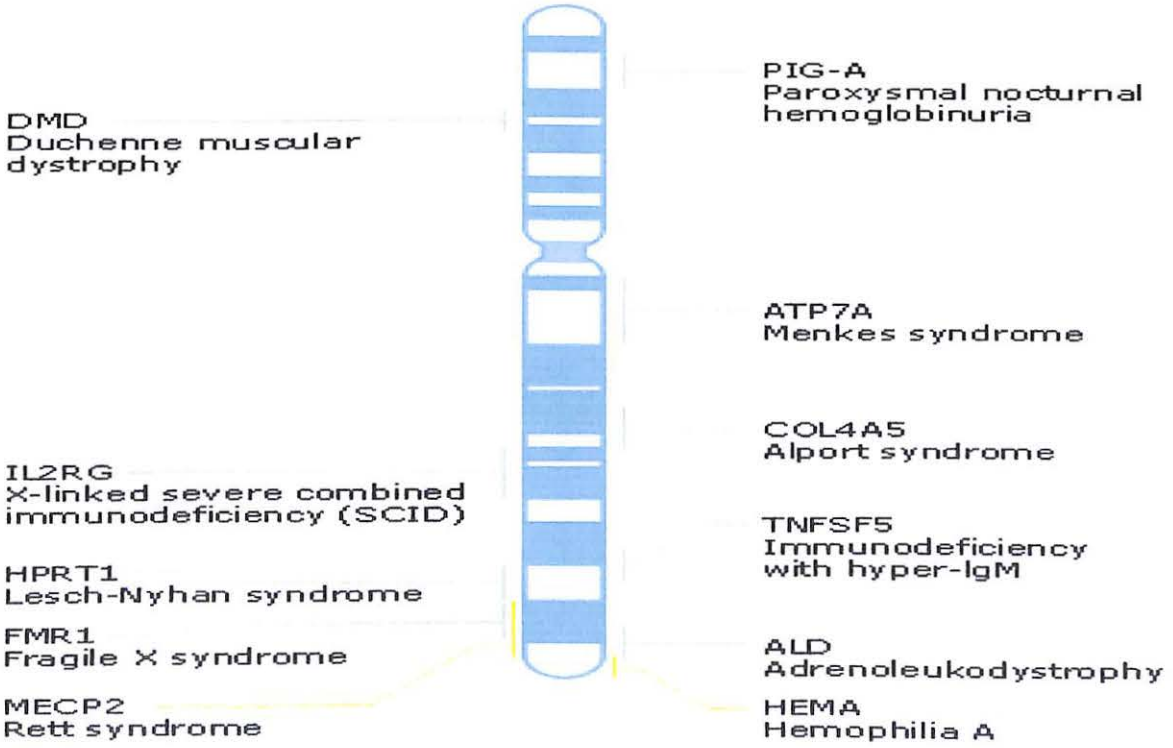

Figure 4: The structure of X chromosome.

2.0 LITERATURE REVIEW

2.1 Duchenne Muscular Dystrophy

2.1.1 Features

Duchenne Muscular Dystrophy (DMD) is one of the most prevalent types of muscular dystrophy and characterized by rapid progression of muscle degradation that occurs early in life. DMD is also known as pseudohypertrophic muscular dystrophy. In common, DMD patients started to show the sign as early as age two. By the aged of twelve, most of the patients need a wheelchair to support them to move around. The sign shown by DMD patients is called the Gower's sign.

Chandrasoma and Tylor 1995

2.1.2 Ratio

All are X-linked recessive and affect mainly males,an estimated 1 in 3500 boys worldwide.

http://www.ncbi.nlm.nih.gov/books/bv.fcgi

2.1.3 Dystrophin protein

Dystrophin protein links actin filaments to a complex of a transmembrane protein hence to the extracellular matrix. Mutations that affected the C-terminus or actin-binding region cause Duchenne muscular dystrophy while mutations that shorten α helix cause

Becker muscular dystrophy. Absent of dystrophin protein will lead to muscle damage due to produce of permeable membrane.

http://www.mda.org.au/specific/mdadmd.html

2.2 Molecular Analysis

2.2.1 X-chromosome

Dystrophin gene located on the X chromosome with specific locus Xp21.1, is the largest gene in human being with 2.3 million base pair, comprises of 79 exons.

Emery *et al.*, 1991

2.2.2 Types of mutation

There are several types of mutation in DMD patients, which are deletion, duplication and point mutation. Within this mutations, deletion accounted for two third of all DMD mutations, followed by large duplication and point mutation. Deletion is the commonest causes of DMD account for the 60% of DMD cases.

Read *et al.*, 1988

2.2.3 Previous study

Based on the deletion type of mutation, 98% can de detected by polymerase chain reaction (PCR).

Beggs *et al.*, 1990; Chamberlain et al., 1990