The Egyptian Journal of Medical Human Genetics (2014) **15**, 235–240

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com

ORIGINAL ARTICLE

Non-deletion mutations in Egyptian patients with Duchenne muscular dystrophy

Rabah M. Shawky [a,*], Solaf M. Elsayed [a], Theodor Todorov [b], Andree Zibert [b], Salem Alawbathani [a], Hartmut H.-J. Schmidt [b]

[a] *Genetics Unit, Children's Hospital, Ain Shams University, Egypt*
[b] *Klinik für Transplantationsmedizin, Universitätsklinikum Münster, Albert-Schweitzer-Campus, Gebäude A14, D-48149 Münster, Germany*

Received 9 March 2014; accepted 29 March 2014
Available online 19 April 2014

KEYWORDS

Duchenne muscular dystrophy;
Deletion;
Duplication;
Egypt;
MLPA;
Sequencing

Abstract Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophies affecting approximately 1:3500 male live births. Deletion of the dystrophin gene accounts for approximately 65% of mutations, duplications occur in 6–10% while the remaining 20–30% are point mutations, small deletion/insertions, or splicing mutations.

Aim: To study non-deletion mutations in a sample of Egyptian patients with DMD as most previous studies focused on deletion mutations.

Patients and methods: The study included 25 patients with DMD from 18 different families from the genetics clinic, Children's Hospital, Ain Shams University. Diagnosis was made based on typical clinical findings, high CPK and EMG result. Molecular analysis included Polymerase Chain Reaction (PCR) followed by multiplex ligation-dependent probe amplification (MLPA) to those patients with no deletion by PCR. Direct sequencing of the whole dystrophin gene was done to those patients who had no deletion or duplication by the previous 2 methods.

Results: Non-deletion mutation included duplications (5 families (27.8%)) which are higher than previously reported and point mutation (c.583C > T) in only one family. Deletion mutations were found in 9 families (50%) and no mutation found in 3 families (16.7%). Interestingly, 60% of the duplications were located in the distal region of the dystrophin gene. A frame shift mutation

* Corresponding author. Address: Children's Hospital, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Tel.: +20 22661717; fax: +20 22585577.
E-mail address: shawkyrabah@yahoo.com (R.M. Shawky).
Peer review under responsibility of Ain Shams University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.ejmhg.2014.03.004

was identified in most patients (93%) except one with duplication of exons 50–51 who had an unexpected severe disease with an early age of onset. Also, an intragenic deletion involving the 5′ end of the dystrophin gene (deletion of muscle protomor and exon 1) was found in another patient with severe disease without cardiac involvement.

Conclusion: The relative higher frequency of duplication mutations in Egyptian patients with DMD may indicate that MLPA and not PCR should be preferred for molecular testing of Egyptian patients with DMD.

1. Introduction

Duchenne muscular dystrophy (DMD; MIM# 310200) is the most common form of all muscular dystrophies caused by mutations within the dystrophin gene and is inherited as X-linked recessive. It is a serious condition with progressive muscle wasting and weakness with most affected boys becoming wheelchair-bound by the age of 12 years and dead by their 20s. A similar but milder condition (known as Becker muscular dystrophy (BMD; MIM# 300376) is caused by mutation in the same gene. The incidence of DMD is approximately 1:3500 male live births [1].

The DMD gene is structurally complex, with 79 exons and 7 promoters, comprising 2.4 million base pairs, making it one of the largest genes known to date [2]. Previous reports suggested that large deletions account for approximately 65% of DMD mutations and 85% of BMD mutations. Duplications occur in roughly 6–10% of males with either DMD or BMD. The remaining 20–30% of mutations are point mutations, small deletion/insertions, or splicing mutations [3]. Most of point mutations lead to premature translational termination due to nonsense (34%), frameshift (33%), splice site (29%), and missense (4%) mutations in the dystrophin gene [4]. Unlike the large deletions that cluster in just two regions, point mutations are more randomly distributed throughout the dystrophin gene [3]. To date, 2556 unique point mutations have been documented in the dystrophin gene [5].

The identifications of the causing mutation in the dystrophin gene is considered very important because it may provide new insights into the function of dystrophin and direct information for genetic counseling, prenatal diagnosis and carrier studies [3,6]. Furthermore accurate molecular diagnosis is essential for different recent mutation specific therapeutic modalities [7].

The aim of this work was to study the non-deletion mutation spectrum in Egyptian patients with DMD because all previous studies focused on deletions only [8–11] and to find the most appropriate molecular method for accurately diagnosing the largest number of our patients.

2. Patients and methods

The study included 25 patients with DMD from 18 different families from the genetics clinic, Children's Hospital, Ain Shams University. Diagnosis was made based on typical clinical findings (progressive symmetric muscular weakness starting $\leqslant$5 years (proximal greater than distal) often with calf hypertrophy), high CPK ($>10\times$ normal) and EMG showing myopathic pattern.

Molecular analysis included

1. Detection of deletion mutations by using Polymerase Chain Reaction (PCR) and amplifying the 79 exons and the Dp427m promoter of the dystrophin gene [12,13].
2. Multiplex ligation-dependent probe amplification (MLPA) was done to those patients with no deletion by PCR using commercial MLPA kits (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's instructions (http://www.mrc-holland.com) [14].
3. Sequencing of the whole dystrophin gene was performed in those patients who had no deletion or duplication by the 2 previous methods using ABI 3037 [15].

The study was approved by the ethics committee of the institute and informed consent was obtained from the parents. The study was carried out in accordance to the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

3. Results

3.1. Clinical findings

Patients' ages ranged between 4 and 20 years. The mean age at diagnosis was 4.5 ± 1.8 years (range 2–9 years). The age of onset was before 5 years in 16 (64%) patients and at the age of 5–10 years in 9 (36%). The main presenting symptoms at the time of diagnosis was abnormal gait in 9 cases (36%), frequent falls in 7 cases (28%), difficulty in climbing up stairs in 6 cases (24%), standing up difficulties in 2 cases (8%) and delayed motor milestones in one case (4%). The mean age of walking was 18 months (range 12–30 months). Six patients (24%) were wheelchair-bound at the time of the study; they lost independent ambulation at a mean age of 10 years (range 9–12 years). Mild to moderate mental retardation was detected in 10 DMD cases (40%). Family history revealed a similar affected member in 9 families (3 sibs and 6 maternal cousins).

3.2. Laboratory investigations

a. Serum CPK levels ranged between 2134 IU/L and 24,000 IU/L with a mean of 11,273 ± 5426 IU/L (normal: 38–173 IU/L).
b. Echocardiography revealed cardiomyopathy in four patients (16%), all of them were above the age of 10 years.
c. Mutation analysis, Table 1: