

The Impact of Prenatal Diagnosis in Egyptian Families with Duchenne Muscular Dystrophy

Laila K. Effat¹, Khalda S. Amr¹, Ashraf A. El-Harouni², Khalid R. Gaber³

¹Departement of Medical Molecular Genetics, ²Departement of Clinical Genetics, ³Departement of Prenatal Diagnosis and Fetal Medicine, National Research Center, Giza, Egypt.

ABSTRACT

Background: Duchenne Muscular Dystrophy (DMD) is one of the most common lethal X-linked recessive genetic muscle disorders. It is caused by various mutations in the dystrophin gene. Due to the lack of efficient rehabilitation and treatment, prenatal diagnosis and proper genetic counseling of families with DMD are of great importance.

Aim of the Work: This study aimed to evaluate an Egyptian prenatal molecular experience considering the impact of molecular information, the availability of prenatal diagnosis, and the changing attitude and choices of the Egyptian families with DMD.

Patients and Methods: The study characterized the deletion patterns in 85 Egyptian patients with DMD and 32 fetuses from 27 mothers with previous history of DMD deletion mutations. Multiplex PCR amplification of 18 exons covering the two hotspots within the dystrophin gene was pursued to detect deletions in the probands. Detection of deletions in the fetal DNA was performed by using the targeted multiplex containing the deleted exons.

Results: Forty-six out of eighty-five probands (55%) had deletion mutations. Twenty-four out of the forty-six (52%) probands had multiple exons deletion and twenty-two (48%) showed single exon deletion. Out of the thirty-two amniotic fetal samples, fourteen fetuses inherited the same deletions present in the index cases while eighteen were normal. An emerging awareness of genetic information was observed and an apparent higher number of mothers seeking prenatal diagnosis was noticed. A change of attitude in favor of choosing the decision of abortion was apparent.

Conclusion: Molecular diagnosis is an important tool for preventive medicine. It has an obvious impact on prenatal diagnosis and accurate genetic counseling. It also seems to have an impact on the attitude and choices of families in particular and society in general.

Key Words:

DMD, dystrophin gene, deletion mutations.

Corresponding Author:

Laila Effat,
Tahrir st., Dokki, Giza, Egypt 12311
Tel.: + (202) 3748 5232
Fax: + (202) 3337 0931
E-mail: lailaeffat@yahoo.com

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is one of the most common lethal X-linked recessive genetic muscle disorders with an incidence of approximately 1/3500 male live births.¹ The affected patient is almost always a boy presenting with proximal muscle weakness before the age of five years, accompanied by remarkably elevated serum Creatine Kinase (CK) enzyme levels, myopathic changes on electromyography and reduced or absent expression of dystrophin in muscle histochemical preparations. Half of patients fail to walk at the age of 18 months, and others are confined to a wheelchair at 7-11 years of age. DMD is a severely handicapping disease with relatively rapid progression. Death due to respiratory failure occurs by the end of the second decades of life.²

DMD is caused by mutations within the dystrophin gene. The dystrophin gene is a large gene (2.4 Mb), located at Xp21 and comprises 79 exons. It codes for the dystrophin protein.³ Deletions, duplications and point mutations have been reported in most exons of the gene, especially the two hot-spot regions.⁴ One third of mutations are de novo.⁵ Conventional molecular diagnosis of DMD is carried out by using multiplex PCR amplifications of the two major and minor hot spots within the dystrophin gene. Recently, a new technique, the multiplex ligation probe amplification (MLPA) has been described.^{6,7} MLPA is an accurate and sensitive technique in the detection of DMD rearrangements including deletions and duplications.^{8,9}

A previous Egyptian molecular study revealed that the most common deletions were within the two main hotspots with

a 55% deletion frequency.¹⁰ This study reports the molecular diagnosis data 85 probands and prenatal diagnosis data of 27 mothers with a previous history of DMD deletion mutations. The molecular diagnosis has facilitated the provision of proper prenatal diagnosis based on mutational genetic data for families, thus opening the door for decision making and counseling of Egyptian families with DMD. An interesting change in the acceptability and attitude of the counseled families considering the social values and religious background was observed.

PATIENTS AND METHODS

Subjects

Between the period of 2004 and 2007 eighty five unrelated patients were referred to the Medical Molecular Genetic Department at the National Research Center for detection of the dystrophin gene deletion mutations. Patients were diagnosed to have DMD based on clinical findings, electromyography (EMG) and elevated serum Creatine Kinase activity. During this period thirty two fetuses delivered from twenty seven mothers were analyzed. One mother sought prenatal diagnosis 3 times and another three requested it twice. Mothers received genetic counseling entailing family history and complete diagnostic data of the probands and prenatal counseling regarding ultrasound guided amniocentesis and its risks. Between 14-18 weeks pregnancy 25ml amniotic fluid was obtained for fetal DNA extraction. The acceptability, response and attitudes of mothers were recorded during the follow up prenatal counseling session.

Methods

Genomic and fetal DNA was extracted from blood and amniotic fluid respectively using QIAamp® DNA Mini Kit (QIAGEN, Germany). Detection of deletions in the probands was carried out by multiplex PCR (mPCR) using 18 exons in six multiplex reactions according to the modified technique of Chaimberlain and Biggs.¹⁰ Detection of deletions in the fetal DNA was pursued using mPCR amplification containing both the targeted deleted exons and non deleted exons as internal control.

RESULTS

Molecular analysis of probands

A total number of 85 probands revealed that 46 (55%) have deletion mutations. Twenty-four out of the forty-six (52%) probands had 13 multiple exons deletion while twenty-two (48%) showed 8 different single exon deletion (Table 1). The most common single exon deletion was exon 48 (10.8%) and the most common multiple exons deletion was 45-52 (10.8%).

Molecular results of prenatal diagnosis

Thirty two fetuses delivered from twenty seven mothers were analyzed. Fourteen fetuses inherited the same deletions present in the index cases while eighteen were normal. Deletions detected in the fetal DNA: [(1) del 8-12, (2) del 45, (2) del 45-52, (1) del 47-50, (1) del 51-52, (2) del 47, (1) del 4-6, (1) del 45-48, (1) del 19, (1) del 43, (1) del 48]. Postnatal samples were available from 10 out of the 18 fetuses with normal outcome proving the reliability of the test.

Acceptability and Attitude

The 27 mothers received the counseling eagerly and did not hesitate to pursue amniocentesis and obtain prenatal diagnosis. A notable increase in the number of mothers seeking amniocentesis in the last year was observed. All mothers with affected fetuses responded to the molecular results and did not hesitate to terminate pregnancy once informed that their fetuses were affected.

DISCUSSION

Genetic counseling and prenatal diagnosis is a blooming field in obstetrics. This unique medical specialty provides clinical health care, education, and

Table 1: Different types and frequencies of deletions found in patients with DMD.

Type of deletion	Number of patients	
Exon 19	2	4.3%
Exon 43	2	4.3%
Exon 45	4	8.6%
Exon 47	3	6.5%
Exon 48	5	10.8%
Exon 50	1	2.1%
Exon 51	4	8.6%
Exon 52	1	2.1%
Exons 3-19	1	2.1%
Exons 4-6	1	2.1%
Exons 8-12	3	6.5%
Exons 8-13	1	2.1%
Exons 43-51	1	2.1%
Exons 45-48	2	4.3%
Exons 45-50	2	4.3%
Exons 45-52	5	10.8%
Exons 47-50	2	4.3%
Exons 48-50	2	4.3%
Exons 48-51	1	2.1%
Exons 48-52	1	2.1%
Exons 51-52	2	4.3%

emotional support to individuals and families facing genetic and inherited diseases¹¹. Prenatal diagnosis depends on accurate molecular techniques, which have become simpler and more accurate, so transfer of technology is no more a barrier.¹²

The implementation of prenatal diagnosis of DMD was based on the identification of the molecular make up of DMD in Egyptian patients. The data was collected from a prior study comprising 165 Egyptian patients with DMD and the current study. Both studies revealed that the most common deletions were within the two main hotspots.¹⁰ Table (1) illustrates the breakdown pattern of deletion frequencies found in families with deletions (55%). The compilation of the breakdown of the different frequencies of deletions of DMD in different countries is still an ongoing issue in attempt of completing the molecular deletion patterns and gaining a deeper understanding of exon deletion distribution of DMD worldwide.^{13,14}

In this study eight single exons deletion (19, 43, 45, 47, 48, 50, 51, 52) were found, the most common was exon 48; thirteen multiple exon deletions, the most was common 45-52. Identifying the breakdown pattern helped the development of a molecular guideline for targeting and screening the most common deletions in our population thus saving time effort and expense. Also identifying the different mutations in these Egyptian studies encouraged the initiation of the prenatal molecular diagnosis clinic, which turned into one of the first specialized referral centers reflecting a true molecular prenatal experience in Egypt. The use of multiplex PCR was sufficient in this study for the

provision of molecular diagnosis (Refer for details to prenatal results), although in families with no deletion mutations indirect analysis of CA repeats and polymorphic restriction sites are needed for completeness.^{15,16,17} Confirmation of the accuracy of the prenatal molecular data was compared with the results postnatal, finding no discrepancies. This accuracy of molecular work up encouraged the determination of pursuing successful counseling of mothers in Egypt. A notable increase in the number of mothers seeking amniocentesis during the last three years was observed. An Egyptian study indicated that the leading cause for seeking amniocentesis was a previous history with a genetic disease.¹⁸ Not only the increasing number of mothers but also the repeated seeking of the same mother in repeated pregnancies indicated the high acceptability of mothers in seeking prenatal diagnosis.

Identifying the molecular defect in the fetus, whether affected or not, is a major concern for the Egyptian mothers. Knowing that they are conceiving an affected child, they considered the decision of terminating rather than endure the pain of watching the affected offspring deteriorate. In the past, mothers seemed hesitant in pursuing the option of abortion as their first decision. It was a difficult step to pursue, due to lack of molecular diagnosis and proper genetic counseling. In addition, no clear religious and social support was available. Although seeking termination is a touchy subject, it is a serious choice to consider. In this study mothers with affected fetuses considered immediate termination, as they became aware of the option of different choices and that they could choose to live without the

burdens of raising a child with severe handicapping abilities. So an obvious new concept of acceptance and confronting choices was observed. The attitude of mothers and society has shown a change regarding the active choice of abortion rather than the passive choice of leaving matters to fate. This change might be due to the exposure of continuous new molecular genetic information. Time will unfold the depth of impact of this molecular information especially when recommendations of religious scholars reevaluate the terms of abortion, considering the available molecular data. No doubt, prenatal diagnosis has become an important tool for preventive medicine and genetic counseling. Molecular prenatal diagnosis provides accurate data for proper genetic counseling and psychological support especially for anxious pregnant women with a history of a particular genetic disease. It also has an apparent impact on awareness, attitudes and choices of families with genetic disorders in particular and society in general.

REFERENCES

1. Emery AE. Population frequencies of inherited neuromuscular diseases--a world survey. *Neuromuscul. Disord.* 1991; 1 (1): 19-29.
2. Brooke MH, Fenichel GM, Griggs RC, Mendell JR, Moxley R, Florence J, et al. Duchenne Muscular Dystrophy: Patterns of clinical progression and effects of supportive therapy. *Neurology* 1989; 39 (4): 475-81.
3. Roberts RG, Coffey AJ, Bobrow M, Bentley DR. Exon structure of the human dystrophin gene. *Genomics* 1993; 16 (2): 536-8.
4. Den Dunnen JT, Grootsholten PM, Bakker E, Blonden LA, Ginjaar HB, Wapenaar MC, et al. Topography of the Duchenne Muscular Dystrophy (DMD) gene: FIGE and cDNA analysis of 194 cases reveals 115 deletions and 13 duplications. *Am. J. Hum. Genet.* 1989; 45 (6): 835-47.
5. Mukherjee M, Chaturvedi LS, Srivastava S, Mittal RD, Mittal B. De novo mutations in sporadic deletional Duchenne Muscular Dystrophy (DMD) cases. *Exp. Mol. Med.* 2003; 30; 35 (2): 113-7.
6. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by Multiplex Ligation-dependent Probe Amplification. *Nucleic Acids Res.* 2002; 15; 30 (12): e57.
7. Sellner LN, Taylor GR. MLPA and MAPH: New techniques for detection of gene deletions. *Hum. Mutat.* 2004; 23 (5): 413-9.
8. Lai KK, Lo IF, Tong TM, Cheng LY, Lam ST. Detecting exon deletions and duplications of the DMD gene using Multiplex Ligation-dependent Probe Amplification (MLPA). *Clin. Biochem.* 2006; 39(4):36772.
9. Lalic T, Vossen RH, Coffa J, Schouten JP, Guc Scekic M, Radivojevic D, et al. Deletion and duplication screening in the DMD gene using MLPA. *Eur. J. Hum. Genet.* 2005; 13 (11): 1231-4.
10. Effat LK, El Harouni NA, Amr KS, El Minisi TI, Abdel Meguid N, El Awady M. Screening of dystrophin gene deletions in Egyptian patients with DMD/BMD muscular dystrophies. *Dis. Markers* 2000; 16 (3-4): 125-9.

11. Ciarleglio LJ, Bennett RL, Williamson J, Mandell JB, Marks JH. Genetic counseling throughout the life cycle. *J. Clin. Invest.* 2003; 112 (9): 1280-6.
12. Beroud C, Karlova M, Bonnefont JP, Benachi A, Munnich A, Dumez Y, et al. Prenatal diagnosis of spinal muscular atrophy by genetic analysis of circulating fetal cells. *Lancet* 2003; 22;361(9362):1013-4.
13. Sbiti A, El Kerch F, Sefiani A. Analysis of dystrophin gene deletions by Multiplex PCR in Moroccan patients. *J. Biomed. Biotechnol.* 2002; 2 (3): 158-60.
14. Hallwirth Pillay KD, Bill PL, Madurai S, Mubaiwa L, Rapiti P. Molecular deletion patterns in Duchenne and Becker Muscular Dystrophy patients from KwaZulu Natal. *J. Neurol. Sci.* 2007; 15; 252 (1): 1-3.
15. Chakraborty R, Zhong Y, de Andrade M, Clemens PR, Fenwick RG, Caskey CT. Linkage disequilibria among (CA)_n polymorphisms in the human dystrophin gene and their implications in carrier detection and prenatal diagnosis in Duchenne and Becker Muscular Dystrophies. *Genomics* 1994; 21 (3): 567-70.
16. Percesepe A, Ferrari M, Coviello D, Zanussi M, Castagni M, Neri I, et al. Detection of a novel dystrophin gene mutation through carrier analysis performed during prenatal diagnosis in a case with intragenic recombination. *Prenat. Diagn.* 2005; 25 (11): 1011-4.
17. Prior TW, Bridgeman SJ. Experience and strategy for the molecular testing of Duchenne Muscular Dystrophy. *J. Mol. Diagn.* 2005; 7 (3): 317-26.
18. Gaber K. Preliminary report on genetic amniocentesis: Single operator. 2007; 6; *Fetal Medicine*, Cairo, Egypt.