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Case Report

Mosaicism in carrier of Duchenne muscular dystrophy mutation – Implication for prenatal diagnosis

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ABSTRACT

Objective: Duchenne muscular dystrophy (DMD) is a severe disorder caused by mutation in the X-linked *dystrophin* gene, therefor carrier testing is required for all female family members. However, there are cases mutation analysis cannot detect any mutation due to a phenomenon called mosaicism. The case report describes a case of mosaicism in a DMD carrier and discusses the approach in diagnosis and counseling of familial disorder.

Case report: The proband was diagnosed with DMD at age six. Sequencing of *Dystrophin* gene identified a 2-nucleotide deletion c.2032_2033delCA, p.Q678DfsX41. Family investigation suggested that the mother was an obligate carrier of *Dystrophin* mutation. Sequencing of DNA sample from the mother's peripheral blood did not reveal any mutation, there for we take sample from hair follicle for analysis. The result indicated that the mother was a carrier but was masked from initial analysis by mosaicsism.

Conclusion: We suggested that more care need to be taken in identifying cases when no mutation was detected in probable or obligate carrier and prenatal diagnosis should remain an option.

Introduction

Duchene Muscular Dystrophy (DMD) is the most prevalent neuromuscular disorders with a rate of 1 out of 3500 male infants [1]. The patients suffer from proximal-to-distal and progressive muscular weakness and degeneration, pseudo-hypertrophy (i.e., enlarged calve muscles), and cognitive impairment [2]. While newborn patients rarely exhibit any symptom, they quickly develop muscle weakness at the age of learning to walk, face difficulties in running and climbing stairs, and become wheelchair-dependent at the age of 12. DMD patients have short life expectancy (i.e., about 20 years) due to further complications such as respiratory failure and cardiac disease [3]. Since reliable treatments for DMD are not yet available, prenatal testing is the primary tool to reduce the disease incidence [4]. While the disease follows an X-linked inheritance pattern of the dystrophy gene, thirty percent of the mutations are *de novo* [5]. However, later evidences have shown that there are discrepancy between the rate of sporadic cases and recurrence rate, suggesting the occurrence of mosaicism [6].

Mosaicism has been proven to present in almost all inheritance pattern and across many heritable disorders [7]. Failure to detect mosaicism in carriers could lead to serious consequences, as their next child would have an increased risk of carrying the mutation. In addition, mosaic cell absence from hematopoietic cell line would further complicate the diagnosis processes, making mosaicism an elusive and often overlooked condition. Consequently, early recommendation suggested performing prenatal diagnosis for parents of children with seemingly "*de novo*" mutation [8].

This case report present a case of mosaic in a carrier of DMD, the diagnostic process were described in detail and through this we discuss how to facilitate better detection of mosaic in the future.

Case presentation

The proband was admitted to the hospital at age six, with phenotypic presentation of DMD. The boy was presented with large calf, staggering gait, mild proximal muscles weakness (Gower's

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sign). Further investigation revealed the child had a history of slow motor development, no behavior or cognitive problem was noted. Blood test return with high serum creatine phosphokinase (CPK) concentration (47.000 UI/L), further confirms the clinical diagnosis of DMD.

MLPA were used to rule out any duplication or deletion of the *Dystrophin* gene in the patient. Then sequencing of 79 exon of dystrophin gene were performed which identified a 2-nucleotides deletion c.2032_2033delCA, p. Q678DfsX41 (Fig. 1A). The case appeared to be *de novo* because sequencing of the mother's Dystrophin gene using DNA extracted from peripheral blood returned a normal variant (Fig. 1B). However, according to the family pedigree the proband had an uncle who deceased at the age of 25 from DMD (Fig. 2). Therefore, it is evidence that the mother should be an obligate carrier of DMD, heterozygous for *Dystrophin* mutation, which prompted us to investigate further. Blood sample was taken from the grandmother (maternal side) for analysis, which confirmed that she was a carrier with the same mutation as the proband (c.2032_2033delCA, p. Q678DfsX41) (Fig. 1C).

We hypothesized that in this case the mother was a mosaic case, in which the cell line from the blood did not carry any mutation of *Dystrophin*. Hair follicle sample was then taken from the mother for analysis which confirmed the hypothesis that she was mosaic for *Dystrophin* mutation (Fig. 1D).

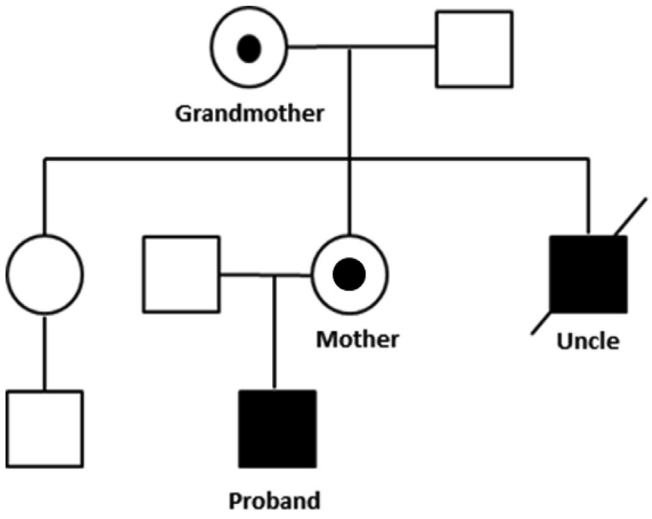

Fig. 2. Family pedigree.

Discussion

Hypothesis of the mosaicism case

This study highlighted a carrier with mosaicism, a condition often overlooked by clinician and geneticist in many genetic disorders. There are various mechanisms leading to mosaic, therefore we can only draw hypothesis as of why the mosaicism occur in the carrier mother. From the result and pedigree, it shown that the mother is an obligate carrier and sequencing result shown she was a mosaic carrier of DMD, thus prompted a question of the mechanism leading to her mosaicism. One widely accepted mechanism is that mosaicism arise in the embryonic state, due to chromosomes unable to separate correctly during mitosis, which give rise to two or more different cell lines in the subject [9]. This type of mosaicism is common in the pre-implantation development stages of the embryo, which can be confined during embryonic development leading to the cell line only express in specific tissues and organs. In this case, we did not detect any mutation from the mother's blood sample can be attributed to the low level of mosaicism in the

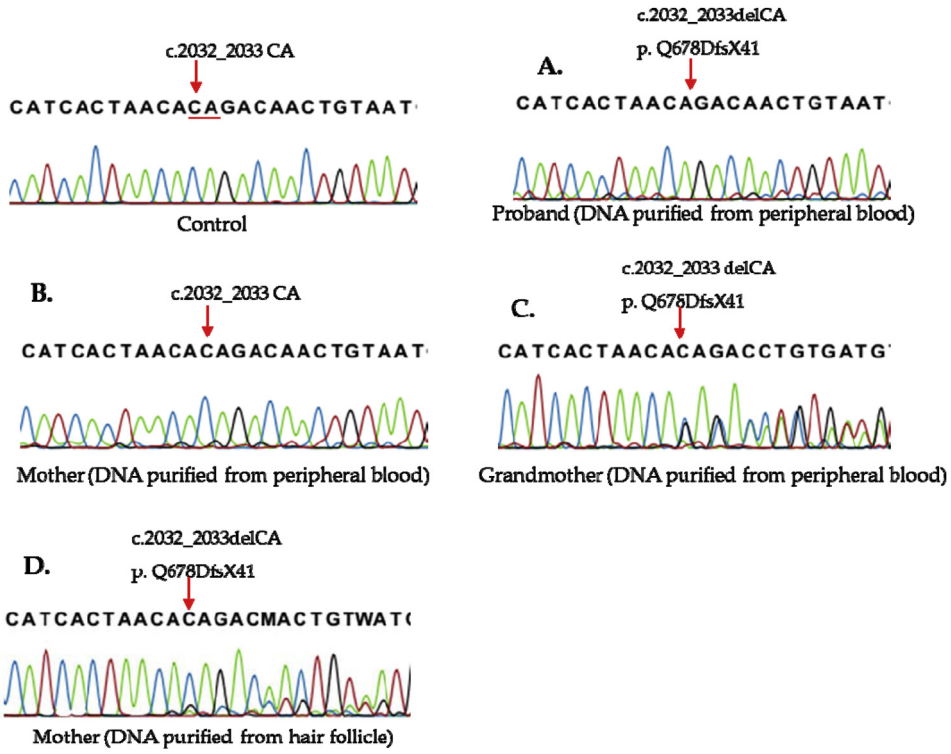

Fig. 1. Sequencing results of the *Dystrophin* gene of the proband DNA; mother DNA (purified from peripheral blood) -mother DNA (purified from hair follicle) and the grandmother DNA (maternal side).

hematopoietic stem cell population. We were able to confirm mutation in the mother through DNA from hair follicle sample. The other mechanism is hypothesized to have been gene conversion resulting in a normal cell line, which have lost one pathogenic mutation.

Recommendation for diagnosis of DMD Carrier and consideration for mosaicism in DMD

In clinical setting, genetic testing mostly focuses on the proband and parents, negative finding of mutation in the parents would lead to a conclusion that the mutation is *de novo*. Recent study has revealed that in such cases, further investigation identified a high percentage of mosaicism in apparently *de novo* cases [7]. This case report prompted an important clinical consideration in genetic testing of Duchenne muscular dystrophy and other genetic disorder. In fact, recent evidence and study have called for changes in diagnosis guideline and policy toward a wider set of criteria for prenatal diagnosis and carrier identification [10].

Counseling and prenatal diagnosis for DMD

Previous evidence have shown that parents initially identified as non-carriers have higher and expected recurrence risk (i.e. 14% recurrence rate), which imply the presence of mosaicism [11]. Carrier detection and counseling are important steps in management of DMD [4], effort need to be focus on preventing new incidence of DMD as there are currently no effective therapy to the disorder as they are currently in the development stages [12]. Consequently, it is important to discuss this aspect in counseling, to consider prenatal diagnosis for female members of affected families in case of mosaicism.

Conclusion

This case report highlighted multiple aspects of mosaicism in DMD, from diagnosis to mechanism and counseling consideration. Mosaicism is a common but often overlooked condition, which requires higher awareness from clinicians and geneticists. Further study and changes in guideline should be implemented to better diagnose and provide counseling to affected families.

Conflicts of interest

The authors declare no conflict of interest.

Methods

1. Samples

The proband, mother, grandmother: 2 ml EDTA anticoagulated peripheral blood were obtained, hair follicle was collected and DNA was extracted for analysis.

2. DNA extraction

DNA was extracted using Qiagen DNA mini kit (Qiagen DNA mini kit, Venlo, The Netherlands).

3. MLPA

Multiplex Ligation-dependent Probe Amplification (MLPA), a high-throughput and straightforward technique for quantification of gene copy number was performed using the MLPA Kit P050B2 (MRC- Holland) according to the manufacturer's protocol. Products from amplification were analyzed on gen 3100-Avant Genetic Analyzer ABI-PRISM (ThermoFisher, US). The kit contains probes for Dystrophin (…) and is utilized to detect deletions and duplications of one or more exons of CYP21A2.

4. Dystrophin gene sequencing and result analysis

Dystophin gene sequencing is used to detect mutations after screening for common mutations. This was done on the ABI 3500 (Applied Biosystems, Life Technologies Corporation, Foster city, Ca, USA). The results were analyzed using Chromas and Chromas Pro software (Technelysium Pty Ltd, South Brisbane, Australia) and then compared to the reference sequence on GenBank (ref.no).

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