

DMD Phenotype in Girls with a *de novo* Balanced X;3 Autosome Translocation: A Case Report

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Introduction

Mutations in the DMD gene, which encodes dystrophin, can result in a complete absence of the protein product leading to Duchenne muscular dystrophy phenotype (DMD) or in a reduced amount of the protein leading to the milder Becker muscular dystrophy (BMD) [1], or to the X-linked dilated cardiomyopathy phenotype (XLDC) [2].

Most heterozygous female carriers of DMD mutations are asymptomatic; however, 2.5-7.8% of them are *manifesting* carriers (MCs) who develop symptoms ranging from mild muscle weakness to a rapidly progressive DMD-like muscular dystrophy [3,4]. Female carriers are also at risk of developing cardiomyopathy [5,6]. Dystrophin labeling in muscle biopsies from MCs shows a mosaic pattern, with some fibers having continuous membrane immunostaining, other fibers appearing unstained, and some fibers showing a partial dystrophin staining [5,7-9].

A DMD-like phenotype in DMD carriers may result in presence of a) mutation in both Xp21 alleles [10,11]; b) loss of one X chromosome (Turner Syndrome) [11-13]; c) structural abnormalities of the X chromosome such as deletions, duplications and unbalanced X;autosome translocations [14-16]; or d) an extremely skewed X chromosome inactivation (XCI) [17-20].

Balanced X;autosome translocations are rare; females carrying them are a clinically heterogeneous group of patients, ranging from phenotypically normal women to history of recurrent miscarriage, gonadal dysfunction, congenital abnormalities or developmental delay. Abnormal phenotypes associated with *de novo* balanced chromosomal rearrangements may be the result of disruption of a gene at one of the breakpoints, submicroscopic deletion/ duplication, or of a positional effect.

Moreover X;autosomal translocations are associated with additional unique risk factors such as X linked disorders, functional autosomal monosomy, or functional X chromosome disomy resulting from the complex X-inactivation process [10]. In these conditions the inactivation preferentially involves the X chromosome not involved in translocation, because the inactivation of the translocated X chromosome, will produce an autosomal monosomy, life incompatible.

We report the clinical case of a young female, prenatally diagnosed as carrying an apparently balanced X;3 chromosome translocation, who developed a Duchenne-like phenotype.

Case Report

The proband is a 13 years old girl, the second child of non-consanguineous Italian healthy parents. There was no family history of autoimmune or musculoskeletal disorders. The mother, 40 years old at the time of her conception, performed a prenatal diagnosis resulting in a *de novo* balanced X;3 chromosomal translocation. The parents decided to continue the pregnancy.

The birth was planned at 38 weeks by caesarean delivery; birth weight was 2620 g and length 50 cm. The baby presented neonatal

jaundice. The patient achieved all developmental milestones appropriately and attended regular school. At the age of 4, because of poor growth, she was investigated for celiac disorder that was excluded by specific tests. In this occasion a moderate increase of ALT (324 IU/L) and AST (301 IU/L) was found, confirmed many times. Because this elevation she was referred to a pediatric hepatology service. Although the initial clinical impression was of a chronic hepatitis, the laboratory investigations for infectious or autoimmune hepatitis and/or Wilson's disease were negative. The child was addressed to the department of pediatric neurology, where she performed muscle laboratory tests that showed elevated CK levels (5500 IU/L) and an electromyography whose results were consistent with a myopathic process.

Muscle biopsy revealed severe variability of fiber size, occasional internal nuclei and an increase in perimysial and endomysial connective tissue; no glycogen or lipid accumulation, fiber necrosis and inflammatory changes were found. Enzymatic studies for metabolic myopathies were normal.

Immunoreactivity with antibodies against alpha-, beta-, and delta-sarcoglycan, alpha dystroglycan was normal, while no immunoreactivity was found to antibodies for NH2, Rod and COOH domains of dystrophin (Figure 1), suggesting a primary deficiency in dystrophin.

The parents were advised to perform a new karyotype and the analysis of X chromosome inactivation. Cytogenetic analysis, performed at the age of 5.6 years, in the same laboratory where the prenatal diagnosis was made, confirmed the presence of a *de novo* translocation t(X;3)(p21;p24). This time, however, a FISH analysis was performed showing that the breakpoint on the X chromosome

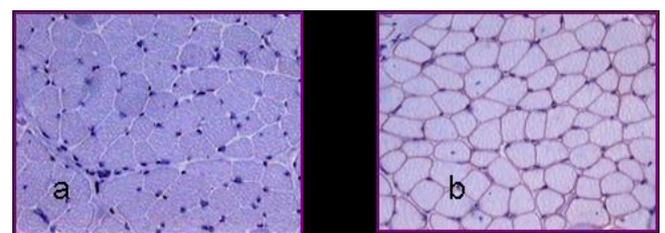


Figure 1: Immunostaining for dystrophin with COOH antibody in the case report (a) compared to the control (b).

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included DMD gene, with the consequent interruption of its sequence, and that the active X chromosome was involved in the translocation.

The girl was addressed to our Service to perform the molecular analysis of the dystrophin gene and for the taking care. At the first admission, at age 5,6 years, the clinical examination revealed a verbal communicative girl, able to interact with the examiner, a physiological gait and a mild enlargement of both calves. She was able to run 10 meters in 3,70 sec and climbing stairs in 2,03 sec. Gowers times ranged from 1,89 sec to 4,06 sec. ECG showed tachycardia (115b/m), a short PQ interval, with increased Cardiomyopathic Index and posterolateral fibrosis (Figure 2), a picture typical of dystrophinopathic cardiomyopathy [21,22]; echocardiography showed normal diameters and volumes of cardiac chambers, normal thickness of septum and left posterior wall and a normal ejection fraction.

The analysis of DMD gene, by 80-plex PCR analysis, failed to demonstrate any deletion of the dystrophin gene, while the X inactivation analysis showed a pattern moderately skewed (72.5:27.5), identical in both lymphocytes and muscles (Table 1).

Oral deflazacort (DFZ) was started when the patient was 6 years old, according to our protocol in cases where a deterioration of the timed tests is observed [23]. DFZ administration was able to improve muscular weakness for about 4 years, followed by stabilization and then a worsening in motor performance. In fact among functional tests, 6MWT passed from 301 to 315 and then to 200 meters, while North Star test declined from 21/34 to 17/34 to 10/34 [24-26]. The ability to stand up from the floor was lost at the age of 11 years. Ecg and echocardiography remained unchanged. Spirometry showed normal vital capacity values during the entire period.

In 2012, at the age of 13 years, because the worsening of the motor performance, we decided to re-assess the X chromosome inactivation (XCI) on peripheral blood lymphocytes. The analysis was performed through the HUMARA test [27] followed by capillary electrophoresis by ABI Prism 3100 analyzer. The degree of the XCI in the digested DNA was calculated as follows: peak area of (XCm digested/non digested)/(XCm digested/non digested)+(XCn digested/non digested) x 100. The results showed 2 peaks of different size indicating a different numbers of CAG repeats between the 2 alleles on the two X chromosome; in non-digested sample, the 2 alleles had a different number of CAG repeats, while in the digested sample only 1 peak was found, suggesting a pattern of extremely skewed inactivation (100:0) (Figure 3). This result was discordant from that obtained by the first analysis, 6 years before, and justifies the DMD-like phenotype observed in the girl.

Discussion

DMD carriers are usually asymptomatic at the muscle level, because the normal copy of the DMD gene is usually able to produce sufficient dystrophin. Symptomatic DMD carriers are reported in less than 10% of cases. Factors responsible of a muscular dystrophy in manifesting carriers include the presence of mutation in both Xp21 alleles, Turner Syndrome, unbalanced X;autosome translocation, usually rare, and the more frequent preferential inactivation of the wild X chromosome.

A high prevalence of cardiomyopathy due to lack of dystrophin in the myocardium has also been reported. Cardiomyopathy starts in a preclinical manner, that can be diagnosed by instrumental investigation (ecg and echocardiography), and evolves toward pictures of dilated cardiomyopathy [5,21,28,29]. The severe DMD phenotype observed in our case cannot depend only on the X;autosome translocation



Figure 2: Characteristic features of ECG in DMD: On the left a diagrammatic representation of a single complex; on the right the ECG registered in the case report. Note in the boxes the short PQ segment, prolonged QT interval and tall R wave, expression of the posterior wall fibrosis.

Translocation	Clinical Features	X Chromosome Breakpoint	Autosomal Breakpoint	X Inactivation Pattern (Xw.Xm)
X;1		Xp21-2	1p34 or 34-3	100:0 (L)*
X;2		Xp21	2q14	
X;2	Moderate mental retardation	Xp21-2	2q37-3	
X;3		Xp21	3q27	
X;3	Mental retardation, dysmorphism	Xp21-2	3q13-2 or 3q13-32	95:5 (L)*
X;4		Xp21-1	4q26	100:0 (L)*
X;4				
X;4				
X;5	Moderate mental retardation	Xp21-2	5q31-1	100:0 (L)*
X;5		Xp21-1	5q35-3	100:0 (F)*
X;5		Xp22	q32	95:5 (L)*
X;6		Xp11	q21	100:0 (L)*
X;6		Xp21	6q16	
X;6		Xp21-2	6q21	99:1 (L)*
X;7		Xp22	p11.1	99:5 (L)*
X;8	Mild	Xp21-1	8q24-3	80:20 (L)*
X;9	Tumer S; Epilepsy; mental retardation	Xp21 (Extreme proximal)	9p21 (Extreme proximal)	100:0 (L)*
X;9	Moderate mental retardation	Xp21-2	9p22-3	98:2*
X;11		Xp21-1	11q13-5	
X;11	Mild	Xp21-2	11q23-3	Xn inactivated (L)*
X;15		Xp21	15q26	93:7 (L); 95:5 (F)*
X;21	Mild	Xp21-1	21p12	99:1 (L); 95:5 (F)*
X;22		Xp21	22q13	
X;22		Xp22.1	p11.1	100:0 (L)*
X;3	DMD phenotype	Xp21	p24	72.5:27.5 (L; M); 100:0 (L)

*XCI evaluated by BrdU technique of Hagemeijer et al. (1976) and following modifications (Panasiuk et al. 1997) L, F, M= X inactivation analysis on fibroblasts, lymphocytes or muscle cells respectively

Table 1: Inactivation Analysis on Fibroblasts, Lymphocytes or Muscle Cells.

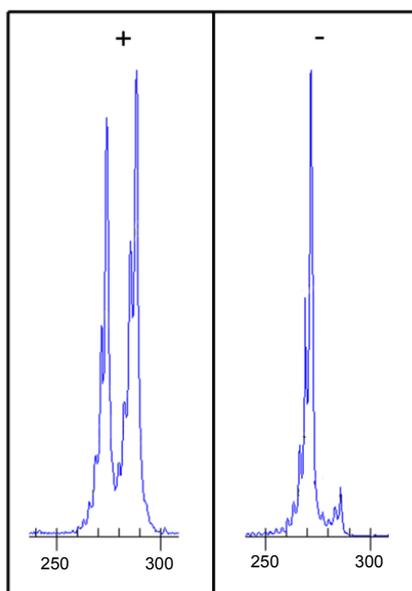


Figure 3: XCI in the case report. In the non-digested sample (on the left), the 2 alleles had different numbers of CAG repeats, while in the digested sample (on the right) only 1 peak is present, indicating a pattern of extremely skewed inactivation (100:0).

determining the rupture of the dystrophin gene, because this condition should result in a heterozygous condition and a DMD carrier status, but probably relies on the extremely skewed X inactivation (100:0). In fact while in unbalanced translocations the abnormal X is usually completely or partially inactivated, the apparently balanced X;autosome translocations are usually associated with a non random XCI [30].

DMD carriers - symptomatic at the muscle level - usually show in muscle biopsies a mosaic pattern of dystrophin positive/negative fibres which reflect the proportion of myonuclei bearing the X chromosome with the wild allele versus the X chromosome with the mutant allele [31].

A correlation between XCI in the peripheral blood and clinical

symptoms has been observed, the higher inactivation of the X chromosome carrying the normal allele determining a more severe clinical manifestation [18,19].

In the case here reported, the analysis of X inactivation was performed twice; in the former – performed at the age of 5 years - the pattern of inactivation was moderately skewed (72.5:27.5) in both muscle cells and lymphocytes; in the latter, performed at the age of 12 years on lymphocytes only, it was extremely skewed (100:0). The differences in these results are likely related to the older age of the patient, as a positive correlation between XCI pattern and age has been reported [32,33].

This case report reinforces the need for a side by side collaboration between molecular biologists and cytogeneticists during the prenatal diagnostic process and counseling. Furthermore we suggest to perform FISH and XCI analysis in all cases of *de novo* apparently balanced X;autosome translocations that involved the dystrophin gene.

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