Marked Hemiatriphy in Carriers of Duchenne Muscular Dystrophy

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Objective: To describe the clinical and molecular genetic findings in 2 carriers of Duchenne muscular dystrophy (DMD) who exhibited marked hemiatrophy. Duchenne muscular dystrophy is an X-linked disorder in which affected male patients harbor mutations in the dystrophin gene. Female patients with heterozygous mutations may be manifesting carriers.

Design: Case study.

Setting: Neurology clinic.

Patients: Two manifesting carriers of DMD.

Interventions: Clinical and radiologic examinations along with histologic and molecular investigations.

Results: Both patients had marked right-sided hemiatrophy on examination with radiologic evidence of muscle atrophy and fatty replacement on the affected side. In each case, histologic analysis revealed a reduction in dystrophin staining on the right side. Genetic analysis of the dystrophin gene revealed a tandem exonic duplication in patient 1 and a multixenic deletion in patient 2 with no further point mutations identified on the other chromosome.

Conclusions: Marked hemiatrophy can occur in DMD manifesting carriers. This is likely to result from a combination of skewed X-inactivation and somatic mosaicism.

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MANIFESTING CARRIERS OF Duchenne muscular dystrophy (DMD) are heterozygous symptomatic female patients who generally present with a milder phenotype than affected male patients. The phenotypic spectrum of the manifesting carrier is broad, encompassing cramps, myalgia, muscle weakness, dilated cardiomyopathy, and, rarely, progressive proximal weakness. This report describes 2 manifesting carriers of DMD who presented with striking right-sided asymmetrical hemiatrophy in the presence of normal left-sided musculature. We present clinical, genetic, radiologic, and histologic data and suggest that a combination of skewed X-inactivation and somatic mosaicism are the likely underlying mechanisms accounting for this unusual presentation.

Consent was obtained from both patients. Genomic DNA was extracted from leukocytes via a Chemagic Magnetic Separation Module I using the 3-mL Chemagic DNA blood kit special (chemagen Biotechnologie AG, Baesweiler, Germany) or via a Nucleon BACC3 kit (GE Healthcare, Piscataway, New Jersey). Multiplex ligation-dependent probe amplification (MLPA) analysis using probe sets P034 and P035 (MRC-Holland, Amsterdam, the Netherlands) was performed according to the manufacturer’s instructions. Muscle RNA was extracted from frozen muscle biopsy specimens using the RNaseq Fibrous Tissue Mini kit (Qiagen, Düsseldorf, Germany). Muscle DNA was extracted from frozen muscle biopsy specimens using organic extraction. To amplify the exon 10-11 duplication in patient 1, reverse transcriptase–polymerase chain reaction (RT-PCR) amplification was performed, as previously described.

Open muscle biopsy specimens were frozen in 2-methyl-butane or isopentane, cooled by liquid nitrogen, and stored at −80°C. Ten-micrometer-thick sections were stained with hematoxylin-eosin, periodic acid–Schiff reagent, oil red O, Congo red, and a modified Gomori stain. Enzyme histochemical analysis with NADH (nicotinamide adenine dinucleotide)-tetrazolium-reductase, and adenosine triphosphatase at pH 4.2 and 4.6 was performed according to standard techniques. For immunohistochemical analysis, the
following antiserum solutions were used: ubiquitin (rabbit polyclonal antibody, code No. Z.0458, 1:200; Dako Corp, Carpinteria, Calif.); spectrin (mouse monoclonal antibody [mAb], clone SPEC1, 1:200 Novocastra Laboratories Ltd, Newcastle upon Tyne, England); dystrophin I, II, and III (mouse mAb, clones DYS1, DYS2, and DYS3, 1:20; Novocastra); and utrophin I and II (mouse mAb, clones DRP1/12B6 and DRP2/20C5, 1:20; Novocastra).

**REPORT OF CASES**

**CASE 1**

Patient 1 presented at age 50 years with an 18-year history of progressive wasting and weakness in her right arm and right leg. There was no family history of muscular dystrophy. The musculature in the left arm and leg was unaffected as were her facial muscles. She denied any cardiac symptoms.

On examination we found marked wasting of her right arm and leg with weakness proximally (Medical Research Council [MRC] grade 3/5) more than distally (Figure 1A and B). The biceps, supinator, knee, and ankle reflexes on the right were absent but present on the left side. The left arm and leg were entirely normal on examination. The creatinine kinase level was elevated (1448 U/L; reference range, <200 U/L). (To convert creatinine kinase to microkatal per liter, multiply by 0.0167.) An echocardiogram showed left ventricular dilatation. A magnetic resonance imaging (MRI) scan of her legs revealed clear right-sided muscle atrophy with fatty replacement compared with the left, which was only slightly infiltrated in the posterior compartment (Figure 1D).

Histologic examination of the affected right quadriceps muscle demonstrated reduced staining for dystrophin in scattered fibers (Figure 2A-D). DNA sequencing of the 79 coding exons of the **DMD** gene (OMIM 300377) did not identify any point mutations, but MLPA analysis revealed a heterozygous duplication of exons 10 and 11, which is predicted to cause a frame shift (Figure 3A). X-inactivation studies from genomic DNA, according to the previously published method, revealed a ratio of 0.88, a result suggestive of nonrandom X-inactivation. However, clearly the inactivation pattern, at least in the lymphocytes, was not completely skewed.

To investigate the effect of this duplication on the patient’s dystrophin in muscle messenger RNA (mRNA), RT-PCR analysis was performed using primers located in the coding sequence of exons 9 and 13 of the **DMD** gene and with primers designed to amplify across the duplication breakpoint at the RNA level. In both cases, no mutant RT-PCR products were detected. Sequence analysis of the patient’s dystrophin complementary DNA tran-
script showed that she was heterozygous for the exon 53 coding sequencing polymorphism r.7728u→c, which had been previously detected in her genomic DNA.

**CASE 2**

Patient 2 presented at age 35 years with progressive asymmetrical wasting and weakness of her right arm and leg with exertion-dependent myalgia and cramps but with no cardiac symptoms. Examination revealed wasting and weakness of her right arm (Figure 1C) and leg. Her left side was unaffected. Deep tendon reflexes were absent on the right side and normal on the left. The creatinine kinase level was elevated at 958 U/L. An MRI scan of her thighs revealed fatty degeneration of the muscles on the right, while the left side showed slight but clear fatty infiltration of muscles from the posterior compartment (Figure 1E). Her echocardiogram showed no abnormalities.

Immunostaining of affected deltoid muscle from the right demonstrated a reduced expression of dystrophin compared with the clinically unaffected left side (Figures 2E-H); this was further confirmed by Western blot analysis, which showed a profound reduction in dystrophin expression in the right deltoid. Patient 2 was the mother of a boy diagnosed with DMD who was found to have a deletion of exons 3 through 13 of the DMD gene (Figure 3B). Although, this mutation is predicted to maintain the translation reading frame, it is likely that it has a severe disruptive affect on the function of the F-actin domain of the affected son’s truncated dystrophin protein, explaining his DMD phenotype. Genetic analysis of the DMD gene of patient 2 showed that she was a carrier of this mutation (Figure 3B). X-inactivation studies on lymphocyte DNA revealed no difference in methylation between the 2 X chromosomes. Cytogenetic analysis revealed a normal karyotype, thus excluding Turner syndrome as a possible cause.

**COMMENT**

The identification of a pathogenic duplication of DMD exons 10 and 11 in patient 1 and a deletion of exons 3 through 13 in patient 2 are consistent with both patients being manifesting carriers of DMD. Immunohistochemical studies on affected muscle further sup-
ported their carrier status: the biopsy specimens from both patients clearly demonstrated a reduction in dystrophin staining with compensatory upregulation of utrophin. Patient 1 was also found to be heterozygous for a synonymous polymorphism in the coding sequence of exon 53 (c.7728t→c and r.7728u→c) at both the genomic DNA and RNA levels, indicating that she expressed dystrophin mRNA from both of her X chromosomes. A sensitive RT-PCR assay was used to amplify the mutant mRNA transcript from affected muscle, but we were nonetheless unable to detect the presence of the duplication. This suggests either that the mutant mRNA was not present at a sufficient level for detection, or that the mutation is more complex than a straightforward tandem duplication, in which case our RT-PCR assay would not be able to detect it.

The striking clinical feature in both patients was marked asymmetrical wastling. Although mild asymmetrical weakness is a common occurrence in DMD carriers, marked hemiatrophy is very unusual. In addition, patient 1 had a dilated cardiomyopathy, which is described in approximately 8% of manifesting carriers. The MRI studies correlated with the marked asymmetrical phenotype but also indicated subclinical involvement of left-sided muscles. The manifestation of X-linked recessive disorders in female patients is generally considered to arise from skewed X-inactivation, a process that is normally random and ensures dosage equalization of X-linked genes. However, the skewed X-inactivation patterns is transmitted to offspring in humans. Since no second DMD mutation was detected in the other chromosome in either patient, their phenotype is predicted to arise from nonrandom or skewed X-inactivation resulting in overexpression of the mutant X chromosome. Although X-inactivation ratios in lymphocyte DNA were normal in case 2 and suggestive of skewing in case 1, the pattern is likely to be tissue specific; hence, the result does not exclude higher levels of skewed X-inactivation in the affected muscle tissue. It is possible that the higher 0.88 ratio seen in patient 1 may be the result of age-related skewing of X-inactivation.

Skewed X-inactivation alone would not account for the very marked asymmetrical presentation. It is possible that the marked asymmetry reflects a disproportionately high expression of the X-chromosome carrying the mutant DMD gene on the more affected side, with preferential inactivation of the X chromosome harboring wild-type DMD. This may have arisen from an event early in embryogenesis when the X-chromosome–carrying wild-type and affected muscle tissue. This event may have been either stochastic or genetic, involving for example the XIST gene, a key player in the X-inactivation pathway. One can speculate that mosaicism of the XIST gene could account for the marked asymmetrical skewed inactivation.

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