PERGAMON

Neuromuscular Disorders 13 (2003) 317–321

www.elsevier.com/locate/nmd

Case Report

Somatic mosaicism of a point mutation in the dystrophin gene in a patient presenting with an asymmetrical muscle weakness and contractures

A.T.J.M. Helderman-van den Enden[a,*], H.B. Ginjaar[a], A.L.J. Kneppers[a], E. Bakker[a], M.H. Breuning[a], M. de Visser[b]

[a]*Center for Human and Clinical Genetics, Leiden University Medical Center K5-R, PO box 9600, 2300 RC Leiden, The Netherlands*
[b]*Department of Neurology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands*

Received 17 July 2002; received in revised form 6 November 2002; accepted 25 November 2002

Abstract

We describe a patient with somatic mosaicism of a point mutation in the dystrophin gene causing benign muscular dystrophy with an unusual asymmetrical distribution of muscle weakness and contractures. To our knowledge this is the first patient with asymmetrical weakness and contractures in an ambulatory patient with a dystrophinopathy.

Keywords: Somatic mosaicism; Point mutation; Dystrophin gene; Asymmetrical muscle weakness; Contracture

1. Introduction

Duchenne muscular dystrophy (DMD) is a progressive muscular dystrophy leading to death in early adulthood. In the Netherlands the prevalence is about one in 4000 boys [1]. Signs and symptoms due to symmetrical weakness of the hip muscles and lower proximal limb muscles occur in early childhood. Before the age of 13 DMD patients are wheelchair-bound. They usually die due to cardiac arrest or respiratory failure [2] although of late a much more protracted course is observed since artificial ventilation is increasingly employed. About one third of the patients presents non-progressive intellectual impairment. The diagnosis is usually suspected in boys with symmetrical weakness and the serum creatine kinase (CK) activity of more than ten times the upper limit of normal. DMD is caused by mutations in the dystrophin gene on the X chromosome band p21. Most patients have a deletion of part of the gene (60%), about 5% have a duplication, the remaining 35% have a frameshift, a nonsense or a splice site mutation [3].

Becker muscular dystrophy (BMD) is also caused by mutations in the dystrophin gene. Usually these mutations retain the reading frame and generate a shortened and reduced amount of the protein. Mutations that disrupt the reading frame cause a premature termination and a loss of dystrophin and lead to DMD [2]. The phenotype of BMD is similar to that of DMD but in terms of skeletal muscle and cardiac involvement, the course is much milder.

One third of the patients have the disease as the result of a new mutation. Mothers of patients with apparent de novo mutations, were shown to transmit the mutation a second time, while these mothers were not carrying the mutation in lymphocytes. This phenomenon is known as germinal mosaicism. Empirical data revealed a recurrence risk for male pregnancies of around 14–20%, associated with transmission of the X chromosome of their affected son [4,5]. Somatic mosaicism has been described in a number of mothers of Duchenne patients and in one Duchenne patient and possibly in a maternal grandfather, who has three DMD grandsons from his three daughters [3,4,6–11].

Here we describe a patient with an unusual phenotype (asymmetrical muscle weakness and contractures) caused by somatic mosaicism of a point mutation in the dystrophin gene.

* Corresponding author. Fax: +31-71-5266749.
E-mail address: helderman@lumc.nl (A.T.J.M. Helderman-van den Enden).

0960-8966/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0960-8966(02)00285-7

2. Patient and methods

2.1. Case report

The patient was born after a normal pregnancy, birth weight was 2700 g. He walked at the age of 18 months. At 6 years of age he was diagnosed with psychomotor retardation of unknown cause. He attended a school for mildly intellectually impaired children and he never learned to read or write. Currently he lives 'semi independently' in a small group with supervision.

At the age of 8 years an abnormal gait was observed. Gradually, increasing atrophy and weakness of the muscles of the right leg, scoliosis and contractures became apparent prompting referral to a neurologist who ascribed these symptoms to a presumed perinatal trauma despite a normal electro-encephalogram (EEG) and brain computed tomography (CT).

Neurological examination at the age of 30 showed atrophy of the muscles of the upper arms, pectoralis major right more than left and the thighs, right more than left (Fig. 1). The left calf was hypertrophic (Fig. 2). There was moderate weakness of the shoulder girdle muscles and right peroneal muscles and severe weakness of the upper arm, pelvic girdle, thigh and calf muscles. Remarkably, Gowers' phenomenon was negative. He was not able to walk on tip-toe nor on his right heel. Contractures at the right shoulder, elbows (left more than right, Fig. 1) and of the finger flexors were noticed. Reflexes were negative except the left knee and left Achilles tendon. A depigmented nevus was present at the back on the left side of the chest.

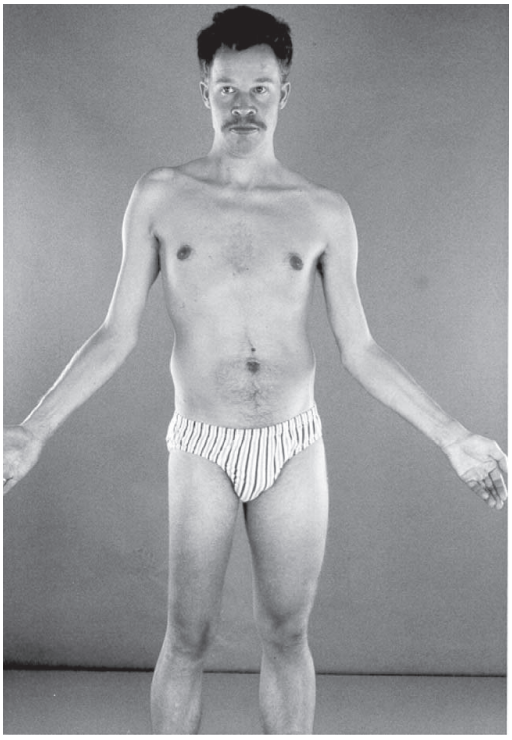

Fig. 1. Note the atrophy of the upper arms (left more than right), of the right leg and of the elbow contractures.

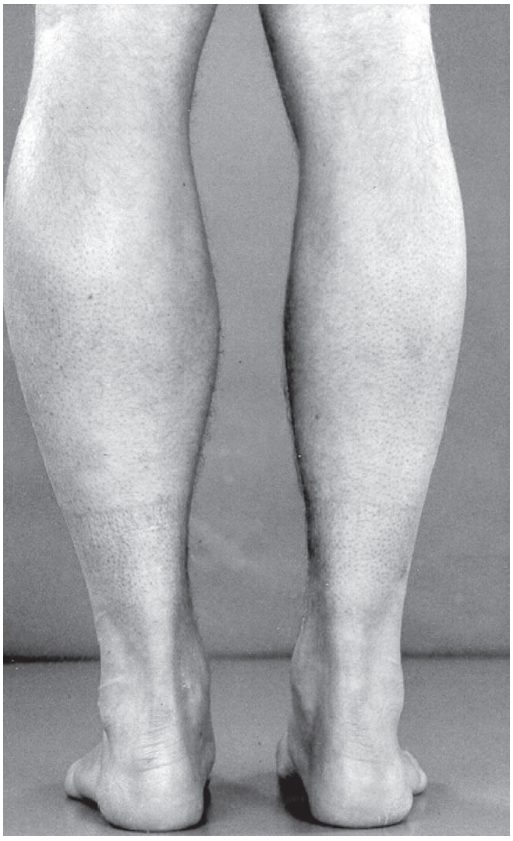

Fig. 2. Note the hypertrophy of the left calf.

Creatine kinase (CK) activity was 3000 IU/l (normal $<$ 190 IU/l). Electromyography (EMG) showed myopathic changes. A muscle biopsy taken from the vastus lateralis muscle of the left leg showed necrotic fibers, occasional regenerating fibers and a marked variation in the size of muscle fibers. There were numerous pycnotic nuclear clumps, and atrophic fibers showed an increase in non-specific esterase activity. There was endomysial fibrosis and liposis. The histological pattern was consistent with muscular dystrophy.

The cardiologist was consulted once the diagnosis was made. Ultrasound investigation of the heart was normal. The electrocardiogram (ECG) showed an electric semivertical

heart axis, QRS time of 0.08 s with a high RS ratio right precordial and in the caudal and lateral leads rather deep Q's. This finding is compatible with abnormalities observed in patients with Becker muscular dystrophy.

Karyotyping showed a normal male pattern, 46, XY.

2.2. DNA analysis

DNA was extracted from the patient's whole blood, according to the method of Miller [12]. Multiplex polymerase chain reaction (PCR) was performed according to Chamberlain and Beggs [2] and Southern blotting was after Bakker and den Dunnen [13]. Approximately 200 ng of the PCR product generating the truncated translation product was used for sequence analysis. Sequencing was performed using the Big Dye Terminator Sequencing kit (Applied Biosystems, Nieuwekerk a/d IJssel, the Netherlands) on ABI PRISM 310. Since a PstI restriction site present in exon 64 is abolished by the mutation, the percentage of lymphocytes containing the mutation could be determined by a quantitative PCR analysis for exon 64 on genomic DNA. After quantitative PCR using a fluorescently labelled primer, the PCR product was digested with PstI followed by electrophoresis of the PCR products on an ALF DNA sequencer analyser (Amersham Pharmacia Biotech). The relative peak areas were measured.

2.3. PTT assay

RNA was isolated from muscle tissue specimens and whole blood using RNAzol (Campro Scientific, Veenendaal, the Netherlands) according to the manufacturer's protocol. Reverse transcriptase-polymerase chain reaction (RT-PCR) and the protein truncation test (PTT) was performed as described by Roest [14].

2.4. Immunohistochemistry

Serial unfixed cryostat sections of the muscle specimen of the patient were studied for dystrophin and spectrin expression. The following antibodies were used against dystrophin: NCL-DYS1, NCL-DYS3 (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK), Mandys108 [15] and spectrin (NCL-SPEC1 Novocastra). The immunohistochemical stainings were done as described previously [16].

3. Results

Immunohistochemical analysis of dystrophin revealed a mosaic pattern of positive and negative staining muscle fibers using several dystrophin antibodies (Fig. 3). Western blotting showed a reduced amount of dystrophin (not shown).

In genomic DNA extracted from lymphocytes no abnormality was found by multiplex PCR and quantitative Southern blot analysis. mRNA from the muscle biopsy was studied using the PTT analysis yielding two bands of one fragment corresponding to exons 59–68 as can be seen in Fig. 4, indicating mosaicism. Sequencing of the RT-PCR product showed a possible point mutation, in exon 64 leading to a premature stopcodon of the dystrophin gene, although the band was weak. Thereupon genomic DNA was sequenced and the mutation was confirmed at position 9554C → T; Q3116X.

The percentage of the mutated allele in DNA from lymphocytes was calculated to be 75% according to the method described in Section 2.2 of patient and methods.

DNA analysis in lymphocytes of the patient's mother did not show the point mutation.

4. Discussion

The patient reported by us is remarkable because of the peculiar phenotype and the mosaicism. As stated before, patients with BMD have progressive symmetrical muscular weakness and contractures only appear in due course, namely when patients become wheelchair-bound. Our case had asymmetric weakness and prominent contractures while he was still ambulatory. In addition, muscle biopsy showed a combination of dystrophy and neurogenic changes. Therefore the differential diagnosis was the following: facioscapulohumeral dystrophy, spinal muscular atrophy and dystrophinopathy. DNA analysis for these muscle disorders did not show any abnormality. However, only quantitative PCR and Southern blotting of the dystrophin gene were done, which only reveals large deletions or duplications. Subsequent immunohistochemical dystrophin analysis showing a mosaic of dystrophin negative and positive fibers, led way to the diagnosis.

It is known that the skeletal muscles of DMD patients may have some dystrophin positive fibers [17]. The most likely explanation of these so-called revertant fibers, is a second site in-frame deletion. In our patient the wild type allele as well as the point mutation were present both in lymphocytes and in skeletal muscle, indicating that we are dealing with a true somatic mosaicism.

About one third of DMD and BMD patients are intellectually impaired, as is our patient. The cause of the impairment is not known. A correlation was found with the loss of Dp140 regulatory sequences. Dp140 is a dystrophin isoform with predominant expression during foetal brain development. The promoter and first exon lie in the intron between exon 44 and 45 [18]. In our patient the mutation is located in exon 64. The point mutation in his dystrophin gene causes premature translation termination leading to absence of dystrophin in the affected cells, which is typical for DMD. The most likely explanation of the mental impairment in our patient is that the proportion of brain cells with the mutation is rather high. Family history and physical

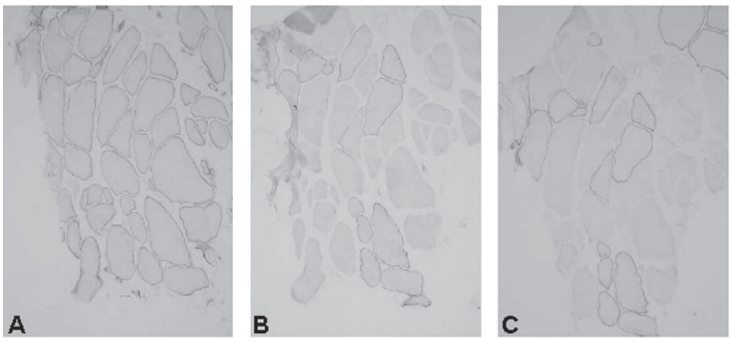

Fig. 3. Immunohistochemical labeling of spectrin and dystrophin in vastus lateralis muscle of the left leg. Serial transverse sections (10 μm) were immunostained with anti-spectrin monoclonal antibody (A) and with anti-dystrophin monoclonal antibody NCL-DYS1 (B) and Mandys108 (C) (× 100).

examination did not point to another cause for the intellectual impairment.

Somatic mosaicism has been previously described in six carriers [3,4,6–9] and in two male patients with a dystrophinopathy [10,11].

Lebo et al. [10] described a grandfather with an elevated CK, striking muscle weakness and atrophy in the right arm and shoulder in the C5–6 innervated myotomes. Each of his three daughters had a son with DMD. Molecular analysis showed the three grandsons had inherited the grand paternal X chromosome. Certainly the grandfather must have had germinal mosaicism, a somatic mosaicism is possible because of his clinical picture. Unfortunately, this could not be confirmed at the molecular-genetic level since the family was further uncooperative. Saito et al. [9] reported on a classical DMD patient who had a deletion of exon 1–7 in the lymphocytes. His mother and the patients' sister did not carry the deletion. Post mortem DNA investigation showed the presence of the deletion in ectodermal and endodermal tissues. In some mesodermal tissues (temporalis, sterno-cleidomastoid, diaphragm muscles and the kidney) the deletion was absent at the DNA level whereas in other mesodermal tissues the deletion was present, indicating a somatic mosaicism. Dystrophin expression in muscles showed no dystrophin in some muscles, in a few muscles a mosaic pattern was found and in the diaphragm dystrophin was detected in every fiber. They suggested that the deletion might have occurred early during embryogenesis because all cells of the ectoderm and the endoderm carried the deletion while in the mesoderm a somatic mosaicism was found.

Our patient illustrates a somatic mosaicism of a point mutation in the dystrophin gene which made the phenotype of the dystrophinopathy (with a disruption of the reading frame), milder. The spectrum of dystrophinopathies can yet be extended to asymmetrical weakness and early contractures.

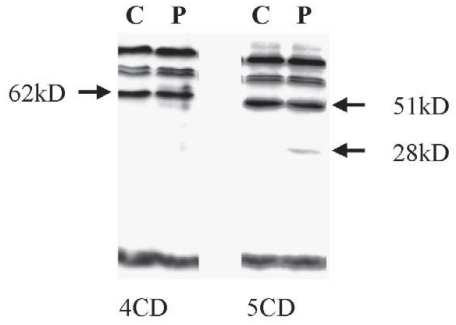

Fig. 4. Study of mRNA from the muscle biopsy with the PTT analysis yielding two bands in fragment 5 CD corresponding to exons 59–68, indicating mosaicism. C means control and P patient. The upper protein bands observed in all lanes are endogenous biotinylated proteins present in the rabbit reticulocyte lysate used for translation detection.

Acknowledgements

We thank Dennis Verbove for his expert technical assistance.

References

[1] van Essen AJ, Busch HF, te Meerman GJ, ten Kate LP. Birth and population prevalence of Duchenne muscular dystrophy in The Netherlands. Hum Genet 1992;88:258–66.
[2] Bakker E, van Ommen GJB. Duchenne and Becker muscular dystrophy (DMD and BMD). In: Emery AEH, editor. Neuromuscular disorders: clinical and molecular genetics. Chicester: Wiley, 1998. p. 59–85.
[3] Bunyan DJ, Crolla JA, Collins AL, Robinson DO. Fluorescence in situ hybridisation studies provide evidence for somatic mosaicism in de novo dystrophin gene deletions. Hum Genet 1995;95:43–5.
[4] Bakker E, Veenema H, Den Dunnen JT, et al. Germinal mosaicism increases the recurrence risk for 'new' Duchenne muscular dystrophy mutations. J Med Genet 1989;26:553–9.
[5] van Essen AJ, Abbs S, Baiget M, et al. Parental origin and germline mosaicism of deletions and duplications of the dystrophin gene: a European study. Hum Genet 1992;88:249–57.

[6] Voit T, Neuen-Jacob E, Mahler V, Jauch A, Cremer M. Somatic mosaicism for a deletion of the dystrophin gene in a carrier of Becker muscular dystrophy. Eur J Pediatr 1992;151:112–6.
[7] Bunyan DJ, Robinson DO, Collins AL, Cockwell AE, Bullman HM, Whittaker PA. Germline and somatic mosaicism in a female carrier of Duchenne muscular dystrophy. Hum Genet 1994;93:541–4.
[8] Van Essen AJ, Mulder IM, van der Vlies PJ, Hofstra RMW, Buys CHCM. Somatic and germline mosaicism of a point mutation in the dystrophin gene. Eur J Hum Genet 1998;6(4):140.
[9] Smith TA, Yau SC, Bobrow M, Abbs SJ. Identification and quantification of somatic mosaicism for a point mutation in a Duchenne muscular dystrophy family. J Med Genet 1999;36:313–5.
[10] Lebo RV, Olney RK, Golbus MS. Somatic mosaicism at the Duchenne locus. Am J Med Genet 1990;37:187–90.
[11] Saito K, Ikeya K, Kondo E, et al. Somatic mosaicism for a DMD gene deletion. Am J Med Genet 1995;56:80–6.
[12] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215.
[13] den Dunnen, J.T., Bakker, E:http//www.dmd.nl.
[14] Roest PA, Roberts RG, van der Tuijn AC, Heikoop JC, van Ommen GJ, den Dunnen JT. Protein truncation test (PTT) to rapidly screen the DMD gene for translation-terminating mutations. Neuromuscul Disord 1993;3:49–52.
[15] Nguyen TM, Ellis JM, Ginjaar IB, et al. Monoclonal antibody evidence for structural similarities between the central rod regions of actinin and dystrophin. FEBS Lett 1990;272:109–12.
[16] Ginjaar IB, van der Kooi AJ, Ceelie H, et al. Sarcoglycanopthies in Dutch patients with autosomal recessive limb girdle muscular dystrophy. J Neurol 2000;247:524–9.
[17] Klein CJ, Coovert DD, Bulman DE, Ray PN, Mendell JR, Burghes AH. Somatic reversion/suppression in Duchenne muscular dystrophy (DMD): evidence supporting a frame-restoring mechanism in rare dystrophin-positive fibers. Am J Hum Genet 1992;50:950–9.
[18] Bardoni A, Felisari G, Sironi M, et al. Loss of Dp140 regulatory sequences is associated with the cognitive impairment in dystrophinopathies. Neuromuscul Disord 2000;10:194–9.