A cysteine 3340 substitution in the dystroglycan-binding domain of dystrophin associated with Duchenne muscular dystrophy, mental retardation and absence of the ERG b-wave

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Received February 12, 1996; Revised and Accepted March 29, 1996

We report the first C-terminal missense mutation in a Duchenne muscular dystrophy patient. A G10227A transition of the dystrophin gene was found which resulted in the substitution of a highly conserved cysteine at position 3340 within the second half of the dystroglycan-binding domain. Residual amounts of 427 kDa dystrophin were detected in western blot analysis of the patient’s muscle tissue, and immunohistological examination revealed weak traces of dystrophin on all fibers. Sarcolemmal staining intensity of 43 kDa β-dystroglycan was also reduced. Mental retardation in our patient and absence of the b-wave in his electroretinogram indicate that central nervous functions of dystrophin isoforms also depend on the presence of cysteine 3340.

INTRODUCTION

The majority of dystrophin gene mutations that cause Duchenne muscular dystrophy (DMD) lead to premature stop codons. These mutations (out-of-frame deletions or nonsense mutations) offer few clues about the relationship between protein structure and function. Only the small set of missense mutations may contribute to the functional analysis of dystrophin and its isoforms (1). This paper reports the first C-terminal missense mutation in a DMD patient indicating functional relevance for the cysteine residue at position 3340.

RESULTS

Clinical data

The affected child is the only case of DMD in the kindred. CK activities of his mother and maternal grandmother were normal. The patient presented with muscular hypotonia during the first year of life. Calf enlargement was noticed at the age of 2 years. Language development and motor milestones were delayed. The patient did not use two-word sentences before the age of 4 years. At that time, Gowers’ sign was positive and CK activity in serum was 7000 U/l. Urinary glycerol excretion was normal. At the age of 8 years, CK activity was 1700 U/l and the patient could no longer stand up from the floor without assistance. At the age of 9 years he was wheelchair bound. Dark-adapted electroretinography revealed absence of the b-wave. Visually evoked potentials, echocardiography and ultrasound of the adrenal glands were normal.

Immunohistology and western blot

Traces of dystrophin were found on all muscle fibers (m. vastus lateralis) by labeling with dys 1 and dys 2 antibodies (Fig. 1). As in other cases of markedly reduced dystrophin expression, staining with dys 3 was negative. Staining intensity for β-dystroglycan was also reduced compared with normal controls (Fig. 1). Utrophin expression was found at the sarcolemma of most fibers. Spectrin labeling was normal. Western blot analysis using dys 2 antibody revealed markedly reduced amounts (10–20%) of 427 kDa dystrophin and a degradation product (data not shown). The detection of normal sized dystrophin with the dys 2 antibody that binds to the last 17 C-terminal amino acids made large deletions and chain-terminating mutations unlikely.

Dystrophin gene analysis

Southern blot analysis of the dystrophin gene using cDNA probes 1a–2a, 2b–3, 4–5a, 5b–7, 8 and 9–10 (2) did not show any deletion. Hence, point mutation screening (brain- and muscle-specific promoters, exons 3, 4, 6, 8, 12, 13, 17, 19, 55a–79) was performed as described before (3,4). A G10227A transition (Fig. 2) was found within exon 69 resulting in a cysteine to tyrosine substitution at position 3340.

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Figure 1. Cryosections of m. vastus lateralis (negatives). Immunofluorescence staining of dystrophin (dys 2 antibody) and 43 kDa β-dystroglycan. Comparison of the patient’s muscle tissue with a normal control indicating reduced expression of both proteins.

Figure 2. Dystrophin DNA sequence (2) between A10221 and T10229 (top) indicating a G to A transition at position 10227 of the patient’s gene.

DISCUSSION

All available DMD/Becker muscular dystrophy (BMD) point mutation data (1,4–7) as well as 118 cases screened by our group suggest that C3340Y is not a polymorphism. In fact, C3340 is highly conserved (8). Four dystrophin missense mutations have been described to date. They affected the N-terminus (5) or rod domain of dystrophin (6,7) or were found in BMD patients (4,7). Our report on a DMD patient with a missense mutation in the first half of the C-terminus is of special interest since it may help to elucidate the interaction of dystrophin with the dystroglycan complex.

Binding to intracellular dystrophin and extracellular laminin, the dystroglycan complex is a transmembrane linker between the subsarcolemmal cytoskeleton and the extracellular matrix and may be involved in mechanical protection of the sarcolemma (9,10). The first half of the C-terminus and the cysteine-rich domain form the dystroglycan-binding domain of dystrophin (D-domain; amino acid residues 3080–3408) (10–13) which is present in all dystrophin isoforms including the non-muscle isoforms Dp71, Dp116, Dp140 and DP260 (14–16). Deletions or chain-terminating nonsense mutations involving the D-domain result in DMD (1,17–19). In particular, the well-conserved cysteine residues concentrated in the D-domain are important for binding to β-dystroglycan (12). The mutation found in our patient emphasizes the functional importance of cysteine 3340. In vitro studies have shown that the first half of the D-domain binds directly to dystroglycan while the second half (= first half of the C-terminus) confers a 5- to 20-fold increase of the binding affinity (12,19,20). In accordance with the results of these studies, we found residual traces of dystrophin and β-dystroglycan in our DMD patient. How cysteine 3340 influences the binding to dystroglycan deserves further attention. Cysteine palmitoylation affects membrane binding of cytosolic proteins (21,22). Palmitoylation of a tightly membrane-associated subpopulation of spectrin, a close cytoskeletal relative of dystrophin (14), has been found in erythrocytes (23). To our knowledge, however, palmitoylation of dystrophin has not been described as yet.

Finally, this case report is another example of the association between mental retardation and C-terminal dystrophin mutations (4). Absence of the b-wave in the patient’s electroretinogram indicates that the neurophysiological functions of dystrophin isoforms such as retinal Dp260 (16) also depend on the presence of cysteine 3340, that is, on adequate function of the dystroglycan binding domain.

MATERIALS AND METHODS

Immunofluorescence labeling of muscle tissue was performed according to standard procedures. Antibodies against the N-terminal (dys 3), C-terminal (dys 2) and rod-like domain (dys 1) of dystrophin, against 43 kDa β-dystroglycan, utrophin and spectrin were purchased from Novocastra (Newcastle upon Tyne, UK). Southern blot analysis of dystrophin gene deletions, SSCP point mutation screening, and direct sequencing from PCR products were performed as described before (3,4).
ACKNOWLEDGEMENTS
This work was supported by the parents action group ‘Helft dem muskellkranken Kind’, Hamburg, and by the Deutsche Forschungsgemeinschaft (Vo 392/2-3).

REFERENCES