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Different early pathogenesis in myotilinopathy compared to primary desminopathy

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Abstract

Mutations in the human myotilin gene may cause limb-girdle muscular dystrophy 1A and myofibrillar myopathy. Here, we describe a German patient with the clinically distinct disease phenotype of late adult onset distal anterior leg myopathy caused by a heterozygous S55F myotilin mutation. In addition to a thorough morphological and clinical analysis, we performed for the first time a protein chemical analysis and transient transfections. Morphological analysis revealed an inclusion body myopathy with myotilin- and desmin-positive aggregates. The clinical and pathological phenotype considerably overlaps with late onset distal anterior leg myopathy of the Markesbery–Griggs type. Interestingly, all three analyzed myotilin missense mutations (S55F, S60F and S60C) do not lead to gross changes in the total amount of myotilin or to aberrant posttranslational modifications in diseased muscle, as observed in a number of muscular dystrophies. Transiently transfected wild-type and S55F mutant myotilin similarly colocalised with actin-containing stress fibers in BHK-21 cells. Like the wild-type protein, mutated myotilin did not disrupt the endogenous desmin cytoskeleton or lead to pathological protein aggregation in these cells. This lack of an obvious dominant negative effect sharply contrasts to transfections with, for instance, the disease-causing A357P desmin mutant. In conclusion our data indicate that the disorganization of the extrasarcomeric cytoskeleton and the presence of desmin-positive aggregates are in fact late secondary events in the pathogenesis of primary myotilinopathies, rather than directly related. These findings suggest that unrelated molecular pathways may result in seemingly similar disease phenotypes at late disease stages.

Keywords: Myotilin; Titin; Limb-girdle muscular dystrophy; Distal myopathy; Tibial muscular dystrophy; Myofibrillar myopathy; Inclusion body myopathy; Desmin

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1. Introduction

Mutations in the human myotilin gene on chromosome 5q31 have been shown to cause limb-girdle muscular dystrophy 1A (LGMD1A) and myofibrillar myopathy (MFM) [1–4]. LGMD1A is an autosomal dominant disorder presenting with adult onset proximal muscular weakness [1,2,5], while patients with MFM caused by myotilin mutations clinically present more often with distal lower leg weakness [3,4].

Myotilin is composed of a unique serine-rich N-terminus and a C-terminus containing two Ig-like domains, which show a high degree of sequence similarity to Ig-domains of palladin, myopalladin, and Z-disc Ig-domains 7 and 8 of the giant protein titin [6,7]. In cardiac and skeletal muscle, myotilin has a distinct localization at the level of myofibrillar Z-bands, where it directly binds α-actinin via its N-terminal region and filamin C via its C-terminal region [6–8]. Furthermore, a direct interaction with the Z-disc protein FATZ-1 and with monomeric and filamentous actin has recently been described [9,10], indicating that myotilin acts as an actin-cross-linking protein playing an important role in the stabilization of myofibrillar Z-discs [7].

Here, we report on the clinical and myopathological findings in a 61-year-old German patient presenting with a late adult onset distal anterior leg myopathy due to a heterozygous S55F myotilin mutation. It is noteworthy that this particular mutation has previously been identified in patients with LGMD1A and MFM. In addition to protein expression studies using muscle specimens from patients with three distinct myotilin missense mutations, we studied the functional consequences of the S55F myotilin and the A357P desmin mutants by means of transient transfection studies in BHK-21 cells.

2. Materials and methods

2.1. Patients and muscle biopsies

Two diagnostic muscle specimens (M1a, M. rectus femoris, 2004; M1b, M. biceps brachii, 1994) of the patient and muscle biopsy specimens from three Spanish patients harboring S60F, S55F, and S60C myotilin mutations (M2–M4) were analyzed [3].

2.2. Molecular genetic analysis

DNA extraction from blood samples and myotilin mutation analysis were performed by standard procedures using previously published primers [1,2].

2.3. Muscle MRI

Magnetic resonance imaging was performed on a 3.0 T whole body MRI system (Gyroscan Intera, Philips, Best, The Netherlands) using the standard body coil including axial T1-weighted TSE sections (TR 500 ms, TE 12 ms). Evaluation was performed as described [11].

Morphological analysis and immunohistochemistry were performed as described [12–14]. The following primary antibodies were used: (1) rabbit polyclonal antiserum 151 raised against the first 150 mouse myotilin amino acids [15,16], (2) a mouse monoclonal anti-filamin C antibody RR90 [8], (3) rabbit polyclonal anti ZASP serum [17], (4) rabbit polyclonal antiserum raised against αB-crystallin (Chemicon, USA), (5) monoclonal mouse α-actin and α-actinin antibodies AC-40 and EA-53 (Sigma–Aldrich, Germany); and (6) the mouse monoclonal desmin antibody D33 (DAKO, Germany). For standard electron microscopy muscle tissue was fixed in 3% glutaraldehyde with HEPES buffer (pH 7.5), postfixed in 1% osmium tetroxide, dehydrated and embedded in Spurr. Ultrathin sections were contrasted with uranyl acetate and lead citrate, and examined using a Zeiss 900 electron microscope (Zeiss, Germany).

3. 1D- and 2D- gel electrophoresis, and mass spectrometry

1D- and 2D-gel electrophoresis, immunoblotting, and mass spectrometry were performed as described [12,13,18]. For 2D-gel electrophoresis, protein samples were loaded at the anodic end of rehydrated 18 cm linear IPG-strips of pH 6–11; instead of 20 mM DTT the rehydration buffer contained 100 mM hydroxyethyl disulfide (HED). To investigate putative phoshorylation, samples of the normal control specimen were subjected to in vitro dephosphorylation. Briefly, 100 μg (25 μl) of the total protein extract solubilised in 2D-lysis buffer were mixed with 25 μl dephosphorylation buffer (50 mM Tris pH 8.5, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 2× Roche Complete proteinase inhibitor mix), 5 U (5 μl) calf intestinal phosphatase (CIP), and 5 μl H_2O. As a negative control, 5 μl of CIP were replaced by dephosphorylation buffer. For a photometrical positive control, the 5 μl of H_2O were replaced by pNPP (*para*-nitrophenylphenole, 1 mg/ml stock solution). The mixtures were incubated at 30 °C for 45 min, precipitated by chloroform–methanol, and subjected to 2D-gel electrophoresis.

3.1. Site-directed mutagenesis and transfection studies

Mutations were introduced into full-length human myotilin (S55F) and for control purposes into desmin (A357P, [19]) cDNA using QuickChange Site-Directed Mutagenesis Kit (Stratagene) as described [10,20]. Cells were cultured, trypsinized, transiently transfected and analyzed as described [20].