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CORRESPONDENCE

Kyphoscoliosis peptidase (*KY*) mutation causes a novel congenital myopathy with core targetoid defects

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Congenital myopathies generally manifest during early infancy and may be caused by a wide range of genetic abnormalities. Their common pathogenic concepts touch excitation–contraction coupling, mitochondrial function, myofibrillar force generation, atrophy, and autophagy [7]. In many patients, genetic defects could be discovered only after the advent of next generation sequencing. Here we present a mutation in the *Kyphoscoliosis peptidase* (*KY*) gene of human individuals as a novel cause of a slowly progressive congenital myopathy with characteristic neuropathological features.

A deleterious recessive mutation in the *Ky*-gene has first been described in a mouse mutant with thoracolumbar kyphoscoliosis [3]. In these *Ky*-mice, scoliosis is the result of early onset progressive muscle dystrophy and atrophy, which mainly affects the postural muscles [4]. Light microscopy indicated a myopathic-dystrophic pattern with central nuclei, necrosis, and regeneration followed by myofiber atrophy with myofibrillar disorganization and Z-disc thickening on electron microscopy [4]. The KY-protein is essential for muscle growth and function [3] and interacts with different muscle specific sarcomeric cytoskeletal proteins such as filamin-C and titin [2]. A protein complex of KY-protein/filamin-C/KY-interacting protein IGFN1 is associated with the Z-disc and thought to be relevant for stabilization of the sarcomere [1].

We report on two Arab–Israeli brothers from a first-cousin marriage with slowly progressive congenital myopathy due to a homozygous nonsense mutation in the *KY*-gene. Six further siblings are healthy (Supplementary Fig. 1).

The now 23-year-old patient IV:07 was born with bilateral equinovarus foot deformity that was surgically corrected. He displayed progressive weakness and atrophy of the lower limbs since early infancy. Contractures had to be treated several times by tendon release. His intellectual development was globally impaired and he suffered from anxiety and impulsive behavior. Muscle weakness and atrophy extended to the upper limbs mainly affecting the biceps and triceps muscles with preservation of the deltoid, forearm, and hand muscles. He developed elbow contractures,

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facial weakness, and kyphosis with rigid spine (Fig. 1a–c). His tongue is atrophic at the lateral margins. Presently, he is able to walk only short distances on crutches, has difficulties speaking, and suffers from severe recurrent muscle cramps, especially in cold weather. CPK was elevated up to 1215 U/L. Electromyography pointed to myopathic changes with small and polyphasic action potentials with early recruitment, but also some interspersed large potentials. Peroneal and tibial motor neurography revealed reduced amplitudes of the muscle action potential, but normal conduction velocities, while median and ulnar nerves were entirely normal. All sensory and F-wave conduction velocities were normal, thereby excluding a general neuropathy or significant α-motoneuron decay. Results of cranial and spinal MRIs as well as cardiologic investigations were normal.

Quadriceps muscle biopsy at age 17 years showed signs of myopathy with fiber-size variation, rounded, atrophied and necrotic fibers, internalized nuclei, occasional myophagocytosis, and non-rimmed vacuoles. ATPase treatment (pH 9.4) revealed fiber-type II uniformity (Supplementary Fig. 2). NADH-staining showed numerous myofibers with central mitochondrial depletion. Electron microscopy revealed these as unstructured core targetoid defects and detected streaming and thickening of Z-discs, as well as an enlarged endoplasmic reticulum (Fig. 1e–h).

The 34-year-old patient IV:05 is less severely affected than his younger brother. A congenital bilateral equinovarus foot deformity was surgically corrected. During early infancy, he also suffered from slowly progressive muscle weakness and mild atrophy of the lower limbs. Achilles tendon contractures had to be corrected by Z-plasty. He maintained full muscle strength of his upper limbs but developed bilateral elbow contractures, mild facial weakness, and kyphosis with rigid spine. His cognitive and speech development is normal. He also showed atrophy of his lateral tongue margins (Fig. 1d). Presently, he can walk with the help of a walker and his CPK activity is only mildly elevated (248 U/L). As in his younger brother, results of cardiologic investigations and spinal MRI were normal. A comparison of the phenotypes is presented in Supplementary Table 1.

A combination of autozygosity mapping and whole exome sequencing (Supplementary material) revealed a homozygous variant, which constitutes a premature termination codon in *KY* exon 6 (Chr3:g.134,343,973G>T [hg19]; c.405C>A [NM_178554]; p.Y135*) and possibly leads to nonsense mediated mRNA-decay. This variant is absent in ExAC and 1000 Genome project databases. Homozygosity of the mutation, confirmed by Sanger sequencing, co-segregated with the disease phenotype. Along the AMCG criteria [8], evidence of pathogenicity for the c.405C>A variant can be classified as "strong". Other potentially disease-causing variants in the autozygous regions are discussed in Supplementary Table 2.

To verify the genetic defect at the protein level, we performed western blots but were unsuccessful using three different antibodies (Atlas-antibodies: HPA036492/HPA036668; Antibodies-online: ABIN654368). Even in control muscle we did not detect any bands of the correct size. Others have reported this problem as well, which could be attributed to impeded extractability, protein aggregation or failure of the protein to run on polyacrylamide gels [2]. However, immunostaining with an antibody directed against the N-terminus of KY (clone AA104-133, ABIN654368) verified the absence of the KY-protein in the patient. In normal muscle, strongest anti-KY staining was found at the sarcolemma (Supplementary Fig. 4), while sarcoplasmatic staining seemed to be fiber-type dependent and increased in type II fibers (Supplementary Fig. 3).

While most of the histological features of patient IV:07 corresponded well to those seen in the *ky*-mouse, core targetoid defects have not been reported in the mouse [4]. The clinical phenotype of our patients was not so much dominated by kyphoscoliosis, as by kyphosis, rigid spine, contractures, and patterned progressive muscle atrophy. The characteristic tongue atrophy of both patients was also described in the *Ky*-mouse [4].

The finding of core targetoid defects points towards a neurogenic process. In favor of this hypothesis: the reports on the *Ky*-mouse mention prolific nerve terminal axonal sprouting and reshaping of the neuromotor endplates in the absence of hallmarks for motoneuron decay such as grouped fiber atrophy [4]. Given the small muscle specimen we were unable to search for axon sprouting in our patient. However, we hypothesize that the fiber-type II uniformity or large fiber-type grouping depicted in Supplementary Fig. 2 might be the result of a slow and chronic reinnervation process of an entire muscle fascicle [12]. As in the mouse, we did neither find grouped fiber atrophy nor F-wave or nerve conduction abnormalities as signs for neuropathy. Axonal sprouting might thus have occurred independently of α-motoneuron decay. In this case, the KY-protein could function as a suppressor of uncontrolled axon sprouting on the level of the muscle.

On the other hand we found obvious myopathic signs such as split fibers, internalized nuclei, myophagocytosis, necrotic and regenerating fibers. Such combinations of myopathic and neurogenic abnormalities occur in myofibrillar myopathies [10], and electron microscopy of our patient showed clear myofibrillar derangement and Z-disc streaming. Mutations in the *filamin-C* (*FLNC*) gene are known to cause myofibrillar myopathy. Since the KY-protein and filamin-C interact and filamin-mislocalization was demonstrated both in *Ky*-muscle [1, 2] and in our patient (Fig. 1k), the pathophysiology of KY-mutations

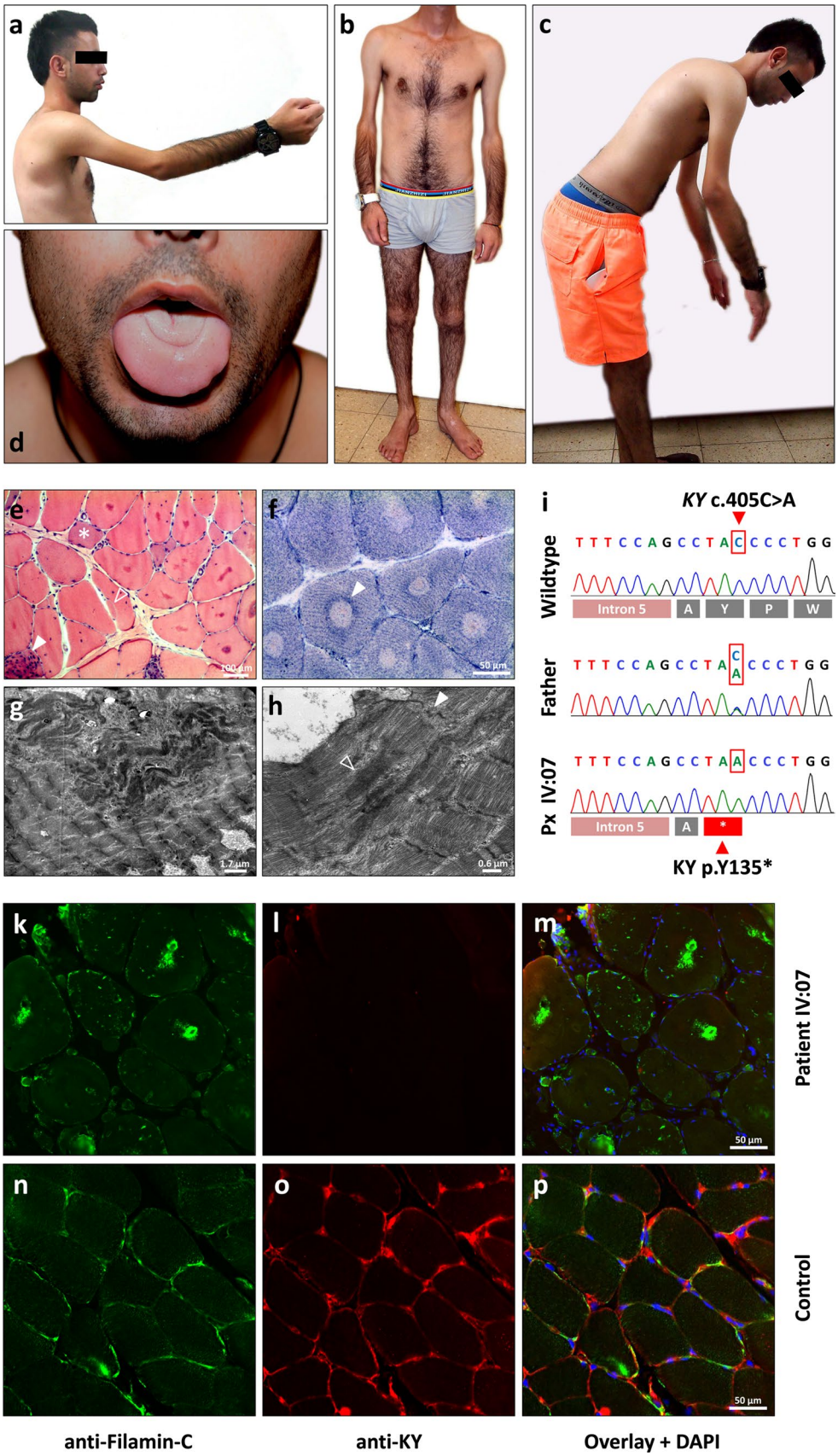

Fig. 1 **a** Elbow contracture, **b** predominant muscle atrophy of the biceps, triceps and lower limb muscles, as well as (**c**) rigid spine in patient IV:07, **d** Tongue atrophy in patient IV:05. **e**–**h** Muscle histology of patient IV:07: **e** H&E-staining with core targetoid defects, internalized nuclei, rounded and regenerating fibers (*asterisk*), fiber splitting (*open arrowhead*), and myophagocytosis (*closed arrowhead*). **f** NADH-staining with areas of sarcoplasmatic disorganization with central depletion and peripheral walling of mitochondria (*closed arrowhead*) that impose as core targetoid defects on electron microscopy (**g**). **h** Higher magnification shows Z-disc streaming (*closed arrowhead*) and myofibrillar disarray/aggregation (*open arrowhead*). **i** Sanger electropherograms of wildtype, heterozygous parent, and homozygous patient. **k**–**p** Co-immunostaining with anti-filamin-C (1:100, clone RR90 [11]) and N-terminal anti-KY (1:10, ABIN654368) antibodies shows absence of the KY-protein in the patient (**l**) and accumulation of filamin-C in the core targetoid defects (**k**) while being present in a predominant sarcolemmal location in the healthy controls (**n**). All images were photographed with identical camera settings

might thus, at least partially, be mediated via its interaction with filamin-C. However, protein aggregates as a hallmark for myofibrillar myopathies [9] were not evident in our patient, albeit rare cases with *FLNC*-associated distal myopathies without protein aggregates have been reported [5, 6]. We suggest placing the *KY*-related myopathy close to the myofibrillar myopathies, thereby expanding their phenotypical and molecular genetics spectrum. While the characteristic myofibrillar aggregation and specific clinical phenotype is well known in adults, we provide evidence for a potentially similar pathology in children via the interaction between filamin-C and KY-protein.

However, to grasp the full clinical and morphological spectrum of the disease and to confirm mutations in *KY* as its genetic cause, more patients have to be found. Individuals with slowly progressive congenital myopathy, rigid spine, and core targetoid defects in muscle would be good candidates for mutation screening in *KY*.

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