

Tubular Aggregate Myopathy : A Case Report

We report a first Korean case of presumably dominantly inherited primary tubular aggregate myopathy in a 19-yr-old man, who presented with slowly progressive proximal muscle stiffness and weakness. In hematoxylin and eosin stain, it showed subsarcolemmal, or central pale basophilic granular vacuoles, which stained red with modified Gomori's trichrome and intensive blue with nicotinamide adenonine dinucleotide-tetrazolium reductase, respectively. Ultrastructurally, aggregates of 60 nm-sized hexagonal tubules were found in both type 1 and type 2 fibers. We briefly review the pathologic findings of the previously reported cases of tubular aggregate myopathy and discuss the possible pathogenesis of this disease.

Key Words : *Tubular Aggregates; Muscular Diseases; Sarcoplasmic Reticulum; Genes, Dominant*

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INTRODUCTION

Tubular aggregate myopathy is a rare inherited primary myopathy with an autosomal dominant or recessive pattern (1). It usually presents with slowly progressive proximal muscular weakness or stiffness. Diagnostic hallmark is ultrastructural presence of tubular aggregates. To date, 24 Caucasian patients from 7 families have been reported (1-8). Here, we describe the first Korean case of presumably dominantly inherited primary tubular aggregate myopathy occurring in a 19-yr-old man.

CASE REPORT

A 19-yr-old Korean man presented with progressive weakness and muscle stiffness without atrophy since the age of 13 yr. He was born as a full-term baby with 2.2 kg of birth weight. No perinatal events or developmental delay was documented. He had no history of alcohol intake or medication. The stiffness began in the lower extremities, spreading gradually to the upper parts. It was symmetrical or asymmetrical and the proximal musculature was more affected than the distal. Gower sign was positive, but pseudohypertrophy of the calf muscle was absent. Exertional myalgia, cramps or myotonia were not found. Sensation, coordination and deep tendon reflexes were unaffected. Ranges of movement were normal. No

triggering factors were found prior to the onset of the symptom. His condition continued to deteriorate slowly for 6 yr. Electromyogram of the biceps brachii showed brief small action potentials, indicating a slightly myopathic pattern. Nerve conduction velocity and Jolly test revealed no abnormal findings. Laboratory findings including serum creatine kinase, aldolase, lactate, lactate dehydrogenase, electrolytes and thyroid function test were normal. Forearm ischemic exercise test showed a normal rise of the lactate level. His mother has a history of periodic stiffness once or twice a year for the past several years. However, she declined muscle biopsy and further studies. His father and younger brother had no complaints of neuromuscular symptoms at the time of biopsy from the patient. The initial clinical impression was limb-girdle muscular dystrophy. Muscle biopsy was obtained from the vastus medialis. Muscle samples were treated as follows: (i) snap-frozen in isopentane pre-cooled in liquid nitrogen, and stored at -80°C until use; (ii) placed in formaldehyde fixative and paraffin embedded for routine histology; (iii) for conventional transmission electron microscopy (TEM), specimens were fixed in 2.5% glutaraldehyde in 0.1 M phosphate-buffered saline, post-fixed with 1% osmic acid and embedded in Epikote. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined with a TEM (Hitachi model 7100) at an accelerating voltage of 75 kv. Six- μ m serial cryostat sections of submitted biopsied muscle were processed for a routine battery of usual histologic stains and enzyme

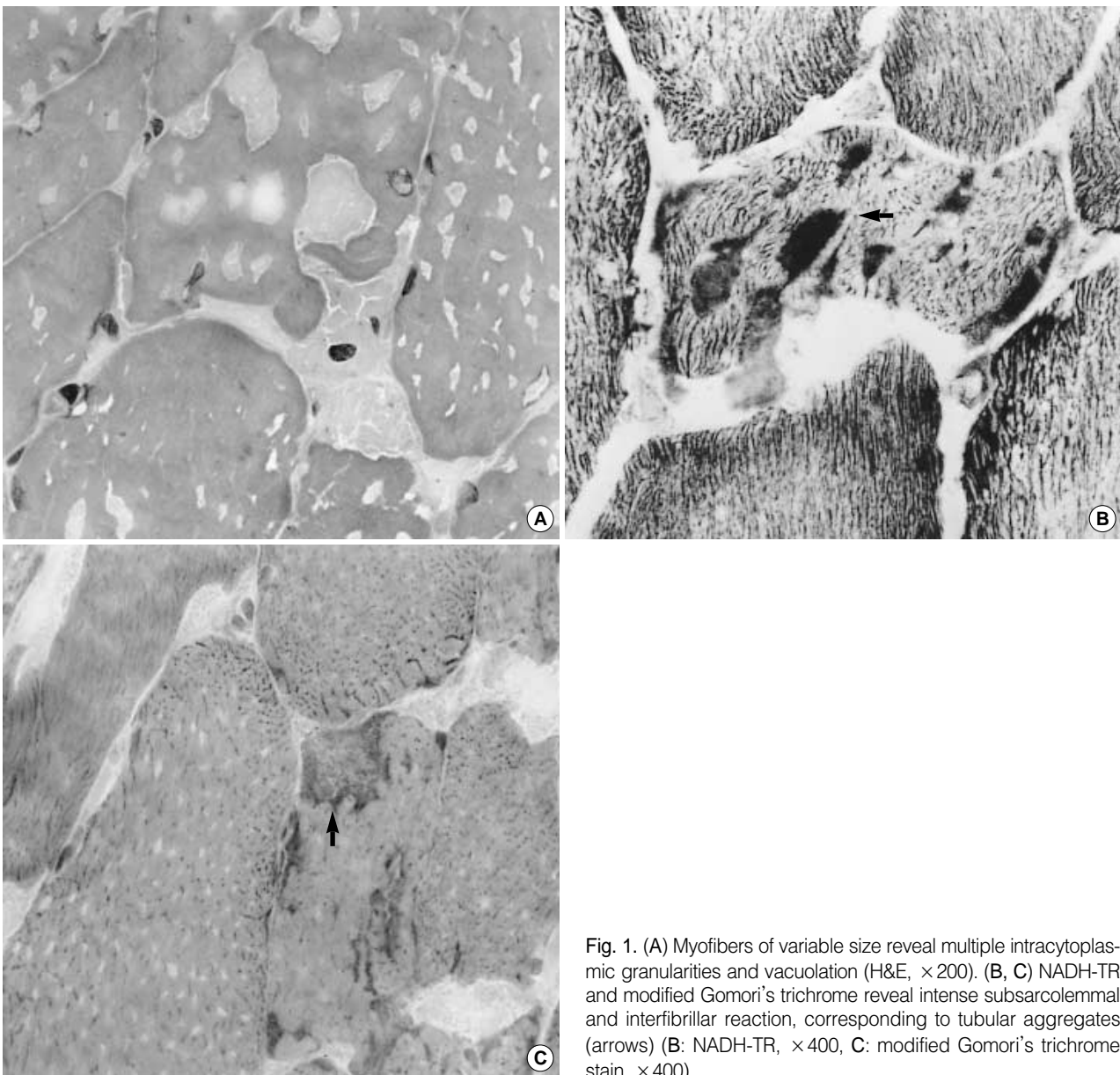


Fig. 1. (A) Myofibers of variable size reveal multiple intracytoplasmic granularities and vacuolation (H&E, $\times 200$). (B, C) NADH-TR and modified Gomori's trichrome reveal intense subsarcolemmal and interfibrillar reaction, corresponding to tubular aggregates (arrows) (B: NADH-TR, $\times 400$, C: modified Gomori's trichrome stain, $\times 400$).

histochemistry. Immunohistochemistry was performed on the paraffin-embedded sections ($5\ \mu\text{m}$ -thick) using the monoclonal antibody against dystrophin (MS/486/P1, Neomarkers, Fremont, Calif, U.S.A., 1:20 dilution). Peripheral blood sampling was done for polymerase chain reaction (PCR) screening of the dystrophin gene at Xp21 (exon 3, 6, 13, 43, 47, 49, 50, 52, and 60). PCR for the dystrophin gene at Xp21 showed amplification of exon 3, 6, 13, 43, 47, 49, 50, 52, and 60 of the dystrophin gene without abnormalities.

PATHOLOGIC FINDINGS

On frozen cryostat sections stained by hematoxylin and

eosin, pale granular sarcoplasmic changes were observed in approximately 30% of myofibers. The pale zones were round or moth-eaten, and varied in size, measuring up to $90\ \mu\text{m}$ in diameter (Fig. 1A). They were single or multiple, and situated in the center and periphery of the myofibers. They showed an intense staining with nicotinamide adenine dinucleotide-tetrazolium reductase [NADH-TR] (Fig. 1B) and periodic acidic Schiff [PAS], stained bright red with Gomori's trichrome (Fig. 1C), but not stained with succinyl dehydrogenase [SDH] and myofibrillar adenosine triphosphatase [ATPase] at pH 9.4, 4.6, and 4.3. They occurred in both type 1 and 2 fibers. In paraffin sections the pale zones appeared to harbor irregular spaces or vacuoles with or without granular materials. There were nonspecific changes, including variation of

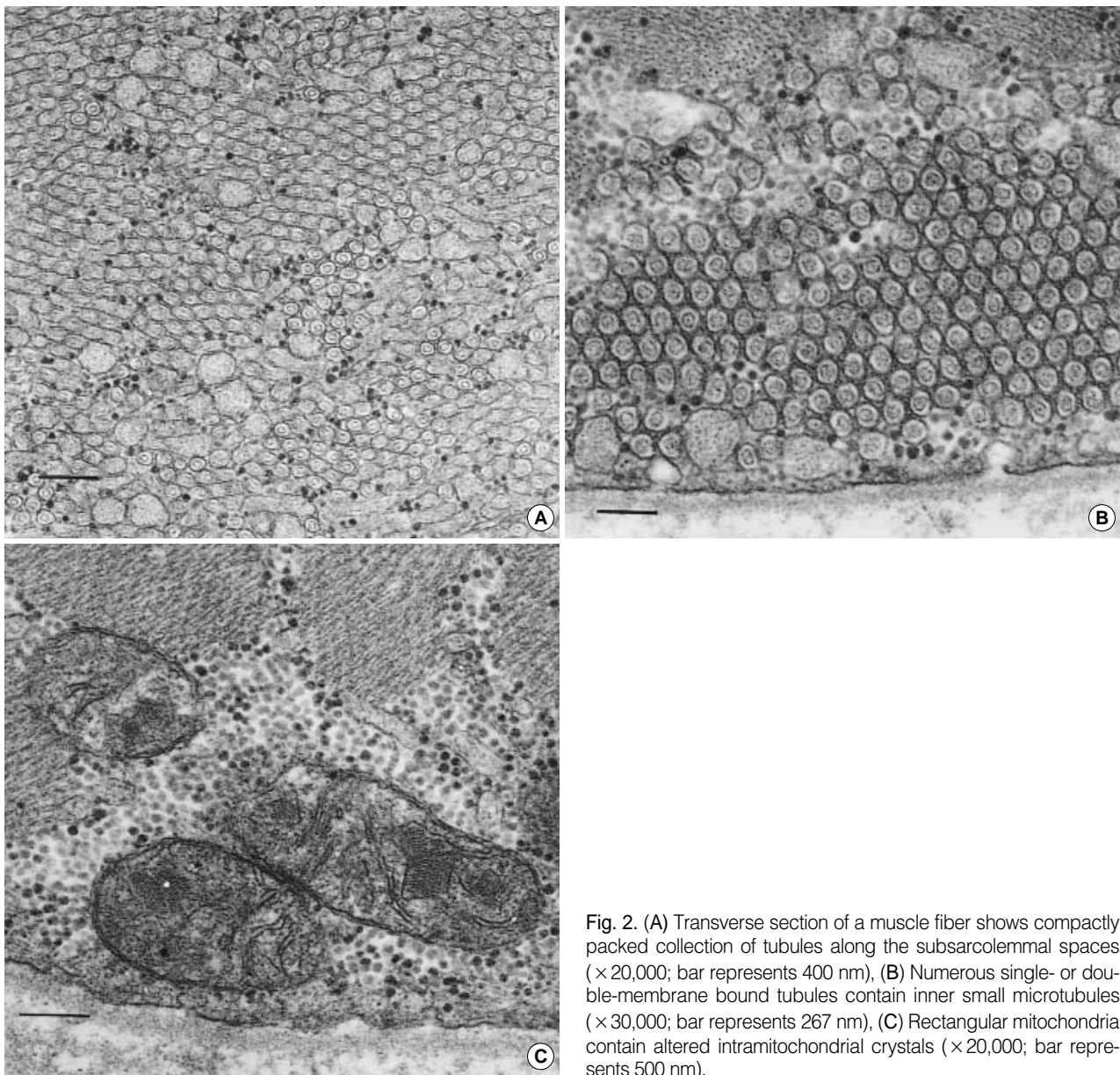


Fig. 2. (A) Transverse section of a muscle fiber shows compactly packed collection of tubules along the subsarcolemmal spaces ($\times 20,000$; bar represents 400 nm), (B) Numerous single- or double-membrane bound tubules contain inner small microtubules ($\times 30,000$; bar represents 267 nm), (C) Rectangular mitochondria contain altered intramitochondrial crystals ($\times 20,000$; bar represents 500 nm).

myofiber size ranging from 15 to 90 μm , atrophic fibers, hypertrophic and split fibers, frequent internal nuclei, and a few inflammatory cells. Neither necrotic nor regenerating changes were found. Type 1 fiber predominance and type 2 fiber atrophy were also observed in ATPase reactions. Immunohistochemistry for dystrophin revealed a normal linear staining along the sarcoplasmic membrane. Ultrathin sections of skeletal muscle fibers showed focal disorganization of myofilaments with a disarray of Z-bands and papillary appearance of sarcolemmal membrane. Tubular aggregates were found in subsarcolemmal and intermyofibrillar areas of almost all myofibers (Fig. 2A). On longitudinal section, they consisted of collections of long, straight tubules, 60 nm in diameter. On transverse section, the tubules were in hexagonal array and contain-

ed a smaller 25 nm-sized central tubule-like structure, i.e., tubule in tubule (Fig. 2B). The central tubule was separated by a clear space from the outer wall of the tubules. There were many dysmorphic mitochondria such as megamitochondria and intramitochondrial crystal inclusions (Fig. 2C). The histologic and electron microscopic findings of the muscle were consistent with primary tubular aggregate myopathy.

DISCUSSION

Tubular aggregates have been observed in many diseased skeletal muscle fibers though minor in proportion. The diseases include periodic paralysis (9), hyperaldosteronism (10),

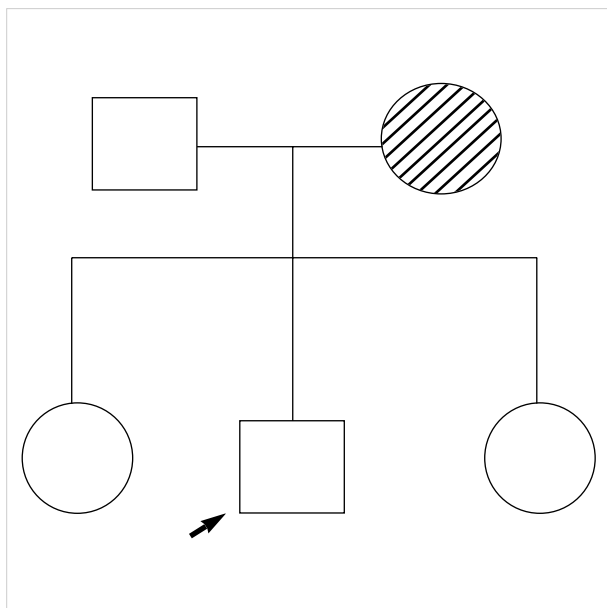


Fig. 3. Pedigree of the family. A shaded circle indicates a suspicious patient and an arrow indicates the present patient.

facioscapulohumeral dystrophy with aminoaciduria (11), myasthenia gravis (12), exercise-induced cramps (13), alcohol, caffeine, or drugs such as zidovudine (14, 15). However, patients with these tubular aggregates may present as a specific type of primary myopathy (1-8). Primary tubular aggregate myopathy of familial occurrence was first reported by De Groot and Arts (1) in 1982. To date, 24 patients from 7 families have been reported (1-8). Clinically, the age of onset ranged from 6 to 45 yr (mean: 25.4 yr). All were Caucasians. Progressive proximal weakness (40%) and periodic paralysis or exertional muscle cramps (40%) were the most common presenting symptom. Entirely asymptomatic family members having primary tubular aggregate myopathy accounted for 8% (6). Although facial muscular weakness (4%) and limb girdle contracture (4%) have been rarely described (1, 3), facial sparing is regarded as an important clue to distinguishing primary tubular aggregate myopathy from facioscapulohumeral dystrophy. Muscle pain was accompanied in 24% of inherited tubular aggregate myopathy. Autosomal dominant inheritance is the most common (2-6, 8), but less commonly autosomal recessive inheritance or rarely sporadic occurrences have been reported (1, 7, 16). Although the histologic confirmative diagnosis of his mother could not be made, the present case suggested mother-to-son transmission (Fig. 3), implying a presumably autosomal dominant inheritance or remote possibility of autosomal recessive inheritance. Molecular genetic studies have not yet been performed.

Light microscopic examination or clinical history alone cannot exclude the presence of the affected family members because ultrastructural study can show tubular aggregates in patients with normal histology and enzyme histochemistry of

the muscle. Therefore, diagnostic hallmark of this disease is the ultrastructural demonstration of tubular aggregates of variable morphology. The tubular aggregates usually consist of closely packed double-membrane tubules often containing a single inner tubular structure or amorphous material (8). Tubular aggregates have been divided into three types (17); type I with intraluminal coaxial inner tubules (70 nm); type II with a granular core (70-400 nm), and type III tubules with subsarcolemmal filaments of approximately 10 nm (130-400 nm). In dominantly inherited tubular aggregate myopathy, all three types of tubules have been observed. Recently, Muller and his colleagues (8) described additional findings of tubulofilamentous structures, proliferated terminal cisternae or tubulovesicular structures, in cases of familial tubular aggregates. Interestingly, tubulofilamentous structures were found in the histologically normal looking myofibers of asymptomatic family members. The present case showed many dysmorphic mitochondria such as megamitochondria and intramitochondrial crystal inclusions, which have been described in other reports (10.5%) (8). Other minor ultrastructural changes described in the reported cases included lipid vacuoles (26.3%), proliferated terminal cisternae (15.8%), increased size and number of mitochondria (10.5%), filamentous bodies (5.3%), lipofuscin pigments (5.3%), dense bodies (5.3%), and large lysosomes (5.3%). It is noteworthy that these minor ultrastructural changes can be found even in asymptomatic family members, whereas tubular aggregates are exclusively observed in the symptomatic patients.

Light microscopically, vacuolated granular to flocculent cytoplasm is the most salient finding of primary tubular aggregate myopathy, and is observed in 64% of previously reported cases including the present patient. Vacuolated pale unstained areas were intensely stained with NADH-TR and modified Gomori's trichrome, but not stained with mitochondrial enzymes such as SDH, phosphorylase or cytochrome *c* oxidase (2, 6, 8, 18). Furthermore, the lanthanum staining of T-system also failed to show stainability (3, 15). Pathologic findings of 19 cases of tubular aggregate myopathy previously reported are summarized in Table 1. Increased internal nuclei and split fibers were commonly found in 73.7% and 52.6% of 19 cases of tubular aggregate myopathy. Inflammatory cell infiltration, fibrosis, and necrotic/regenerative fibers were rarely observed (3, 5, 6). Type I fiber predominance was found in 52.6% of tubular aggregate myopathy. Both type I and II fibers were commonly involved by tubular aggregates (52.6%).

As the origin of the tubules, modified degenerated mitochondria were initially suggested (19, 20). Altered sarcoplasmic reticulum membrane is now widely accepted as the origin of tubular aggregates. Sarcoplasmic reticulum is related to calcium uptake channel during muscle contraction and relaxation. Tubular aggregates are the sites of calcium accumulation for ATP-dependent calcium uptake and increasing the calcium and oxalate loading capacity of the affected fibers. This finding was demonstrated by anti-calcium pump protein IgG

Table 1. Summary of pathologic findings of 19 cases of dominantly inherited tubular aggregate myopathy on which biopsies findings were described

Case No.	Histology					Comments	Enzyme histochemistry	Electron microscopy	
	Central nuclei	Split fibers	Vacuolated cytoplasm	Granular cytoplasm	Involved muscle fiber type		Fiber type predominance	Type of TA*	Other findings
1	-	-	-	-	Type II	Nearly normal sized myofibers	NR	I	
2	-	-	-	-	Type II	A few scattered atrophic fibers	NR	I	Lipofuscin pigment, glycogen pool, lipid vacuoles
3	Increased	Occasional	+	+	Type I, II	Marked size variation of myofibers+	Type I	I	Vacuoles, dense bodies, increased mitochondria and distorted cristae
4	Increased	Occasional	+	+	Type I, II	Marked size variation of myofibers+	Type I	I	
5	Increased	Occasional	+	+	Type I, II	Marked size variation of myofibers +++	Type II	I	
6	3+	1+	-	+	Type I, II	Fibrosis 1+	Type I	I	
7	3+	1+	-	+	Type I, II		Type I	I	
8	3+	3+	-	+	Type I, II	Fibrosis 1+	Type II	I	
9	1+	0	-	+	Type I, II		Type I	I	
10	38%	Some	-	+	Type I, II	Inflammatory cells	Type I	II	Small tubular arrays, proliferation of sarcoplasmic reticulum
11	38%	Some	-	+	Type I, II		Type I	I, II	
12	20%	-	-	+(30%)	Type II	Normal fiber size	Type II (99%) type I (1%)	NR [†]	
13	1+	-	-	+	Type II	Necrotic fibers	NR	I, II	Lipid vacuoles, increased size and number of mitochondria
14	1+	-	-	+	Type II	Necrotic fibers, angulated fibers, many atrophic fibers		I, III>II	Lipid vacuoles, increased size and number of mitochondria
15	2+	-	-	+	Type II			I, II	Filamentous bodies, honeycombing of T-system, lipid vacuoles
16	-	-	-	+	-		Type I	I, II	Tubulofilamentous structures (1-21), proliferated terminal cisternae, tubules with granular filaments, annulate lamellae, rectangular mitochondrial paracrystalline inclusions
17	-	Some	-	+	Type II		Type I	I, II	Tubulofilamentous structures (1-21),
18	-	Some	-	-	Type II		-	I, II	Tubulofilamentous structures (1-21), proliferated terminal cisternae, tubulovesicular structures
19 [‡]	30%	-	+	+	Type I, II		Type I	I	Mitochondrial paracrystalline inclusions, large lysosomes, lipid vacuoles

NR, not recorded; TA, tubular aggregates; *, type according to Cameron et al. (5); †, Electron microscopic examination was done, but the type of tubules was not recorded or illustrated; ‡, The present case.

indirect immunofluorescence technique as well as calsequestrin antibody (4). Tubular aggregates may represent an adaptive mechanism aiming at regulating an increased intracellular

level of calcium in order to prevent the muscle fibers from hypercontraction and fiber necrosis (4). The fact that cases sparing type I fibers are not rare (42.1%) might be explained

by relative abundance of sarcoplasmic reticulum in type II, compared to type I fibers (21). Bendahan et al. (7) demonstrated by magnetic resonance spectroscopy the association between tubular aggregates and hyperacidosis, indicating altered proton handling as a mechanism of pathogenesis. Martin et al. (22) also suggested that the heat shock protein contributes to the formation of tubular aggregates. Although tubular aggregates can be found as a nonspecific change representing an adaptive response, it remains unclear whether the same mechanism is ascribed to the primary inherited tubular aggregate myopathy.

In summary, the diagnosis of this unique tubular aggregate myopathy can be made on the basis of clinical and pathologic examination, using skeletal muscle enzyme histochemistry and electron microscopy including asymptomatic family members. The present case is the first Korean case of presumably dominantly inherited tubular aggregate myopathy.

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