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A Novel Form of Familial Congenital Muscular Dystrophy in Two Adolescents

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Abstract

We report on two brothers (the product of first-degree consanguineous marriage; aged 15 and 12 years) who presented with severe hypotonia at birth, proximal muscle weakness associated with delayed motor milestones but normal cognitive function. Investigations (at 4 years of age) revealed mildly elevated serum creatine kinase (CK) levels (300 and 824 IU/l; $N \leq 210$). Muscle biopsies showed minimal change myopathy, no neurogenic atrophy but remarkable type-1 fibre predominance (up to 85.5%) without fibre-type disproportion.

Clinical examination at 12 and 9 years, respectively, showed mild facial weakness and high-arched palate in both patients. The younger sibling also had ptosis but otherwise normal external ocular muscles. They showed symmetric proximal muscle weakness and wasting associated with calf-muscle hypertrophy. They could walk independently. A repeat muscle biopsy showed advanced dystrophic changes

in the younger patient at the age of 10 years. Virtually all the remaining fibres were type 1.

Immunohistochemistry revealed normal expression of the dystrophin-glycoprotein complex (DGC), including dystrophin, β -dystroglycan, α -(adhalin), β -, γ -, and δ -sarcoglycan, laminin- α 2 chain (merosin) and syntrophin. Mild dystrophic features and type-1 fibre predominance (92.5%) were seen in the biopsy of the older patient, whereas immunohistochemistry showed normal expression of the DGC. Both cases also showed clear expression of integrin α 7 at the muscle fibre surface and in the blood vessels. Three years later, they could still walk, but with difficulty, and the older brother showed enlargement of the tongue and echocardiographic features of left ventricular dilated cardiomyopathy.

Key words: Congenital muscular dystrophy – Adolescent – Laminin – Cardiomyopathy

Introduction

Congenital muscular dystrophy (CMD) encompasses a group of autosomal recessive neuromuscular disorders, characterized by hypotonia, muscle weakness presenting at birth (or within the first 6 months of life) and dystrophic features on muscle histology (6, 27). Several sub-groups of CMD have been defined on the basis of the variable involvement of the brain and eyes (5, 7). In the “classical” type, the eyes are spared, whereas brain imaging abnormalities are either absent or only confined to white matter changes on computerized tomography (CT) or magnetic resonance imaging (MRI). The brain involvement in the other 3 types, namely, *Fukuyama* CMD, *Walker-Warburg* syndrome and *Santavuori’s* Muscle-Eye Brain (MEB) disease, is characterized by consistent structural changes at autopsy or on imaging. Recently (8), the term “cobblestone lissencephaly” has been used to encompass these changes. A fourth variant of this cobblestone lissencephaly syndrome without ocular malformations, which manifests with arthrogryposis multiplex and ends fatally in the first few months of life, has been described (23).

Advances in molecular genetics and immunohistochemistry identified two groups of the “classical” type of CMD which can be distinguished according to the status of the α 2-chain of laminin-2 (formerly merosin) with about half of the cases displaying a

deficiency of this protein (18, 24, 26). Patients with a deficiency of the laminin α 2-chain have a more severe clinical phenotype and white matter changes as compared to those without such a deficiency (18). Children with partial laminin α 2-chain deficiency appear mildly affected compared to others who completely lack the protein (1). Most recently, deficiency of integrin α 7 was found to account for a subgroup of patients who present with congenital myopathy and normal expression of laminin-2 (11, 15).

We report on two brothers (the result of a first degree consanguineous marriage) who presented with hypotonia at birth, proximal muscle weakness and delayed motor milestones. Muscle biopsies showed type 1 fibre predominance at age 4, and in repeat biopsies at 12 and 9 years, dystrophic changes were evident, being more advanced in the younger sibling. At 15 years, the older patient showed echocardiographic features of left ventricular dilated cardiomyopathy. The possibility that the disease in these brothers represents a novel form of CMD is discussed.

Patients and methods

Open muscle biopsy samples were from the vastus lateralis. The second biopsy of Case 1 and the two biopsies of Case 2 were frozen in isopentane, cooled in liquid nitrogen and stored at -80°C . Conventional histological and histochemical techniques were performed on 10- μm thick transverse frozen cryostat section (3). Morphometric analysis of muscle biopsies were done using a semi-automated computer-assisted method (14). Indirect immu-

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no fluorescence analysis of dystrophin and dystrophin-glycoprotein complex (DGC) was performed on 7–10 μm -thick transverse cryosections as previously described (16). All incubations were performed at room temperature. Tissue sections were blocked, using an avidin/biotin-blocking kit (Vector Laboratories, Inc.) followed by incubation with a solution of 3% BSA in PBS for 30 minutes. Primary antibodies were incubated for 90 minutes followed by washing for 3×5 minutes with PBS. Sections were incubated with a biotinylated secondary antibody at a dilution of 1:1000 for 30 minutes. All dilutions were made in a solution of 1% BSA in PBS. After washing, the sections were mounted, using Vectashield mounting medium (Vector Laboratories, Inc.). Sections stained with antibodies to integrin $\alpha 7\text{B}$ were digitized using a Bio Rad 1024 Confocal microscope and all other sections were viewed under a Leitz Diaplan fluorescence microscope with an attached Optronics DEI-750 camera used to digitize the images (Central Microscopy Research Facility, University of Iowa, Iowa City, IA, USA). Monoclonal antibodies (mAbs) against the c-terminal (VIA42) and the rod domain (XIXC2) of dystrophin, and against α -sarcoglycan (IVD3₁) and the polyclonal antibody against integrin $\alpha 7\text{B}$ were previously described (9, 12, 15). MAb against β -dystroglycan (5B1) and β -sarcoglycan (5B1) and γ -sarcoglycan (21B5) were kindly provided by Dr. Louise Anderson (Muscular Dystrophy Research Labs, Newcastle General Hospital, Newcastle upon Tyne NE1 6BE, UK). An mAb against the intact 300 kDa chain of laminin $\alpha 2$ (41I8-2) was a gift from Dr. Lydia M. Sorokin. A polyclonal antibody against residues 1–882 of the human laminin $\alpha 2$ -chain was kindly provided by Dr. Peter D. Yurchenco. An affinity-purified δ -sarcoglycan antibody (rabbit 215) was produced against the N-terminal sequence (MMPQE-QYTHHRSTMPGAA) of human δ -sarcoglycan. This synthetic peptide (Research Genetics) with an additional cysteine at the N terminus was conjugated with the N-terminal cysteine of keyhole limpet hemocyanin (Pierce) using m-maleimidobenzoic acid – N-hydroxysuccinimide ester (Pierce), mixed with Freund's adjuvant (Sigma) and injected into rabbit 215. Polyclonal antibody against the peptide was affinity-purified from crude sera using BSA-conjugated peptide as described previously (13). Monoclonal antibodies against the laminin- $\alpha 2$ chain (clone 5H2) were purchased from Gibco-BRI, and dystrophin amino terminal domain antibodies (NCI-DYS3) were purchased from Novocastra Laboratories Ltd.

Case reports

Case 1

The 15-year-old boy was first admitted to King Khalid University Hospital (KKUH) when aged 4 years with a history of floppiness since birth and delayed motor development. He was the product of an uneventful pregnancy and delivery. Contrary to normal social and speech development, he was noticed by the parents to have delayed motor milestones. He sat at 12 months and walked at 26 months. He could neither run or climb stairs, was easily fatigued and had difficulty in getting up from a lying or sitting position. He also had difficulty in managing solid food. His parents were first-degree Sudanese cousins and he had a younger brother who was similarly affected (Case 2). There were no more siblings since the family decided against further pregnancies and there was no history of miscarriages. There were also no other affected relatives. On examination, hearing, speech and intelligence were normal but he had marked hypotonia of the upper and lower limbs. He could walk independently with a waddling gait and could stand up from a sitting position, using Gowers' maneuver. Reflexes were diminished in upper and lower limbs and plantar reflexes were downgoing.

Routine investigations and electrocardiogram (ECG) were normal. Electromyography (EMG) revealed myopathic changes. Serum creatine kinase (CK) was marginally raised at 300 IU/l ($N \leq 210$). Paraffin sections of a muscle biopsy (stained with H&E, PAS, PASD and HVG) showed an increased number of internal nuclei and mild perimysial fat replacement. Endomysial connective tissue seemed to be normal. There was no variation in fibre size, degenerative changes or evidence of grouping. Instead, PAS stain suggested marked type-1 fibre predominance.

He was enrolled in a physical rehabilitation program, could maintain independent walking and go upstairs but with difficulty. He joined a regular school with good performance. Mild facial weakness, high-arched palate and calf hypertrophy were recorded during one of his visits to the paediatric neurology clinic at the age of 12 years. A second muscle biopsy showed muscle fibres that were speckled with internal nuclei (Fig. 1a). There was variation in fibre sizes with scattered atrophic and hypertrophic fibres. Few split, whorled and moth-eaten fibres were noted, whereas degeneration and regeneration was not prominent. There was no fibre

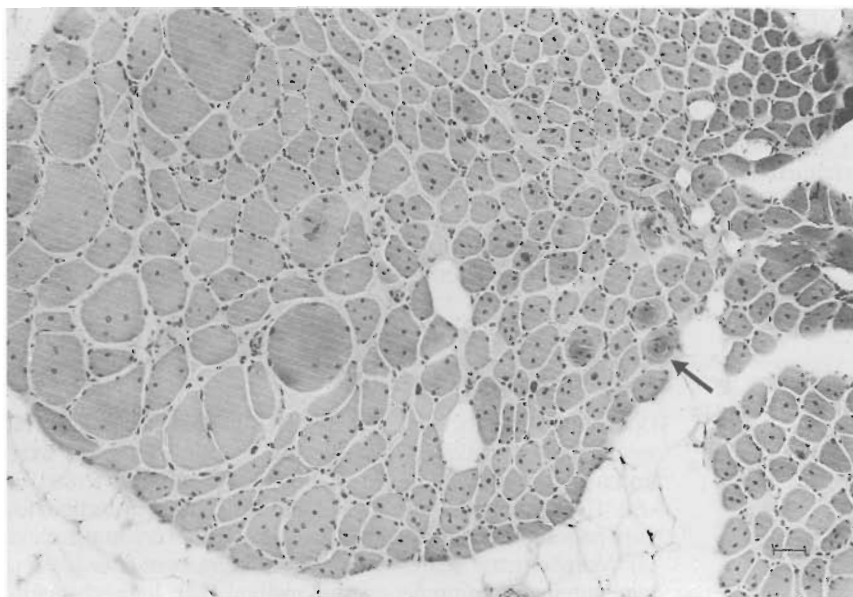


Fig. 1a Muscle histology of Case 1 showing variation in fibre sizes, mild increase in endomysial connective tissue, occasional necrotic and whorled fibres (arrow), fibre splitting and scattered atrophic and hypertrophic fibres. Most muscle fibres were speckled with internal nuclei (hematoxylin and eosin [H & E]; Bar = 50 μm).

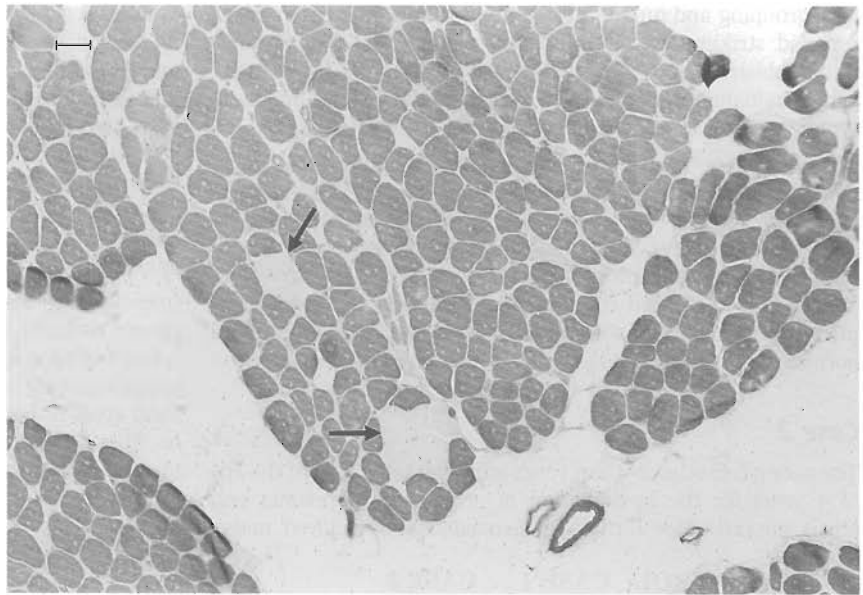


Fig. 1b

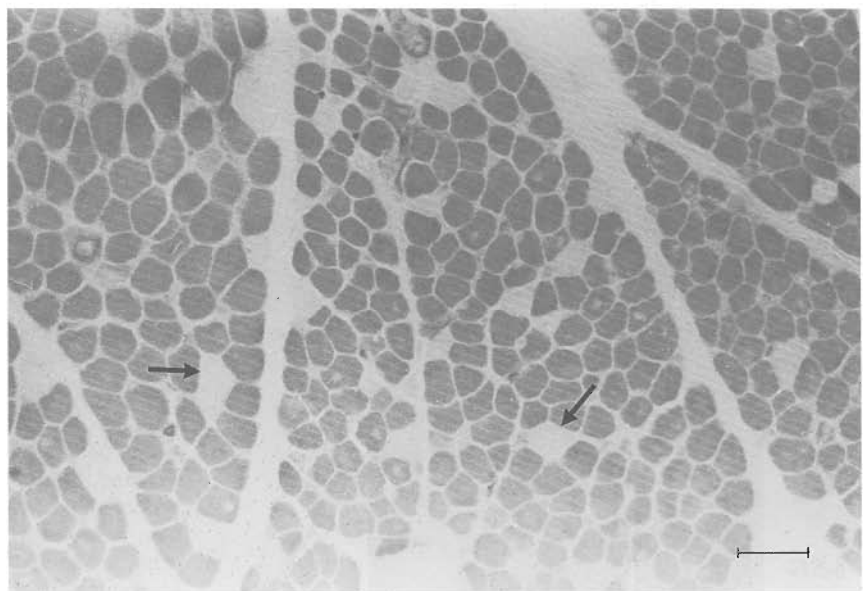


Fig. 1c

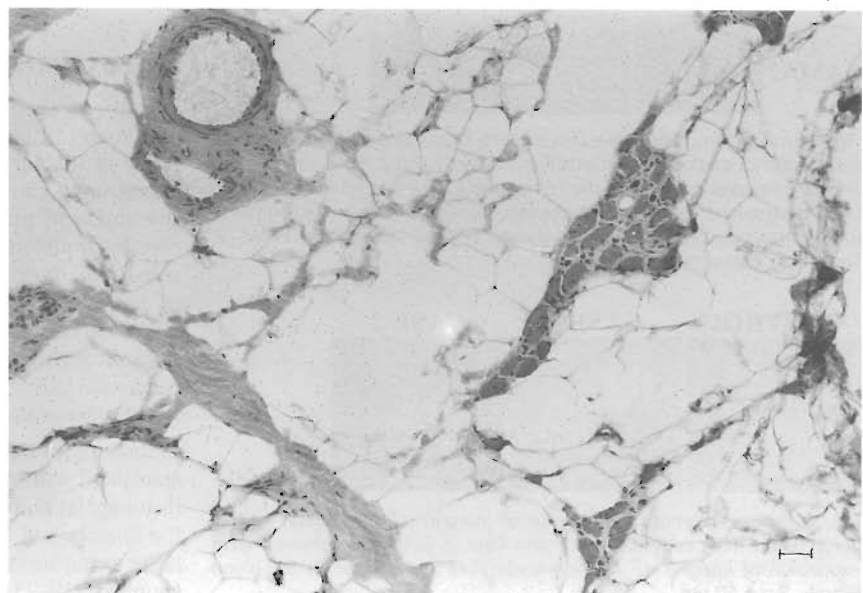


Fig. 1d

Fig. 1b to d Myofibrillar ATPase (4.3) preparation of Case 1 (Bar = 50 μ m) and Case 2 (C; Bar = 200 μ m). Type 1 (dark) fibres predominated the field and scanty type 2 (pale) fibres are shown (arrows). **d)** Muscle histology of Case 2 at the age of 10 years revealed advanced dystrophic changes with marked disruption of muscle architecture by fatty replacement and fibrosis (H & E; original magnification \times 100).

type grouping and quantitative computer-assisted measurements revealed striking type-1 fibre predominance (92.5%, Fig. 1b). Immunohistochemistry showed normal dystrophin, β -dystroglycan, α (adhalin), β -, γ -, and δ -sarcoglycan, the laminin- α 2 chain, syntrophin and integrin α 7 (Figs. 2 and 3).

During a follow-up visit 3 years later, he could still walk but could no longer rise from a sitting position. The tongue was noted to be enlarged. A recent ECG showed left-axis (-60) deviation and an echocardiogram revealed a mildly dilated left ventricle (LV) with severe global dysfunction. An estimated LVEF of 25% was consistent with dilated cardiomyopathy. The right ventricle dimensions were normal with preserved contractility. MRI showed normal white matter on T₂-weighted images (data not shown).

Case 2

The younger brother of Case 1 was admitted to KKUII at the age of 4 years for the investigation of generalized hypotonia and ptosis noticed since birth and associated with delayed motor

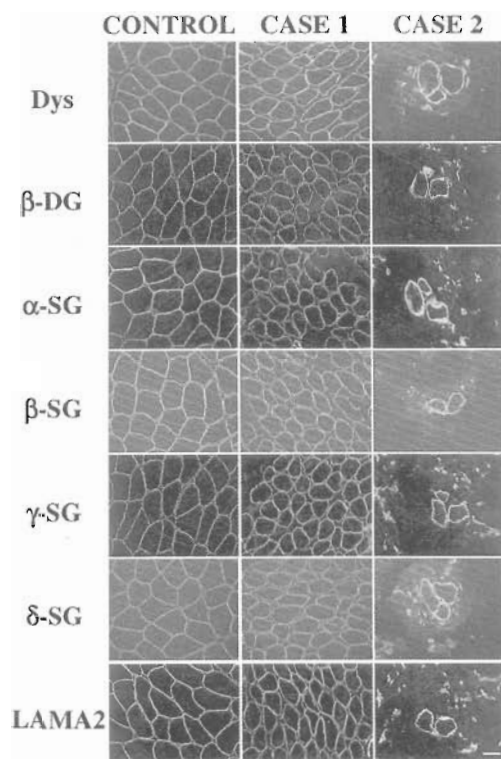


Fig. 2 Indirect immunofluorescence of the DGC components on skeletal-muscle frozen sections from control and Cases 1 and 2. Both patients had normal immunostaining of all the components as compared to the control. DYS: dystrophin; β -DG: β -dystroglycan; α -SG: adhalin (α -sarcoglycan); β -SG: β -sarcoglycan; γ -SG: γ -sarcoglycan; δ -SG: δ -sarcoglycan; LAMA 2: laminin α 2 chain (merosin).

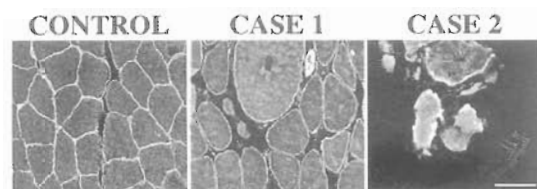


Fig. 3 Indirect immunofluorescence of integrin α 7 on skeletal muscle cryosections from control, Case 1 and Case 2. Both cases showed clear expression of integrin α 7 at the muscle fibre surface and in the blood vessels. Bar = 50 μ m.

development. Pregnancy, delivery and early neonatal life were uneventful. He was able to sit without assistance at 14 months and he could manage to stand with support at 4 years. His speech and social development were normal. Bilateral ptosis (mild on the left), associated with normal eye movements and a myopathic face were noted. Tone was reduced and associated with symmetrical proximal muscle weakness of the upper and lower limbs. He could climb up on himself to stand but could not walk independently. Tendon jerks were hypoactive in the upper and lower limbs and sensation seemed to be intact.

Investigations showed serum CK of 824 IU/l ($N \leq 210$). Muscle biopsy revealed mild variation in fibre size, occasional central nuclei but no evidence of atrophy or degenerative changes. There was no increase in endomysial or perimysial connective tissue but focal areas of increased adipose tissue. ATPase reaction showed no fibre type grouping whereas quantitative computer-assisted measurements revealed that 85.5% of the fibres were type 1 (Fig. 1c).

On follow-up, he showed steady improvement and could walk independently by the end of his fourth year. He also joined a regular school with excellent grades. Clinical assessment (when aged 9 years) noted hypertrophy of the calf muscles. He could rise from a sitting position using *Gowers'* maneuver. Investigations (at 10 years) showed normal brain auditory evoked responses (BAER), visual evoked responses (VER) and electroretinogram (ERG). Nerve conduction studies revealed normal results whereas EMG was myopathic. Level of CK was increased to 1276 IU/l ($N \leq 232$). A repeat muscle biopsy (Fig. 1d) showed advanced dystrophic features with marked disruption of muscle architecture by fatty replacement and fibrosis. The scanty remaining fibres showed variation in size and atrophic changes. Virtually, all of them were of type 1.

Immunohistochemistry showed normal expression of the dystrophin-associated glycoproteins (DAG) including the laminin- α 2 chain (Fig. 2) and also normal expression of integrin α 7 (Fig. 3).

Over the following year, he could no longer stand up from the sitting position. However, he could walk on calipers when helped to stand. Serum CK decreased to 496 IU/l ($N \leq 232$). He still walked at 12 years and could raise his hands to the level of his face. A recently done ECG showed left axis (-65) deviation and echocardiography showed normal cardiac structure and function. His brain MRI revealed no evidence of white-matter attenuation.

Discussion

The two brothers described had a myopathy affecting mainly the girdle and proximal limb muscles, manifesting with hypotonia (early in infancy), delayed motor development and calf muscle hypertrophy. Close consanguinity suggested an autosomal recessive mode of inheritance. Muscle biopsies, at 4 years of age, revealed a picture of minimal change myopathy (6) with remarkable type-1 fibre predominance that evolved into a florid picture of muscular dystrophy (MD) in subsequent years. Following initial clinical improvement within the first decade, the disease attained a slowly progressive course. Whereas one patient had congenital ptosis, both had abnormal ECG and another manifested echocardiographic features of cardiomyopathy.

In these patients, the limb-girdle distribution of muscle weakness associated with calf muscle hypertrophy and dystrophic muscle histology (in biopsies taken after the age of 4 years) might suggest the diagnosis of *Duchenne* MD or limb-girdle MD (LGMD), the latter being more prevalent in Saudi Arabia and North African countries (19–21). However, serum CK was mildly increased (>4

times normal) at the age of 4 years whereas in *Duchenne* MD its level is 100–300 times normal between the ages of 1 and 5 years (10) and 5–116 times in the severe form of LGMD (20). ECG showed left axis deviation in both patients and did not show the right precordial R waves in the lateral and left leads characteristic of *Duchenne* MD, nor did it show the ST segment depression and T-wave changes observed in the severe form of LGMD (20, 21). The older patient had echocardiographic features of left ventricular dilated cardiomyopathy at 15 years. Finally, immunohistochemical analysis revealed normal DGC expression including the sarcoglycans, dystrophin and the laminin $\alpha 2$ chain (25). It is noteworthy that dystrophin deficiency has been reported in a boy presenting with the floppy infant syndrome and a dystrophic pattern in muscle histology (17), and β -dystroglycan deficiency has been found in a Saudi child with early childhood LGMD (22).

On the other hand, the congenital hypotonia, delayed motor development and myopathic facies in the absence of brain malformation or abnormal white matter attenuation suggested the diagnosis of "classical" merosin-positive CMD. However, congenital ptosis in one patient and the ECG and echocardiographic findings are not established associations in this type of CMD. The evolution of the muscle histology from the type-1 fibre predominance, at 4 years of age, to florid dystrophic changes later is also peculiar. It has been postulated that in many chronic myopathies predominance of type-1 fibres might be due to type-2 motor units undergoing conversion to type 1 in response to a change in physiological demands of muscles following severe compromise in their range of activity (2). On the other hand, normal histological picture with routine stains associated with striking type-1 fibre predominance has been reported in a 5-year-old boy with history of hypotonia, delayed milestones (with gradual improvement) and associated facial weakness (4). However, contrary to the situation in the present family, he and his mother showed joint laxity and his mother had mild facial weakness.

The phenotype, histology, histochemistry and molecular pathological features of the disease in these patients may represent a novel form of CMD.

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