

## Fourty-Four Years of Brody Disease: It is Time to Review

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### Abstract

Brody disease is an inherited skeletal muscle disorder clinically characterized by exercise-induced muscle stiffness and delayed relaxation due to a diminished sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase activity. The disease is transmitted as an autosomal recessive trait and results from mutations in the *ATP2A1* gene encoding the sarcoplasmic/endoplasmic reticulum calcium ATPase 1 (SERCA1), a protein that catalyzes the ATP-dependent Ca<sup>2+</sup> uptake from the cytosol to the lumen of sarcoplasmic reticulum. Mutations in the SERCA1-encoding gene are absent in a quote of patients with autosomal recessive pattern and have never been found in patients with an autosomal dominant inheritance suggesting the genetic heterogeneity of the disease. Therefore, the term Brody syndrome has been proposed to designate patients with decreased sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase activity but without *ATP2A1* mutation.

The review aims to summarize the salient clinical, laboratory and biochemical findings in patients with Brody disease and Brody syndrome.

**Keywords:** Brody disease; Brody syndrome; *ATP2A1* gene; SERCA1; Sarcoplasmic reticulum

### Definition and Historical Background

In 1986, Karpati et al. introduced the term Brody disease to indicate an inherited myopathy clinically characterized by increasing impairment of muscle relaxation during exercise due to deficiency of Ca<sup>2+</sup> ATPase in the sarcoplasmic reticulum [1]. The disease was first described in 1969 by Irwin A. Brody who reported a patient suffering from painless contractures since five year of age; muscle contraction was normal but the relaxation phase became increasingly slow during exercise and most muscle groups were involved [2]. Myotonia was ruled out by the absence of muscle action potentials during the slow relaxation [2]. The decreased uptake of calcium by the sarcoplasmic reticulum was identified as a possible cause for the slow muscle relaxation. Based on these features Brody ascribed the disease to a selective defect of “relaxing factor” [2].

In 1996, Odermatt et al. identified mutations in the *ATP2A1* gene which encodes the sarcoplasmic/endoplasmic reticulum Ca<sup>2+</sup> ATPase 1 (SERCA1) in two families with typical symptoms and signs of Brody disease [3].

Six years later, MacLennan introduced the term Brody syndrome to indicate patients with reduced sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (SR Ca<sup>2+</sup>-ATPase) activity but without *ATP2A1* mutation and to distinguish them from patients with decreased activity of SR Ca<sup>2+</sup>-ATPase and mutation in the *ATP2A1* gene [4].

Since the first description, 47 patients belonging to 32 families have been reported in the literature (Table 1) [1-3,5-14]. The disease is considered a rare skeletal muscle disorder with an estimated incidence of 1 in 10000000 new births [15]. Nevertheless the frequency could be underestimated due to its incomplete recognition.

In the present review the term Brody myopathy will be used to designate patients with reduced SR Ca<sup>2+</sup>-ATPase regardless of the association with *ATP2A1* mutation.

### Physiological Function of SERCA1

In skeletal muscle, the contraction-relaxation cycle is mainly

dependent on Ca<sup>2+</sup> release and uptake by the sarcoplasmic reticulum, respectively [16-18]. Sarcoplasmic/endoplasmic reticulum Ca<sup>2+</sup> ATPases (SERCAs) constitute the most important contributors in removing calcium from the sarcoplasm [19-21]. These enzymes are located in longitudinal sarcoplasmic reticulum and catalyze the ATP-dependent transport of calcium from the cytosol to the lumen of the sarcoplasmic reticulum [17,22,23]. The removal of calcium from the myofibrils after muscle contraction is required to restore the tropomyosin mediated inhibition of actin-myosin interaction and, therefore, for muscle relaxation (Figure 1) [18,19,23,24]. At rest, the cytoplasmic free Ca<sup>2+</sup> concentration in muscle fibers is maintained around 20-50 nM whereas Ca<sup>2+</sup> concentration in the lumen of sarcoplasmic reticulum is about 0.1-0.2 mM [20,25]. The activity of SERCAs is fundamental for the establishment and the maintenance of the 10000-fold Ca<sup>2+</sup> gradient across the sarcoplasmic reticulum membrane [20,25].

SERCAs belong to the family of P-type ATPases that are characterized by the formation, during the reaction cycle, of a phosphorylated intermediate in which a terminal phosphate of ATP has been transferred to a conserved aspartic acid located in the catalytic domain [24,26,27]. SERCAs are encoded by three genes, namely *ATP2A1*, *ATP2A2* and *ATP3A3* each producing different alternative splicing isoforms of SERCA1, SERCA2 and SERCA3 proteins, respectively [28-30]. SERCA1a and SERCA2a are the predominant isoforms in adult skeletal muscle [29]. SERCA1a accounts for more

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Patient	Family	Sex	Age	Onset	Clinical features	Pattern of muscle involvement	Inheritance	Genetic analysis	Ref.
1	1	M	26	1st	Painless contractures; slow relaxation during exercise	Generalized		n.p.	[2]
2	2	M	42	1st	Exercise-induced muscle stiffness; pain of exercised muscles; slow relaxation during exercise; muscle cramps; no grip or percussion myotonia	Generalized	AR	Homozygous p.Pro147Arg fsX33 No <i>SLN</i> mutation	[1,10,33]
3		M	42	1st	Exercise-induced muscle stiffness; slow relaxation during exercise; muscle cramps;	Generalized	AR	Homozygous p.Pro147Arg fsX33	[1]
4	3	M	26	1st	Exercise-induced muscle stiffness; slow relaxation during exercise; no percussion myotonia	Generalized	AR	Homozygous p.Cys675X	[1,3]
5		M	36	n.a.	n.a.	n.a.	AR	Homozygous p.Cys675X	[1,3]
6	4	F	28	2nd	Exercise-induced muscle stiffness; slow relaxation during exercise; muscle cramps; mild weakness of facial and proximal limb muscles	Generalized	AD	n.p.	[6]
7		F	30	1st	Exercise-induced muscle stiffness; slow relaxation during exercise; muscle cramps; mild weakness of facial and proximal limb muscles; no percussion myotonia	Generalized	AD	n.p.	
8		M	19	1st	Exercise-induced muscle stiffness; slow relaxation during exercise; no percussion myotonia	Generalized	AD	n.p.	
9		F	54	n.a.	Slow relaxation during exercise; mild proximal limb weakness; no percussion myotonia	Partial	AD	n.p.	
10	5	M	37	3rd	Exercise-induced muscle pain and stiffness; muscle cramps; improvement in hot weather; worsening in cold; well-developed muscles	Generalized		n.p.	[5]
11		F	46	3rd	Exercise-induced muscle pain and stiffness; worsening in cold	Generalized		n.p.	
12	6	M	38	1st	Exercise-induced muscle stiffness; post-exercise muscle weakness; exertional rhabdomyolysis and myalgia; muscle cramps; no grip or percussion myotonia	Partial	S	No <i>ATP2A1</i> mutation	[7,9,13,32]
13	7	F	10	1st	Muscle weakness and pain	n.a.		No <i>ATP2A1</i> mutation	[13,32]
14		M	14	n.a.	Muscle weakness and pain	n.a.		No <i>ATP2A1</i> mutation	
15	8	M	15	1st	Muscle stiffness, weakness and pain; impaired muscle relaxation; muscle cramps	n.a.	AR	Homozygous p.Arg198X	[3,8,32]
16		M	16	1st	Muscle stiffness, weakness and pain; impaired muscle relaxation; muscle cramps	n.a.	AR	Homozygous p.Arg198X	
17	9	F	17	n.a.	Muscle stiffness	n.a.		No <i>ATP2A1</i> mutation	[13,32]
18	10	M	29	2nd	Muscle stiffness, weakness and pain; impaired muscle relaxation; muscle cramps	n.a.	AD	No <i>ATP2A1</i> mutation No <i>SLN</i> mutation	[13,32-33]
19		F	55	2nd	Muscle stiffness, weakness and pain; impaired muscle relaxation; muscle cramps	n.a.	AD	No <i>ATP2A1</i> mutation	[13,32]
20	11	F	29	n.a.	Impaired muscle relaxation; muscle stiffness cramps and pain	n.a.		No <i>ATP2A1</i> mutation	[13,32]
21	12	F	30	3rd	Impaired muscle relaxation; muscle stiffness, weakness and pain; muscle cramps	Generalized	S	No <i>ATP2A1</i> mutation	[13,32]
22	13	M	60	2nd	Painless exercise-induced muscle cramps	Generalized		No <i>ATP2A1</i> mutation No <i>SLN</i> mutation	[10,33]
23	14	F	45	2nd	Exercise-induced muscle stiffness; difficulty in muscle relaxation; no percussion myotonia	Generalized		No <i>ATP2A1</i> mutation No <i>SLN</i> mutation	[10,33]
24	15	M	n.a.	n.a.	Exercise- and cold-induced muscle stiffness; muscle pain and cramps	n.a.	AD	No <i>SLN</i> mutation	[33]

25	16	M	15	1st	Exercise-induced muscle stiffness	n.a.	AR	p.Glu55Lys fsX20	[11]	
26		M	24	1st	Exercise-induced muscle stiffness and cramps; delayed relaxation; no percussion myotonia	Generalized	AR	p.Glu55Lys fsX20		
27	17	F	26	1st	Exercise-induced muscle stiffness; difficulty in muscle relaxation; mild proximal weakness; no percussion myotonia	Generalized	AR	Compound heterozygous: p.Glu34X and p.Tyr389X		
28	18	M		1st	Exercise-induced muscle stiffness	Generalized	AR	Homozygous p.Pro789Leu		
29		M	40	1st	Exercise-induced muscle stiffness	Generalized	AR	Homozygous p.Pro789Leu		
30	19	M	38	2nd	Exercise-induced muscle stiffness and cramps; no grip or percussion myotonia	Generalized	AR	Non causal mutation p.Arg819Cys		
31	20	M	30	1st	Exercise-induced painless muscle stiffness; muscle cramps; rhabdomyolysis after anesthesiological procedure	Generalized	AR	Compound heterozygous p.Leu67Arg and p.Leu59Ser fsX37		[12,14]
32	21	M	19	1st	Exercise-induced muscle stiffness; delayed muscle relaxation	Generalized	AR	Compound heterozygous: p.delLeu65 and delGlu606		[14,31]
33		F	47	2nd	Exercise-induced muscle stiffness; delayed muscle relaxation	Generalized	AR	Compound heterozygous: del p.delLeu65 and p.delGlu606		[31]
34	22	F	30	2nd	Exercise-induced muscle stiffness; muscle cramps; myalgia	Generalized	S	Non causal mutation p.Pro603Pro		[13]
35	23	M	35	2nd	Muscle cramps; myalgia	Partial	S	No ATP2A1 mutation		
36	24	M	37	4th	Exercise-induced muscle stiffness; muscle cramps; myalgia	Generalized	S	No ATP2A1 mutation		
37	25	M	38	4th	Exercise-induced muscle stiffness; myalgia	Partial	S	No ATP2A1 mutation		
38	26	F	42	4th	Exercise-induced muscle stiffness; muscle cramps; myalgia	Partial	S	No ATP2A1 mutation		
39	27	M	48	5th	Exercise-induced muscle stiffness; muscle cramps; myalgia	Partial	AR	Non causal mutation p.Arg604His		
40		F	52	5th	Exercise-induced muscle stiffness; muscle cramps; myalgia	Partial	AR	No ATP2A1 mutation		
41	28	M	49	5th	Exercise-induced muscle stiffness; myalgia	Partial	S	No ATP2A1 mutation		
42	29	M	55	6th	Exercise-induced muscle stiffness; muscle cramps; myalgia	Generalized	S	No ATP2A1 mutation		
43	30	F	62	1st	Exercise-induced muscle stiffness; muscle cramps; myalgia	Partial	AR	No ATP2A1 mutation		
44		F	64	5th	Exercise-induced muscle stiffness; muscle cramps; myalgia	Partial	AR	No ATP2A1 mutation		
45		F	69	1st	Exercise-induced muscle stiffness; muscle cramps; myalgia	Generalized	AD	No ATP2A1 mutation		
46	31	F	68	7th	Exercise-induced muscle stiffness; muscle cramps; myalgia	Generalized	S	No ATP2A1 mutation		
47	32	M	30	1st	Exercise-induced muscle stiffness; muscle cramps, no grip or percussion myotonia	Generalized	S	No ATP2A1 mutation	[14]	

Onset is indicated in decades; AR: Autosomal Recessive; AD: Autosomal Dominant; S: sporadic; n.p.: not performed; n.a.: not available; blank, unknown/not reported \*mother and sister of the patient said to suffer of similar symptoms, suggesting a dominant inheritance of the disease [33]

Table 1: Clinical features and genetic analysis in patients with Brody myopathy described to date.

than 99% of SERCAs expressed in adult fast-twitch muscle fibers whereas SERCA2a is the major isoform present in slow-twitch fibers [18].

SERCA1a, the structurally and functionally best-characterized sarcoplasmic reticulum Ca<sup>2+</sup>-pump, is an integral membrane protein with a relative molecular mass of 110 kDa, consisting of a single polypeptide chain folded into three cytoplasmic domains, a Membranous domain (M) and small luminal loops [22,26,27]. The three cytoplasmic domains include the Nucleotide-binding (N), the Phosphorylation (P) and the Actuator (A) domains [22,27]. The

membranous domain is constituted by 10 transmembrane helices (M1-M10) that harbor the two Ca<sup>2+</sup> binding sites [22,27].

### Clinical Features

The main clinical finding is the exercise-induced delay in muscle relaxation which causes painless or mildly painful muscle stiffness [1-3,6,7,9-11,13,14,31]. Delayed muscle relaxation and muscle stiffness involve predominantly legs, hands, arms and eyelids and usually resolve within a few minutes of rest [1,2,6,7,9,10,13]. Myalgia and cramps both at rest and/or exercise-related are frequently reported [1,3,5-7,13].

Symptoms ease in hot weather and may worsen in the cold [5,11-14]. Recurrent rhabdomyolysis has also been observed in a few patients [9,12,14,32]. Percussion and grip myotonia are absent [6,7,9,13].

Recently, Voermans et al. reviewed the literature data on clinical features of Brody disease and Brody syndrome. In addition, the authors reported the clinical aspects of 17 patients with Brody syndrome, of which 13 were newly described, and highlighted the differences between patients with and without *ATP2A1* mutation [13]. In Brody disease onset is more frequently in the 1<sup>st</sup> decade, the pattern of inheritance is autosomal recessive, muscle stiffness is generalized and relaxation after repetitive contractions is delayed [13]. Although patients with Brody syndrome also suffer from exercise-induced muscle stiffness and delayed relaxation, they more frequently report myalgia and experience a considerable impact on daily life [4,13]. These might be also related to the longer period of follow-up of the patients in Voermans' clinical study. Moreover, often only a few muscle groups are involved and the onset is in adolescence or in adulthood [4,13].

### Laboratory Investigations

Serum creatine kinase level is normal or slightly elevated [1,2,5,6,9,10,13]. Needle electromyography is either normal or discloses myopathic changes consisting of Motor Unit Potentials (MUPs) of reduced amplitude and duration with a higher-than-normal proportion of polyphasic MUPs. No myotonic or complex repetitive discharges are detected. During the phase of delayed relaxation, the strongly contracted muscles are electrically silent; therefore the cramps are named "silent cramps" [1-3,5-7,9-14,31,33]. Nerve conduction studies are normal.

### Histological Findings

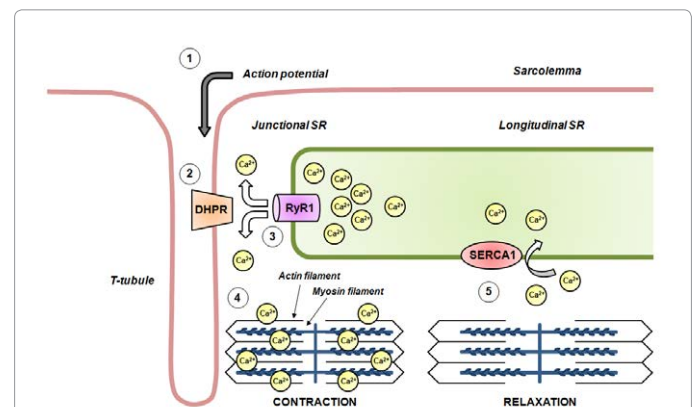
Conventional histochemical studies of muscle specimen commonly document variation in fiber diameter and increased number of fibers with internal nuclei [6,9,12,14,31]. Atrophy of both type I and II muscle fibers [5,31] or selective atrophy of type II fibers is reported [1,6]. The presence in many fibers of basophilic area strongly reactive to Nicotinamide Adenine Dinucleotide-Tetrazolium Reductase (NADH-TR) has been observed in a patient with Brody disease (Figure 2) [31]. The focal areas darker with the modified Gomori trichrome method and display reduced or absent activity for Succinate Dehydrogenase (SDH) [31]. Neither necrotic nor regenerating muscle fibers are usually observed.

Ultrastructural examination may reveal enlargement of lateral cysternae, proliferation of the tubular elements of the sarcoplasmic reticulum and increased number of membranous bodies within sarcoplasmic reticulum vesicles (Figure 3) [6]. The structure of triads and T-tubules are normal [1,6,9,31]. The presence of swollen subsarcolemmal mitochondria containing paracrystalline inclusions has also been reported [9,31].

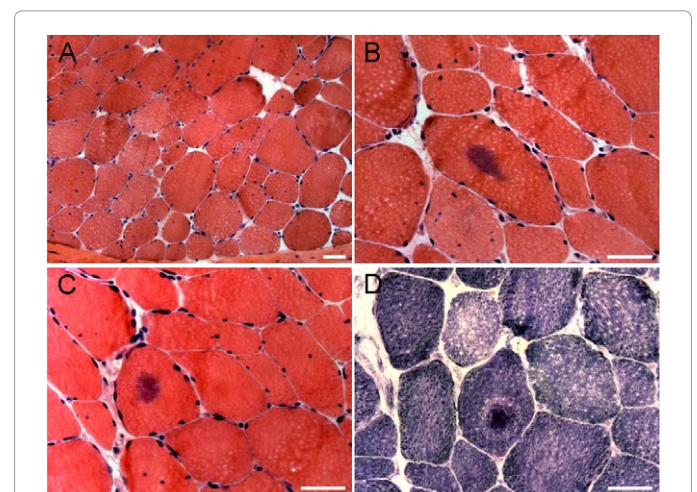
### Biochemistry

SERCA1 activity has been assessed as the compound activity of SR  $Ca^{2+}$ -ATPases including SERCA1, SERCA2 and SERCA3 in isolated microsomal or sarcoplasmic reticulum fractions from muscle or in whole muscle homogenates. Benders et al. reported that SERCA1 contributes at least 80% to the total SERCAs content in quadriceps muscle, thus compound activity of SR  $Ca^{2+}$ -ATPases can be considered a reliable estimation of SERCA1 activity [32]. Since the first description of the disease by Brody who reported the reduction of ATP-dependent

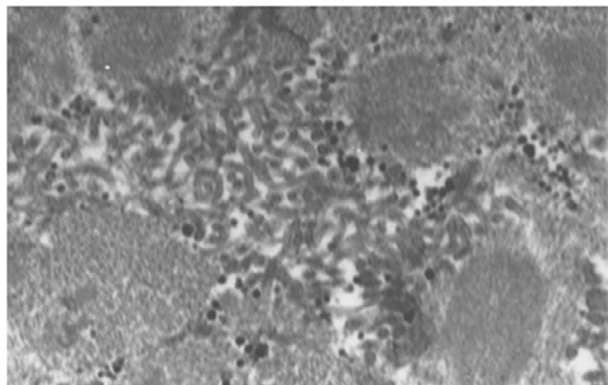
calcium uptake to 25% of control values and a decreased SR  $Ca^{2+}$ -ATPase activity in the microsomal fraction of skeletal muscle from a patient [2], the reduction of the  $Ca^{2+}$ -dependent SR ATPase activity has been reported in muscle of all patients with Brody disease and Brody syndrome [1,2,5,13,14,31,32]. Residual activity of SR  $Ca^{2+}$ -ATPase usually ranges from 2% to 60% of normal.



**Figure 1: Schematic representation of excitation, contraction and relaxation in skeletal muscle.** (1) Motoneuron activity triggers sarcolemmal depolarization generating an action potential that propagates throughout the sarcolemma and enters the T-tubule system. (2) Dihydropyridine receptor (DHPR) acts as a sensor of the action potential and undergoes a voltage-driven conformational change that (3) triggers activation and opening of the ryanodine receptor 1 (RyR1) with the consequent release of calcium from the sarcoplasmic reticulum. DHPR and RyR1 are located in the T-tubules and junctional sarcoplasmic reticulum, respectively. (4) In the sarcoplasm, calcium binding to troponin leads to tropomyosin displacement from myosin-binding sites on actin filaments enabling the formation of cross-bridge interactions between thick (myosin) and thin (actin) filaments with the consequent contraction of the sarcomere. (5) Sarcoplasmic endoplasmic reticulum  $Ca^{2+}$  ATPase 1 (SERCA1) is activated by elevated cytosolic calcium concentration and catalyzes the ATP-dependent transport of calcium from the cytosol to the lumen of the sarcoplasmic reticulum. Calcium withdrawal from the myofibrils leads to the restoration of the tropomyosin mediated inhibition of actin-myosin interaction and therefore to muscle relaxation. SR, sarcoplasmic reticulum; DHPR, dihydropyridine receptor; RyR1, ryanodine receptor 1.



**Figure 2: Light microscopy.** Hematoxylin and eosin stain shows fiber size variation and increased internal nuclei (A). Two fibers display central basophilic areas in hematoxylin and eosin stain (B, C). The focal areas are strongly reactive to nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) (D). Bars, 50  $\mu$ m.



**Figure 3: Electron microscopy.** Proliferation of the tubular components of the sarcoplasmic reticulum. Original magnification: 55,560 $\times$ .

### SERCA1 Protein Expression

SERCA1 protein expression has been investigated in a few cases and conflicting results have been provided by literature. Immunostaining and immunoblot analysis for SERCA1 has been reported to be normal or reduced in muscle of patients with Brody disease and Brody syndrome [1,3,6,13,14,31].

Using polyclonal or monoclonal antibodies against SR Ca<sup>2+</sup>-ATPase isolated from chicken skeletal muscle, Karpati and co-authors observed a barely detectable immunoreactivity in type 2 muscle fibers in two patients with Brody disease and a remarkable reduction of the 100 kDa phosphoprotein by immunoblot on microsomal fraction prepared from skeletal muscle of one of the described patients [1].

In a following study, using the same SR Ca<sup>2+</sup>-ATPase antibodies reported in Karpati et al., a severe reduction of the immunoreactivity limited to type II fibers and a decreased level of 100 kDa band were observed in muscle of two patients with Brody syndrome [6].

Benders et al. determined the total content of SR Ca<sup>2+</sup>-ATPases and of SERCA1 by ELISA using a polyclonal antibody raised against a mixture of type I and II muscle fibers and a monoclonal antibody against SR Ca<sup>2+</sup>-ATPase from fast-twitch muscle, respectively. The authors found that the concentration of SR Ca<sup>2+</sup>-ATPases and of SERCA1 is normal in muscle of patients with Brody disease and Brody syndrome [32]. In addition, immunocytochemical staining did not reveal differences between muscle sections of controls and patients [32,34].

Recently, the expression of SERCA1 has been evaluated in muscle of patients with Brody disease and Brody syndrome using a well-characterized commercial antibody [14,31]. The staining pattern and the intensity of SERCA1 immunoreaction were similar in controls and in patients, regardless of the association with *ATP2A1* mutation (Figure 4) [14,31]. In contrast, immunoblotting revealed a remarkable reduction of SERCA1 level in muscle of patients with Brody disease but not in muscle of patients with Brody syndrome [14,31]. Immunoblot after 2D-PAGE has been used to detect putative changes in SERCA1 protein pattern in muscle of patients due to post-translational modifications [14,31]. SERCA1 was detected as a single spot around pI 5.0 with an apparent molecular weight of 100 kDa in patients and controls, ruling out the presence of specific post-translational modifications [14,31]. Although the discrepancy between the immunohistochemical data and the immunoblotting findings is under debate and according to

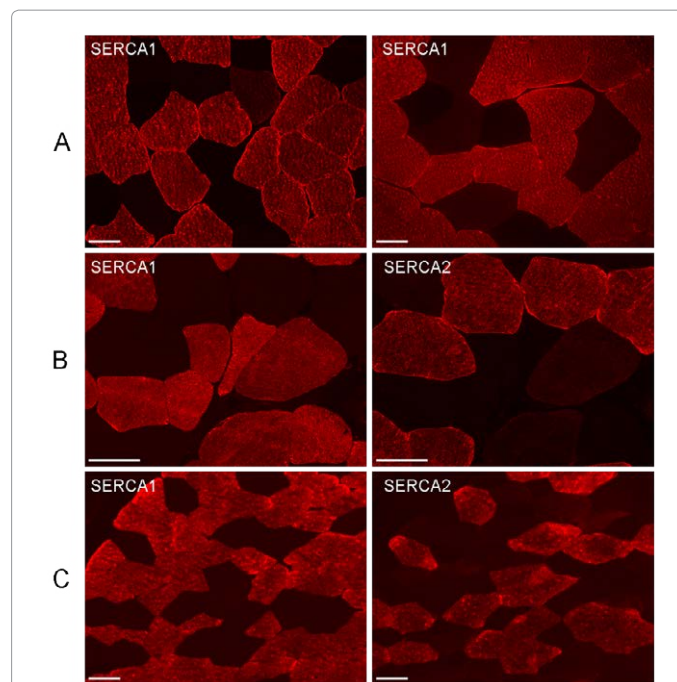
the authors, can be only partially explained by the lower sensitivity of immunohistochemistry, the study unambiguously demonstrates that immunohistochemistry is not a reliable technique for the diagnosis of the disease while provides evidence that immunoblot analysis could be a valuable tool allowing the identification of patients with *ATP2A1* mutation [14].

### Other Investigations

Respiratory chain, glycolytic (myophosphorylase, phosphofructokinase, phosphoglycerate kinase, phosphoglycerate mutase, lactate dehydrogenase) and myoadenylate deaminase enzyme activities are normal in all of the patients assayed [5,32].

The forearm ischemic exercise test causes a normal rise of venous lactate and ammonia [7,9]. An excessive increase of serum potassium has been observed in a patient immediately after the forearm exercise test [7,9]. The normal activity of Na<sup>+</sup>/K<sup>+</sup> ATPase led to exclude a K<sup>+</sup> reuptake disorder suggesting that the excessive K<sup>+</sup> release from working muscle is a consequence of the altered calcium homeostasis on Ca<sup>2+</sup>-activated K<sup>+</sup> channels. Therefore Poels and Wevers proposed that the measurement of serum potassium immediately after the forearm exercise test could be useful for the diagnosis of the disease [7,9].

<sup>31</sup>P-MRS spectroscopy shows rapid and excessive cytoplasmic acidification during aerobic dynamic exercise and a greater than normal drop of the Phosphocreatine (PCr) concentration, which recovers at slower rate [1,5]. Taylor et al. suggested that the excessive intracellular acidification observed during aerobic exercise was due to the prolonged activation of the glycolysis and not to the impairment of oxidative metabolism because the recovery time of ADP, which is a sensitive index of oxidative capacity in muscle, was normal in patients [5].



**Figure 4: Immunohistochemistry for SERCA1 and SERCA2.** Immunostaining for SERCA1 is similar in muscle of controls (A) and of patients with (B) and without (C) *ATP2A1* mutation. Serial sections from muscle biopsy specimen of patients (B, C) show that SERCA1 and SERCA2 antibodies stain type II and type I fibers, respectively. Bars, 50  $\mu$ m.

## Genetic Studies

Brody myopathy is transmitted as an autosomal recessive or dominant trait [13,15]. The recessive inheritance is associated to mutation of the *ATP2A1* encoding the sarcoplasmic/endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase 1 (SERCA1) [3]. Mutation in *ATP2A1* gene causing deletions and missense, frameshift and nonsense mutations in SERCA1 protein have been identified in 14 patients belonging to eight families (Figure 5) [3,11,14,31,33]. *ATP2A1* mutations are missed in some patients with recessive inheritance and have never been found in patients with an autosomal dominant pattern [10,13]. Therefore, beside *ATP2A1* at least two other genes should account for Brody myopathy, one responsible for non-*ATP2A1*-linked autosomal recessive form and the other associated with the autosomal dominant inheritance.

*SLN* gene encoding sarcolipin was considered a prime candidate gene for Brody syndrome [11,33]. Sarcolipin (SLN) is a 31-amino acids transmembrane protein highly expressed in fast-twitch muscle fibers; it was identified as a proteolipid that co-purifies with SERCA1a from rabbit fast-twitch skeletal muscle and experimental data support the ability of SLN to inhibit the SERCAs activity in sarcoplasmic reticulum of fast-twitch, slow-twitch and atrial muscles at low calcium concentration by lowering their apparent affinity for calcium [35-37]. By now, mutations in *SLN* have not been found in any of the four families with symptoms and signs of Brody myopathy [11,33].

## Looking for Compensatory Mechanisms Involved in Muscle Relaxation

In spite of the remarkable reduction of SR  $\text{Ca}^{2+}$ -ATPase activity, patients are still able to relax their fast-twitch muscle fibers, although at decreased rate. Other mechanisms could have a role in the lowering cytoplasmic  $\text{Ca}^{2+}$  concentration and in the re-establishment of calcium homeostasis in muscle of patients.

The hypothesis of increase and/or ectopic expression of SERCA2 protein in type II muscle fibers has been recently excluded (Figure 4) [14].

In physiological conditions the involvement of the mitochondrial compartment through  $\text{Ca}^{2+}$  uniport system in calcium buffering is very unlikely because of the low apparent  $\text{Ca}^{2+}$  affinity of the system ( $K_m > 10 \mu\text{M}$ ) [20,25]. However, mitochondria could have an impact on calcium homeostasis at increased cytoplasmic  $\text{Ca}^{2+}$  concentration, an expected condition in muscle fibers of patients because of the reduced SR  $\text{Ca}^{2+}$ -ATPase activity.

Although their impact in the regulation of intracellular calcium

is considered of minor significance in skeletal muscle [20,25], plasma membrane  $\text{Ca}^{2+}$  ATPases (PMCAs) and  $\text{Na}^+/\text{Ca}^{2+}$  exchangers (NCX) could be involved in the cytoplasmic calcium clearance through calcium extrusion in the extracellular space in muscle of patients.

## Treatment

There is no specific therapy for patients suffering from Brody myopathy even though dantrolene and verapamil are reported to improve exercise tolerance [5,7,9,12,32]. Both drugs were tapered in some patients because of side effects including muscle weakness, palpitations and depression [5,13]. Dantrolene and verapamil are blockers of dihydropyridine receptor-ryanodine receptor complex and act inhibiting the release of calcium from the sarcoplasmic reticulum [32,38,39]. By preventing calcium overload they facilitate the lowering of cytosolic  $\text{Ca}^{2+}$  concentration after muscle contraction; indeed less calcium is released from the sarcoplasmic reticulum, less  $\text{Ca}^{2+}$  pumping capacity is required to restore normal resting calcium concentration [32]. Voermans et al. reported that etoricoxid was effective in two patients with Brody syndrome [13].

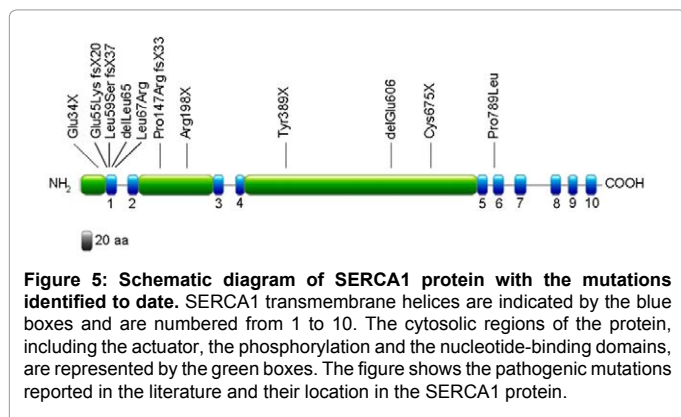
## In vitro Models of Brody Disease: Are Cultured Human Muscle Fibers Reliable?

The expression and the activity of SR  $\text{Ca}^{2+}$ -ATPases have been investigated in primary cultures of human muscle [14,32,34,40]. Benders et al. reported that both content and activity of SR  $\text{Ca}^{2+}$ -ATPases increase in cultured muscle fibers with the maturation grade and that SERCA1 accounts for the total SR  $\text{Ca}^{2+}$ -ATPase content in human muscle cells [32,34]. However, a recent study demonstrated that SERCA2 is strongly expressed in human myoblasts and myotubes grown *in vitro* and that SERCA1b is the main SERCA1 isoform expressed in cultured human muscle fibers at both non confluent myoblast and fully differentiated myotube stages [14]. Guglielmi et al. evaluated the expression of SERCA1 alternative splicing variants using a specific antiserum raised against the C-terminal highly charged octapeptide (DPEDERRK) of SERCA1b [14]. In mammals, *ATP2A1* gene encodes two alternative splicing variants: SERCA1a, the adult isoform which is expressed in fast-twitch muscle fibers, and SERCA1b, the neonatal form expressed in neonatal and developing skeletal muscle. The difference between the two isoforms arises from the alternative splicing of exon 22 and consequently affects the C-terminal region of the proteins [29]. Expression and functional analysis of SERCA1a and SERCA1b in COS-1 cells revealed no differences in either calcium transport activity or affinity between the two isoforms, making the meaning of the developmental-regulated isoform switch an open question [41].

Although cultured human muscle fibers may provide new insight into the pathophysiology of genetically determined neuromuscular disorders, these findings, taken in sum, suggest caution in use primary cell cultures to study the pathogenesis of Brody disease.

## Animal Models

A mouse model of Brody disease has been generated by targeted ablation of *ATP2A1* gene [42]. SERCA1-null mice, unlike human patients, show a severe clinical picture consisting in cyanosis and gasping respiration and die shortly after birth of respiratory failure [42]. Analysis of diaphragm muscle displays increased fiber size variability and hypercontracted regions in scattered fibers suggesting that the absence of SERCA1 severely affects muscle functions [42]. Patients with Brody disease do not develop respiratory problems probably because



**Figure 5: Schematic diagram of SERCA1 protein with the mutations identified to date.** SERCA1 transmembrane helices are indicated by the blue boxes and are numbered from 1 to 10. The cytosolic regions of the protein, including the actuator, the phosphorylation and the nucleotide-binding domains, are represented by the green boxes. The figure shows the pathogenic mutations reported in the literature and their location in the SERCA1 protein.

of a higher percentage of type I muscle fibers in human diaphragm as compared to mice; therefore, SERCA1-null mice are not equivalent to the human disorder.

Spontaneously occurring animals models of Brody disease have been recognized [43-46]. Congenital pseudomyotonia in Chianina and Romagnola cattle closely resembles Brody disease and is clinically characterized by exercise-induced muscle contractures and stiffness after fast movements [44]. SR Ca<sup>2+</sup>-ATPase assay performed on microsomal fraction extracted from fast-twitch skeletal muscle demonstrated a remarkable reduction in SERCA1 activity and genetic analysis revealed *ATP2A1* mutations (c.491G>A, p.Arg164His; c.1675 C>T, p. Arg559Cys; c.632G>T, p. Gly211Val; c.857G>T, p.Gly284Val) that are transmitted with an autosomal recessive pattern [44-46].

A homozygous missense mutation (c.1675 C>T, p. Arg559Cys) in exon 14 of the *ATP2A1* gene has been also described in a Dutch Improved Red and White cross-breed calf; clinical findings include exercise-induced muscle stiffness and electrophysiological examination shows the absence of myotonic discharges [43]. The same genetic defect was previously reported in Belgian Blue cattle with congenital muscular dystonia, a condition characterized by exercised-induced muscle contraction, impairment of swallowing and death few weeks after birth for respiratory failure [47], a phenotypic presentation similar to that observed in SERCA1-null mice [42]. These studies suggest that in cattle SERCA1 defect can result in different phenotypes depending on the genetic background of the breed [43].

The large scale mutagenesis screen in zebrafish led to the identification of *accordion* (*acc*) class mutants [48-50]. In contrast to wild type zebrafish embryos that respond to touch at 24 hour post-fertilization performing two rapid alternating coils of the tail, *acc* mutants show the simultaneous contraction of bilateral trunk muscles resulting in shortening and bending of the trunk [48-49]. *acc* mutants are clinically characterized by muscle stiffness after active contraction and slow relaxation due to the decreased decay in cytosolic Ca<sup>2+</sup> concentration, findings suggesting an impairment of the calcium reuptake in the sarcoplasmic reticulum [48-49]. The *ATP2A1* missense mutations Ile97Asn, Ser766Phe, Thr848Ile and Gly598Val that affect amino acids conserved among vertebrates, have been identified in different *acc* mutants [48,50]. In contrast to the three former mutations which are transmitted with an autosomal recessive pattern, the missense mutation Gly598Val shows a semi-dominant mode of inheritance in zebrafish [49].

## Concluding Remarks

Brody disease and Brody syndrome cannot be diagnosis with confidence by purely clinical criteria. Indeed muscle stiffness, muscle cramps and myalgia is common complaints of several neuromuscular disorders. Despite morphologic alterations are neither sensitive nor specific, muscle biopsy provides further informations because the reduced SERCAs activity is a sensitive tool for patient's recognition and SERCA1 immunoblotting could be useful for the diagnosis of Brody disease. However, the specificity of reduced SERCAs activity is limited because it has been also observed in muscle of patients with myotonic dystrophy type 1 and can be related to other non-muscle pathological conditions including hypothyroidism [51-53]. Therefore, up to now, only mutation analysis of *ATP2A1* allows a definite diagnosis of Brody disease whereas Brody syndrome can be diagnosed on the basis of clinical and biochemical features.

Issues remain unsolved and await further studies: (1) the compensatory mechanisms involved in the lowering of cytoplasmic

Ca<sup>2+</sup> concentration in fast-twitch fibers during muscle relaxation in patients with Brody myopathy and (2) the identification of the pathogenic mechanisms responsible for the reduction of SERCAs activity in patients with Brody syndrome.

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