Aus der Abteilung für Myologie

an Experimental and Clinical Research Center (ECRC)

der Medizinischen Fakultät Charité-Universitätsmedizin Berlin

DISSERTATION

Role of Extracellular ATP in the Progression of Muscle Damage in Sarcoglycanopathies

Rolle von Extrazellulären ATP in der Progression der Muskeldegeneration in Sarkoglykanopathien

zur Erlangung des akademischen Grades

Doktor medicinae (Dr.med.)

vorgelegt der medizinischen Fakultät

Charite-Universitätsmedizin Berlin

von Elisabetta Gazzerro

aus Genova (Italien)

Datum der Promotion: 04.06.2021

Inhaltsverzeichnis

I. Abkürzungsverzeichnis	Seite 3-4
II. Abstract	Seite 5-7
III. Mantel Text	Seite 8-28
IV. Eidesstattliche Versicherung	Seite 29
V. Ausführliche Anteilserklärung an der erfolgten Publikation	Seite 30-31
VI. Auszug aus der Journal Summary List	Seite 32
VII. Publikation	Seite 33
VIII. Lebenslauf	Seite 34
IX. Wissenschaftlichen Veröffentlichungen	Seite 36-41
X. Danksagung	Seite 42

I. Abkürzungsverzeichnis

Activating-Signal-coactivator 1 (Asc1)

Amphyregulin (Areg)

Antigen presenting cells (APC)

α-Sarcoglycan (α-SG)

α-SG knockout model (Sgca)

BenzoylATP (BzATP)

Connective Tissue Growth Factor (CTGF)

Control System (QC)

Creatine Kinase (CK)

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

Damage-associated molecular pattern molecules (DAMPS)

Duchenne Muscular dystrophy (DMD)

Dystrophin-Glycoprotein complex (DGC).

Ecto-5′-nucleotidases (ecto-5′-NT)

Extracellular adenosine triphosphate (eATP)

Forkhead-Box-Protein P3 (Foxp3)

Guanine nucleotide-binding protein (G protein)-coupled receptors (GPCRs)

Induced pluripotent stem cells (iPSCs)

Interferon (IFN)

Knockout (KO)

Limb–girdle muscular dystrophies (LGMDs)

Lipopolysaccharide (LPS)

Loss of function (LOF)

Magnetic resonance Imaging (MRI)

Periodate-oxidase ATP (oATP)

Regulatory T-cells (Treg)

Sarcoglycans (SGs)

Satellite cells (SCs)

Standard Operating Procedures (SOP)

Transforming Growth Factor-α (TGFα)

Transforming Nuclear Factor (TNF)

Wild types (Wt)

II. Abstract

Limb Girdle Muscular Dystrophy 2D (LGMD2D) is an inherited disorder characterized by progressive weakness and degeneration of skeletal muscle, loss of ambulation, respiratory insufficiency and, often, premature death. The disease results from mutations in the alpha-sarcoglycan (αSG) gene, encoding a muscle membrane associated protein. A-SG loss-of-function causes a cell membrane fragility, which ultimately results in a tissue-specific increase of danger-associated molecules (DAMPs) and infiltration of inflammatory cells. The DAMP extracellular adenosine triphosphate (eATP) released by dying myofibers steadily activates muscle and immune purinergic receptors exerting dual negative effects: a direct damage linked to altered intracellular calcium homeostasis in muscle cells and an indirect toxicity through the "triggering" of the immune response and inhibition of regulatory T cells (Tregs). Accordingly, pharmacological and genetic inhibition of eATP signaling improves the phenotype in models of chronic inflammatory diseases.

In α-sarcoglycanopathy (LGMD2D), eATP effects may be further amplified since αSG extracellular domain binds eATP and displays an ecto-ATPase activity, thus controlling eATP concentration at the cell surface and attenuating the magnitude and/or the duration of eATP-induced signals.

Here we show that in vivo blockade of the eATP/P2X purinergic pathway by a broad spectrum P2XR–antagonist delayed the progression of the dystrophic phenotype in α-SG null mice. eATP blockade dampened the muscular inflammatory response and enhanced the recruitment of Forkhead-Box-Protein P3 (Foxp3) positive immunosuppressive regulatory CD4+ T cells. The improvement of the inflammatory features was associated with increased strength, reduced necrosis and limited expression of pro-fibrotic factors, suggesting that pharmacologic purinergic antagonism, altering the innate and adaptive immune component in the muscle infiltrates, might provide a therapeutic approach to slow disease progression in LGMD2D. In this scenario, the consequences of purinoceptor inhibition on the stability and function of Tregs cells are particularly intriguing given the potential clinical value of targeting this T cell subset in muscle diseases. Transition to cure of our approaches is feasible: Tregs immunosuppressive properties have prompted clinical trials in models of autoimmunity and human graft versus host disease; clinical trials with P2X7 antagonists have been completed for other diseases, showing acceptable safety and tolerability.

II. Abstrakt

Die Muskeldystrophie der Gliedmaßengürtel (LGMD2D) ist eine vererbte Erkrankung, die durch fortschreitende Muskelschwäche und Degeneration der Skelettmuskulatur, Verlust der Gehfähigkeit, Ateminsuffizienz und häufig vorzeitigen Tod gekennzeichnet ist. Die Krankheit resultiert aus Mutationen im alpha-Sarkoglycan (αSG) Gen, der für ein muskelmembranassoziiertes Protein kodieret.

A-SG „Loss-of-Function“ verursacht die Schwäche der Muskelmembran, die zu einer gewebespezifischen Erhöhung der danger associated molecules (DAMPs) und zur Infiltration von Entzündungszellen führt. Das DAMP extrazelluläre Adenosintriphosphat (eATP), das durch das Absterben von Myofasern freigesetzt wird, aktiviert die purinergischen Rezeptoren der Muskulatur und des Immunsystems. eATP stellt einen doppelt negativen Effekt im Muskelgewebe: eine direkte Schädigung, mit einer veränderten intrazellulären Kalziumhomöostase in den Muskelzellen, und eine indirekte Toxizität durch das "Auslösen" der Immunantwort mitsamt der Hemmung der regulatorischen T-Zellen (Tregs). Dementsprechend verbessert die pharmakologische und genetische Hemmung der eATP-Signalgebung den klinischen Phänotyp in Modellen chronisch entzündlicher Erkrankungen.

Bei der α-Sarkoglykanopathie, LGMD2D, können die eATP-Effekte weiter verstärkt werden, da die extrazelluläre Domäne der αSG eATP bindet und eine Ecto-ATPase-Aktivität zeigt, wodurch die eATP-Konzentration an der Zelloberfläche und die Stärke und / oder Dauer von eATP- induzierte Signale kontrolliert können werden.

Hier zeigen wir, dass die In-vivo-Hemmung des eATP / P2X-Purinergiewegs durch einen breiten P2XR-Antagonisten das Fortschreiten des dystrophischen Phänotyps in αSG knock-out Mäuse verzögerte. Die eATP-Blockierung dämpfte die muskuläre Entzündungsreaktion und verstärkte die Rekrutierung von Forkhead-Box-Protein P3 (Foxp3) positiven immunsuppressiven regulatorischen CD4 + T-Zellen. Die Verbesserung der Entzündungsmerkmale ging einher mit erhöhter Stärke, verminderter Nekrose und eingeschränkter Expression pro-fibrotischer Faktoren, was darauf schließen lässt, dass ein pharmakologischer purinergischer Antagonismus, der die angeborene und anpassungsfähige Immunkomponente in den Muskelinfiltraten verändert, einen therapeutischen Ansatz zur Verlangsamung des Krankheitsverlaufs in LGMD2D bieten könnte.

In diesem Szenario sind die Konsequenzen der Purinorezeptor-Hemmung auf die Stabilität und Funktion von Tregs-Zellen besonders interessant. Ein Übergang zur Heilung unserer Ansätze

ist möglich: Tregs immunsuppressive Eigenschaften haben klinische Studien mit Modellen von Autoimmunerkrankungen veranlasst; klinische Studien mit P2X7-Antagonisten wurden für andere Krankheiten abgeschlossen und zeigen akzeptable Sicherheit und Verträglichkeit.

III. Mantel Text

Publikation: The danger signal extracellular ATP is involved in the immunomediated damage of alpha-sarcoglycan deficient muscular dystrophy

Introduction

Primary myopathies represent a large group of inherited monogenic disorders that affect skeletal muscle. The clinical and genetic heterogeneity of these diseases is well recognized: some have natal onset, others are typical of adulthood; some are rapidly progressive, others are associated with long periods of stability; some, finally, have a multi-systemic involvement and are associated with a cardiac and central nervous system involvement. Despite this clinical variability, many of these diseases share similar histological characteristics the so defined "dystrophic" changes in the patient muscle biopsies and are thus described as “Muscular Dystrophies”. Histological markers of a dystrophic process are increased myofibral size variability with degeneration/necrosis of muscle cells, central nucleation, regeneration from satellite cells and replacement of muscle tissue by connective and adipose tissue once the prevailing degenerative processes exhaust the pool of regenerative cells (1).

In the last two decades, the significant advances in clinical and genetic research on muscular dystrophies have led to greater diagnostic precision and have allowed the initiation of targeted symptomatic therapy (2).

a) Limb Girdle Muscular Dystrophies

LGMDs are a heterogeneous group of myopathies causing weakness and wasting of the proximal limb (the hip/shoulder girdle) musculature.

The genes, whose mutations cause a LGMD phenotype, encode proteins that are involved in various parts of the muscle fiber physiology including the nucleus, sarcoplasm, sarcomere, sarcolemma and extracellular matrix. With the progressive advancements in next-generation molecular sequencing, it has been demonstrated that the same genetic variant can cause a wide spectrum of symptoms with distinct clinical phenotypes. Taken as a group, LGMDs is the fourth most common muscular dystrophy, with a pooled prevalence of 1.63 per 100,000 (range 0.56–5.75 per 100,000), following Dystrophinopaties, Myotonic Dystrophies and Facioscapulohumeral Muscular Dystrophy (3). Indeed, when the term LGMD was first conceptualized in 1954, it was thought to be a single entity. LGMDs are now classified into 2

main classes based on the inheritance pattern: autosomal dominant (AD-LGMD1) and autosomal recessive (AR-LGMD2) types (according to the last classification in 2018, there are 8 subtypes of LGMD type 1 and 26 subtypes of LGMD type 2).

X-linked disorders that display a limb–girdle pattern of weakness include the Emery–Dreifuss Muscular Dystrophies and the Dystrophinopaties (Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, and manifesting carriers of Dystrophinopaties) and are not traditionally listed in the category of LGMDs.

The clinical presentation and the rate of progression is highly variable also within the same family of LGMD; serum creatine kinase (CK) levels, which in the past were considered a marker of disease progression, vary from normal to greatly elevated, even within the same LGMD subtype. The muscle biopsy often shows nonspecific dystrophic features. The immunohistochemistry on muscle tissue is available only for Sarcoglycanopathies, Dysferlinopathies, Dystroglycanopathies, Caveolinopathies, type VI Collagenopathies and most of the patients are diagnosed through genetic testing. Testing at-risk family members may also assist with medical management and provide information for reproductive decision-making.

b) Sarcoglycanopathies (LGMD 2C-2E)

Sarcoglycans (SGs) are four different heavily glycosylated transmembrane glycoproteins (α, -β, -γ, and -δ) whose loss–of-function (LOF) cause respectively the LGMD-2D, 2E, 2C and 2F. SGs are part of a multimeric structure, the Dystrophin-Glycoprotein complex (DGC). The DGC is a large complex of membrane-associated proteins that further consists of dystrophin, the dystroglycans (α and β), sarcospan, the syntrophins (α1, β1, β2, γ1- and γ2) and α-dystrobrevin. The main function of the DGC is to provide a strong mechanical link from the intracellular cytoskeleton to the extracellular matrix. Dystrophin binds in its N-terminal domain the myofibrillar actin and in its C- terminal portion the sarcoglycans and β-dystroglycan. These in conjunction with α-dystroglycan act as bridge joining components of the extracellular matrix such as laminin-2, agrin and neurexin (Fig.1). The mechanical link provided by the DGC is critical to the preservation of muscle membrane integrity. Each muscle contraction in both heart and skeletal muscle results in cellular deformation and shortening. Throughout this process, the contractile machinery inside the myofibers must remain intimately connected with the membrane and extracellular matrix. Without this tight association, movement would be improperly transmitted and myocytes would risk damage to their membranes (2). The clinical phenotype of LGMD2C-F patients is heterogeneous, and age of onset, rate of progression

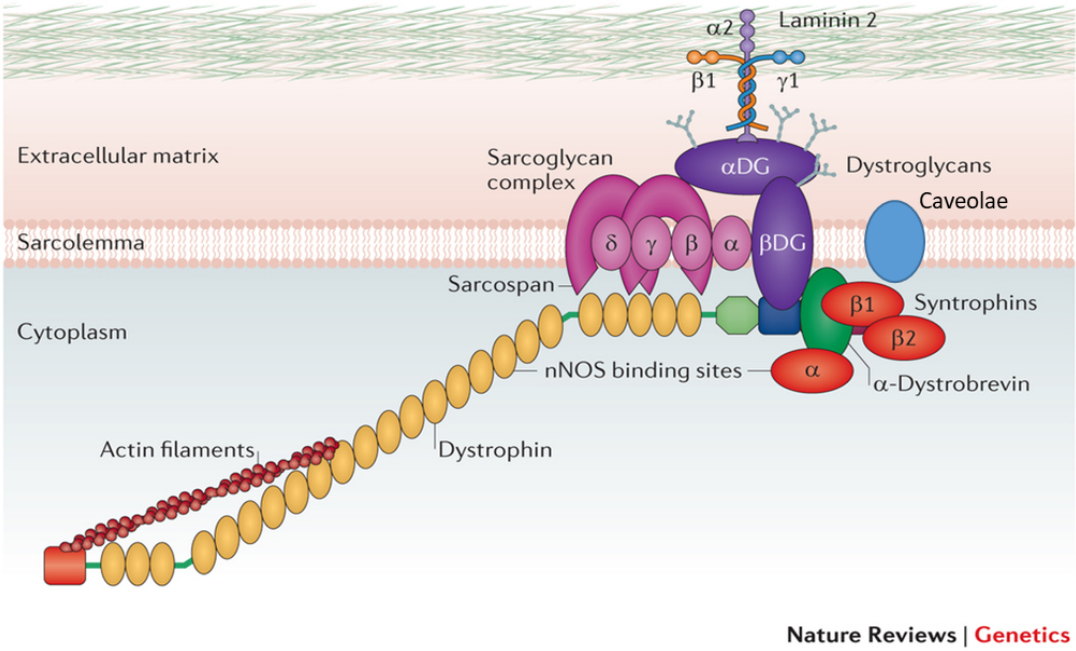

Fig. 1. The skeletal muscle sarcolemma and the DGC. Modified from "Therapy for Duchenne muscular dystrophy: renewed optimism from genetic approaches" Fairclough et al. Nat Rev Gen 2013; 14:373.8

and severity can vary between and within affected families. Patients with stop or severe missense mutations may have a severe clinical presentation characterized by early childhood muscle weakness, wheel chair bound during adolescence and respiratory insufficiency due to the weakness of diaphragm and respiratory muscles and premature death. The patients with a milder clinical presentation may remain ambulatory into adulthood. A small percentage may display an episode of rhabdomyolysis as first clinical symptom (4).

A recent multicenter study suggested that Magnetic Resonance Imaging (MRI) findings can differentiate Sarcoglycanopathies from other LGMDs and Dystrophinopathies. The study showed that thigh adductors, glutei, and posterior thigh muscles are the earliest and most severely affected muscles in sarcoglycan patients. Normal or relatively preserved MRI findings of distal leg muscles and sparing of distal quadriceps are characteristics of all the Sarcoglycanopathies (5).

The muscle biopsy of patients affected by LGMD2C-2E shows dystrophic features with a reduced or absent immunoreactivity for the mutated sarcoglycan and often a decreased signal for dystrophin and the other components of the DGC as a result of a protein destabilization of the whole structure and accelerated proteasomal degradation. With the exception of the

symptomatic physiotherapeutic treatment and the prevention of pulmonary, bone and metabolic complications, no causative therapy is now available for these patients.

However, the research activity working on these disorders has been recently very productive.

In the last few years, by studying the pathogenesis of LGMD2D, it has been established that the loss of function (LOF) condition is the consequence of the activation of the protein quality control system (QC) of the cell. In fact, the majority of LGMD2D genetic defects are originating from a folding-defective protein that is recognized by the endoplasmic reticulum-QC and delivered to degradation through the ubiquitin-proteasome system (6). Sandona et al. reported in 2018 the efficient recovery of αSG missense mutants in muscle cells treated with three different Cystic Fibrosis ΔF508-Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) correctors previously adopted for cystic fibrosis (7). The feasible mechanism of action of these compounds on the αSG mutants is the modulation of the biological capacity of the protein quality control network of the cells, i.e. the proteostasis regulation.

Next to the studies working at the protein level, two clinical trials centered on gene therapy replacement in patients affected by respectively LGMD-2D and LGMD-2E are currently ongoing.

The NCT03652259 “Phase I/II Gene Transfer Clinical Trial for LGMD2E using scAAVrh74.MHCK7.hSGCB by systemic infusion” is currently recruiting (Nationwide Children's Hospital, USA), while, the NCT01976091 “Phase I/IIa Dose escalation study of self-complementary AAVrh74.tMCK.hSGCA delivered to single leg and bilaterally via a major lower limb artery to the whole lower limb” has concluded the recruitment and is currently active (Nationwide Children's Hospital, USA) (8, 9).

Sarcoglycanopathies are also the focus of regenerative medicine. In 2012 Tedesco et al. obtained human induced pluripotent stem cells (iPSCs) from LGMD2D patients and induced them to differentiate into mesoangioblast-like cells that were then genetically corrected in vitro using a viral vector expressing the defective gene *SGCA*, which encodes α-SG. After intramuscular or intra-arterial injection of these genetically corrected, iPSCs-derived mesoangioblasts into mice with LGMD2D (immune-deficient Sgca-null mice), the cells homed to damaged mouse skeletal muscle, engrafted, and formed muscle fibers expressing α-SG (10).

Lastly, it should be underlined that the mechanically weaker plasma membrane lacking SGs, more easily damaged during muscle contraction, allows release of intracellular epitopes and infiltration of immune cells with chronic inflammation (Fig.2).

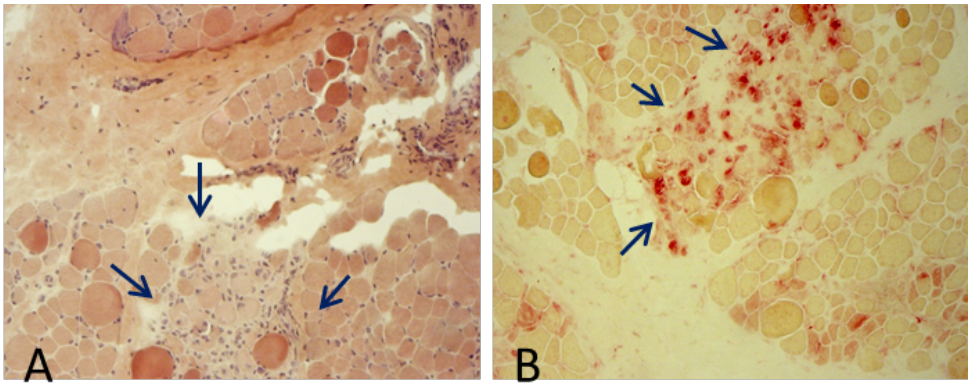

Fig. 2. Hematoxylin & Eosin (A) and Alkaline Phosphatase (B) analysis on a representative muscle biopsy of a patient affected by αSG deficit. Arrows show the inflammatory infiltrates.

It is conceivable that soluble inflammatory factors contribute to the progression of the dystrophic process by direct inhibition of muscle function and /or regeneration and by recruitment of immunological cells to the area of muscle damage. Accordingly, NCX 320, a derivative of ibuprofen, has been evidenced to improve pathogenic features of αSG deficiency in the αSG knockout (KO) model (Sgca) (11).

c) Chronic inflammation influences the evolution of muscle degeneration in sarcoglycan deficient muscular dystrophies.

Intriguingly, in addition to promoting muscle damage, the immune system facilitates also muscle regeneration and repair in muscular dystrophy. During the last decade, innate immunity, in particular, macrophages and their various polarization states M1-M2 have been considered as a central regulator of the tissue healing process in muscle tissue. However, recent evidences suggest that the adaptive immune system and in particular Tregs are a critical actors.

Tregs, particularly those expressing Foxp3+ (a transcription factor involved in their development and function), regulate the immune responses (12). They were originally described as controlling the activities of other T cells but were later recognized to regulate B cells and several innate immune system players. In muscle, Vetrone et al. first described that Treg could play a protective role in Duchenne Muscular Dystrophy (DMD) and showed an increased expression of FoxP3 mRNA in *mdx* mice characterized by a milder dystrophic phenotype (13).

More recently, it has been evidenced that in the muscles from patients affected by dystrophin deficiency Treg accumulate in sites of necrosis and display an activated phenotype with the

expression of IL10, a suppressive cytokine that was previously shown to reduce the pathology of muscular dystrophy in dystrophinopathic mice (14, 15). Next to their action on the myeloid populations infiltrating the degenerative tissue, Tregs can exert a direct stimulatory effect on satellite cells by expressing Amphyregulin (Areg), which enhances satellite cell differentiation in vivo and in vitro (16). Consistently, Tregs ablation following treatment with diphtheria toxin in Foxp3DTR mice or following treatment with the specifically depleting anti-CD25mAb targeting CD4+CD25hi Tregs increases muscle damage in dystrophic mice (15).

While extensive studies have been completed on Dystrophinopathies and innate/adaptive immunity, the role of the Tregs population in sarcoglycanopathies has not been studied yet. Moreover, the molecular mechanisms that on one side initiate and perpetuate inflammation and that on the other recruit and expand the muscle Tregs lymphocyte pool are poorly defined. Plausible candidates for the triggering of the tissue immune-mediated damage are the so-called DAMPS.

d) Extracellular ATP, a DAMP involved in innate and adaptive immunity.

A central tenet of the priming and development of an inflammatory response in the absence of infectious agents are the DAMPs (17). These self-molecules execute precise intracellular task in both innate and adaptive immune system and are able to exert disparate functions when released into the extracellular space. Among DAMPs, eATP and its derivates signal various physiological responses, including platelet activation and aggregation, immune responses, vascular tone, neurotransmission, nociception, cardiac function, tumor growth, renal transport, smooth-muscle contraction and apoptosis (17) (Fig.3). Nucleotides are released from cells into the extracellular fluids as result of cell lysis, exocytosis or efflux from transport/channel proteins. Once outside the cell, eATP actions are exerted through activation of plasma membrane receptors termed P2 receptors. P2X 1−7 receptors open to non-selective ion channels, whereas P2Y1, 2, 4, 6, 11-14 are guanine nucleotide-binding protein (G protein)-coupled receptors (GPCRs), which bind preferentially ADP, UDP, UTP or UDP-glucose. Activation of P2 receptors regulates many cellular functions ranging from survival and proliferation to apoptosis. The final effect of eATP on a given cell depends, therefore, on the composition of P2 receptors expressed on its surface. On the other hand, the activity of ATP in the extracellular milieu is tightly controlled by the combined action of ubiquitous ectoapyrase (CD39) and ecto-5'-nucleotidase (CD73), expressed for example by Tregs, which readily degrade ATP to adenosine (18) (Fig. 4). In the immune system, the purinergic signaling, in

particular P2X7 mediated signal transduction, has been intensely investigated in antigen presenting cells (APC) such as dendritic cells, macrophages and monocytes (19).

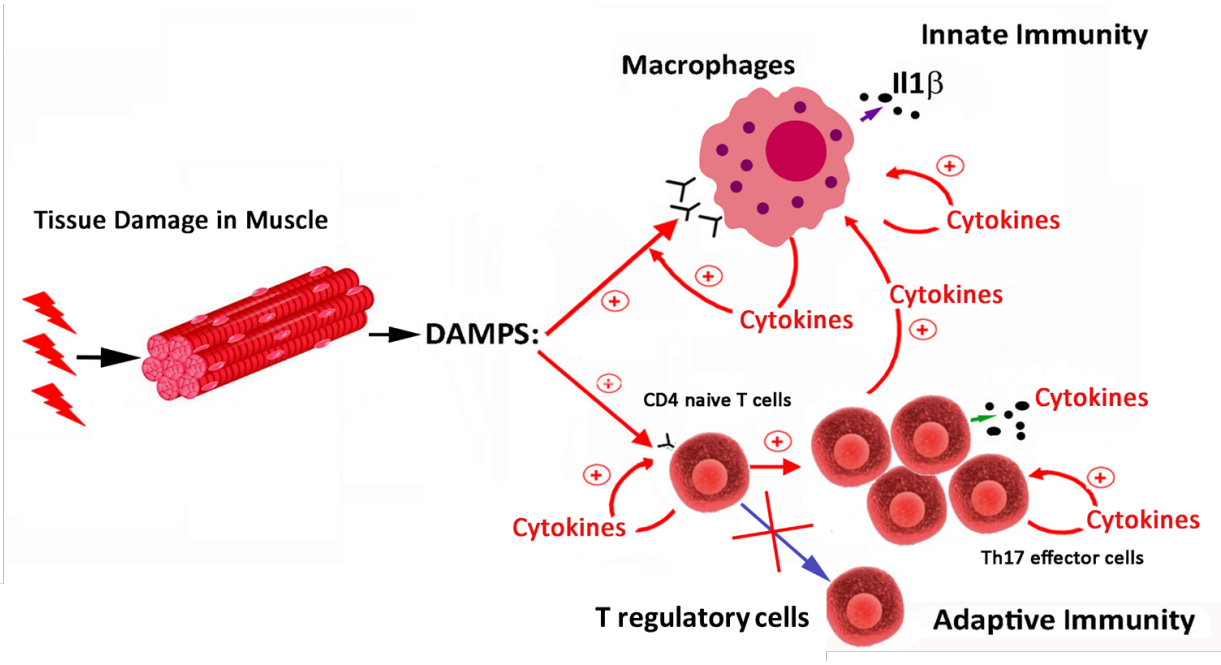

Fig. 3. The DAMP molecule ATP is released by cells in degeneration/necrosis and has the peculiar property to activate both arms of the immune system: (i) the innate immunity with consequent release of Il1β by macrophages and (ii) the adaptive immunity by stimulating the switch from naïve CD4 cells to Th17 lymphocytes and by inhibiting their differentiation in T regs.

eATP, released from the cytosol of dying cells, contributes to an efficient priming of APCs in the initial phase of the immune response and in mouse macrophages activation of P2X7 receptor by exogenous ATP is strictly required as second signal for IL1β processing and secretion in response to extracellular stimuli such as lipopolysaccharide (LPS) (20). Next to the well-documented role in inflammasome activation in cells of the innate immune system, eATP determines effector T cell activation as well as inhibition of Tregs function and stability, underscoring the potential value of P2X antagonism as a pharmacological tool to dampen inflammation (21).

Accordingly, blockade of eATP signaling cascade dramatically ameliorates the outcome of T cell-mediated inflammation in established experimental models of autoimmunity (22-24).

e) P2X receptors mediate inflammasome activation in muscle cells

Muscle cells are not silent actors in the inflammatory reaction that complements the dystrophic process but express P2X receptors and key components of the inflammasome pathway

suggesting that myofibers can exert a direct role in the development and maintenance of the immune-mediated damage

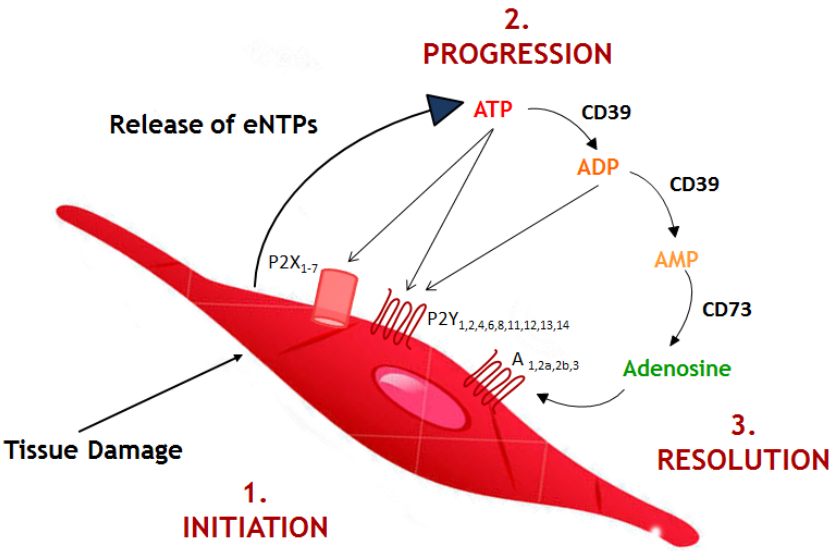

Fig. 4. In the extracellular space, the action of tissue ATP is counteracted by ecto ATPases, which ultimately lead to the formation of adenosine.

In primary myoblasts isolated from dysferlin-deficient mice and LGMD 2B patients, eATP triggers P2X7-mediated secretion of IL-1β (25).

Similarly, significant expression of P2X and, specifically, of the P2X7 receptor has been immunodetected in skeletal muscle from *mdx* mice and primary myoblasts from DMD patients and exposure to eATP in myoblasts of a dystrophin−negative muscle cell line stimulates a strong and sustained increase in cytoplasmic Ca++ concentrations (26, 27).

Rationale of the study

In muscle, ATP is primarily known for its function as an energy source, but, as a DAMP, it displays peculiar properties: availability in high concentrations within the cytoplasm of every cells, virtual absence in the extracellular space in healthy tissue, quick release following cell damage. These features render it an ideal harbinger of tissue damage in muscular dystrophies.

We proposed a model according to which eATP released by necrotic myofibers exerts a dual crucial role in the pathogenesis of SG deficiency or LGMD2D-2E:

1) **an indirect action** through the triggering of the innate and adaptive immunity and the inhibition of Tregs recruitment and 2) **a direct action** on the muscle cell through its own receptors by activating the inflammasome pathway and altering intracellular Ca++ balance.

We first started analyzing a model of αSG deficiency (LGMD2D). Indeed, αSG displays an ATP-binding site in its extracellular domain and is characterized by an ecto-ATPase activity (28). It is thus feasible to hypothesize that αSG can attenuate the magnitude and/or the duration of eATP-induced signals on the P2X receptors present on the muscle membrane, thus modulating its toxic effects.

Methods

In this section, we will describe the methodologies that are peculiar to this work or that required specific procedures. The more standardized techniques already described in the paper will be not further deepened.

a) Choice of the Mouse Model and Treatment Plan

The study was completed in the αSG knockout (KO) mouse model (Sgca) kindly donated by Prof. Giulio Cossu (San Raffaele Institute, Milan, Italy). This mouse line was first described in 1997 and recapitulates many features of the human disease, thus being suitable for preclinical research (29). In these mice the muscle necrosis starts already at 2 weeks of age and in the adult mice the skeletal muscles display a high number of small regenerative fibers and a higher expression of myogenic genes such as MyoD, Myogenin, Myf5 than in wild-type mice. In addition, fibrotic and fat infiltrates accompanied by signs of chronic inflammation progressively accumulates in Sgca muscles and heart. All these dystrophic hallmarks are most pronounced in diaphragm muscle. Functionally, Sgca mice perform significantly worse than the wild types in the two or four limb hanging tests, while the grip strength and the rotarod assessment are less significative. The hanging tests represent therefore the standard procedure in this mouse model for a more direct comparison of pre-clinical drug testing between laboratories (www.treat−nmd.eu/research/preclinical/SOPS).

Our analysis was completed in 4 weeks old male Sgca mice and age -matched wild types (Wt). The experiments were repeated in two separate 4 week trials which both included an n=5 -7 mice for each experimental group. Thus, the results are indicative of at least an n=10 animals per group.

The animals were treated for 4 weeks intraperitoneally with periodate-oxidase ATP (oATP) a compound largely used to realize a wide inhibition of P2X receptors. oATP is a Schiff-base that binds to amino groups on accessible lysine residues of the receptors. The compound is able to exert a wide inhibition of the P2X receptors although its efficacy is minor to the one displayed by new generation specific P2X7 or P2X4 purinergic antagonists. The dose and modalities of the treatment were based on our previous experience in *mdx* mice. Control Sgca mice were treated with vehicle alone (PBS).

At the end of the treatment the analysis included 1) clinical parameters (response to exercise, strength and coordination measurement, serum CK levels), 2) histopathological score (muscle morphology according to Standard Operating Procedures (SOP), 3) immunological characterization i.e. phenotype and function of T cells infiltrating the muscle tissues, 4) evaluation of parameters of fibrosis.

As concerns the muscle functional analysis, the Four Limb Hanging Test was completed at the beginning of the study and at the end of the 2nd and 4th week of treatment. Briefly, oATP-, PBS-treated Sgca and Wt controls mice were subjected to a 180-sec lasting hanging test, during which a "falling score" was recorded. In each time-points, all the mice had to hang for three trials, and the average maximum hanging time of the three trials was measured (SOP, http://www.treat-nmd.eu/research/preclinical/preclinical-efficacy-standards/).

b) Isolation of Primary Satellite Cells

Between the basal lamina that surrounds each muscle fiber and the plasma membrane of the muscle fiber are located mononuclear cells named *satellite cells* (SCs), which are still considered today the main players in skeletal muscle regeneration (30). The isolation of these cells allows realizing cultures of primary myoblasts. The procedure of purification from small muscle tissue fragments can be completed enzymatically or by cell-migration from a small explant of muscle tissue. In this paper we adopted the first methodology using an already standardized cocktail containing solution with Collagenase I (100 μg/ml) Dispase (500 μg/ml), and DNaseI (100 μg/ml) in PBS. Once plated, the cells reach confluence and hence are induced to differentiate in myotubes through a specific Differentiation Medium (DMEM, 10% donor horse serum, 1% L-Glutamine, and 1% Penicillin/Streptomycin, 1‰ gentamicin, 2, 5 ng/ml bFibroblast Growth Factor).

b) Ecto-ATPase Activity

The hydrolysis of ATP is of fundamental importance to the signaling cascades initiated by the presence of extracellular nucleotides.

Indeed, the content of eATP reflects the balance between ATP released from cells and its extracellular degradation, which is tightly regulated by the activity of ectoapyrases referred to as ecto-ATPases. These enzymes are found on the plasma membranes and associate with ATP-binding proteins. They hydrolyze the terminal phosphate residues of nucleoside triphosphates and diphosphates on the extracellular surface of cell. In concert, ecto-5′-nucleotidases (ecto-5′-NT) hydrolyze nucleotide monophosphates to their respective nucleosides. This latter enzyme is bound to the external surface of cell by glycosylphosphatidylinositol anchoring. Ectoapyrase, shown to express the known marker CD39, and ecto-5′-nucleotidase, identified with CD73, can be viewed as converting nontransportable nucleotides to transportable nucleosides. This sequential adenylate scavenger pathway can supply cells with required sources of purines when internal sources are depleted and can act as a feedback control mechanism when the levels of eATP are dangerously increased. Therefore, the action of nucleotide-hydrolyzing enzymes is essential to terminate the signaling, to generate new signaling molecules and to salvage purines.

Sandona et al. showed in 2004 that αSG in C2C12 cells behaves as an ecto-ATPase, whose activity strictly depends on the presence of bivalent cations (28).

In the present study ATP degradation was evaluated in primary myotubes isolated from Sgca and Wt age-matched controls by phosphate HPLC analysis.

In particular, once terminally differentiated, myotubes of each genotype were washed once with 1 ml Hank's balanced salt solution (HBSS) and 0.35 ml HBSS containing 0.3 mM ATP were added. At various times (0, 5, 15 min), 100-ul aliquots of the incubations were withdrawn and incubations were stopped by filtration with a multiscreen vacuum manifold using Immobilon-P membrane plates. ATP degradation was determined by the phosphate HPLC analysis, as previously described (31). Cells were lysed and protein content in each well was determined by Bradford assay.

c) Determination of apoptotic rate

Once differentiated into mature myotubes, the cells were pre-treated with LPS (1μg/mL) for 4 hours and then incubated with ATP (3mM) or BenzoylATP (BzATP) (300 μM), an ATP-analog, which selectively activates P2X7 receptors, for 16 hours. Then, myotubes were analyzed by flow-cytometry according to other studies that have adopted the same technique

(32). Specifically, the cells were stained with Annexin A5 FITC/7-AAD and apoptosis was evaluated by flow-cytometry according to the manufacturer's instructions. Sample analysis was performed using Gallios cytometer and Kaluza 1.1 softwares.

Results and Discussion

Sgca skeletal muscle displays an activation of innate and adaptive immunity.

The first question we addressed in the project is whether muscle tissue devoid of αSG displays an activation of an inflammatory cascade as it is extensively reported in the experimental models of dystrophin deficiency. Indeed, even though the mechanisms of disease are similar, (damage of a mechanically weaker plasma membrane, release of intracellular antigens, infiltration of immune cells, induction of pro-fibrotic cytokines and growth factors), the clinical and histological phenotype of Duchenne patients is in the vast majority of case more dramatic and limited are the clinical studies and the clinical experience on anti-inflammatory strategies in human sarcoglycanopathies. Likewise, although different experimental models have shown how distinct anti-inflammatory approaches may enhance stem cell therapy in Sgca mice, the molecular mechanisms that trigger the immune-mediated damage in this disorder have not been described yet. The first figure of the paper describes that when compared to Wt animals, αSG -deficient muscle tissue displayed higher expression levels of pro-inflammatory cytokines such as IL1β, IFNγ, and IL6. Macrophage surface-proteins as PTPRC (CD45), ADGRE1 (F4/80), and EGR2 were up regulated by respectively 7.5, 4.1 and 10 folds, while in the T cell population we detected a specific increase of CD4+ cells. This process was counteracted by an increase in the number of $Foxp3^{+}$ $CD4^{+}$ Treg, which also showed an activated phenotype as confirmed by the induced levels of the cytokine IL10 (Figure 1). These results were further confirmed by immunoblot and immunohistochemistry data (Figure 7, 8) through which we showed in αSG muscles an augmented content of the pro-inflammatory protein Activating Signal Cointegrator 1 (ASC1), an increased infiltration of CD45 leukocytes, of Ly6C+ macrophages (innate immunity) and of CD3 lymphocytes (adaptive immunity).

The DAMP molecule eATP is involved in Sgca inflammatory process.

1) Sgca muscle cells overexpress the purinergic receptor P2X7, are characterized by a defect in ecto-ATPase activity and undergo apoptosis upon ATP treatment.

Considered the presence of cells and mediators of type 1 and type 2 inflammations in αSG deficient muscle tissue, we aimed to evaluate the possible involvement of the DAMP molecule ATP in this complex cascade.

Sgca mice were characterized by an enhanced expression of P2X4 and P2X7 receptors in muscle tissue, confirming that αSG defects, as Dystrophinopathies, result into a purinergic pathway over activation (Figure 3). While P2X4R was mainly up regulated in $CD45^+$ inflammatory cells infiltrating the muscle, P2X7R was over expressed on the plasma membrane of Sgca muscle fibers. Noteworthy, in dystrophic cells the receptor molecules were found to be organized in specific patches (Figure 4). This same feature was described in the monocytic subset of myeloid derived suppressor cells isolated from neuroblastoma- bearing mice in which an increased activity of the receptor correlated with an increased segmental plasma membrane fluorescence (33).

The P2X7 receptor expressed in Sgca muscle cells is functional as primary myotubes isolated from this mouse model showed an increased susceptibility to ATP- and BzATP- apoptotic induced signal (Figure 2).

Furthermore, we confirmed the previous biochemical data developed in a heterologous cell system (HEK293) on αSG ecto-ATPase activity. Sgca muscle cells were indeed characterized by a decrease in ecto-ATPase activity resulting into a higher accumulation of ATP in the cell medium. This reinforces the hypothesis that the absence of αSG can less efficiently counteract ATP direct toxicity on the muscle cells (Figure 2).

2) Pharmacological inhibition of purinergic signaling *via* oATP led to an improvement of muscular function and structure, a reduction of the innate/adaptive immune response and fibrosis, and an increase in $Foxp3^+$ $CD4^+$Tregs muscle infiltration.

We further verified the relevance of the molecule eATP in the αSG dystrophic model by blocking it with a pharmacological agent and then dissecting the consequences on exercise performance, histological features of inflammation and fibrosis and on the representation of the inflammatory cell subsets in the muscle tissue.

The evaluation of muscle strength completed by Four Limb Hanging Test at the beginning of the treatment (time 0) and at the end of every week showed a stabilization of the limb weakness. The untreated group displayed a progressive worsening of muscle strength along time, while the oATP-Sgca cohort maintained the scores measured at time 0 (Figure 5).

Histological analyses performed on gastrocnemii and diaphragms confirmed a decrease of the area and the intensity of inflammatory reactions in oATP treated mice as evaluated by acid

phosphatase staining, which is positive in activated macrophages and myofibers in degeneration/necrosis (Figure 6). In accordance with this anti-inflammatory picture, P2X blockade led to a reduction of the transcription and protein levels of fibrogenic factors, which ultimately stimulate endomysial fibrosis and connective replacement of muscle tissue such as Osteopontin, Connective Tissue Growth Factor (CTGF) and Transforming Growth Factor-β (TGF−β) (Figure 9, 10).

Finally, the consequences of oATP treatment on innate and adaptive immune response in skeletal muscle of Sgca mice were measured by:
-Quantification of muscular Il-1β, Il-6, Interferon (IFN)γ and Transforming Nuclear Factor (TNF) α transcripts.
-Immunostaining and measurement of the CD45+ and Ly6C+ leucocytes infiltrating the muscle tissue.
-Measurement of the protein levels of Asc1, a co-activator of NF kappa β pathway.
-Immunostaining and measurement of the CD3 lymphocytes infiltrating the muscle tissue.
-Immunostaining and quantification of the transcripts of the Treg molecular marker Foxp3 in muscle lysates.
Altogether, this first immunological profile indicated that the activation of innate and adaptive immunity measurable in the αSG deficient muscle is reduced upon blockade of the DAMP molecule eATP (Figure 7, 8). Nonetheless the decrease in the total number of CD3 lymphocytes, the number of Foxp3+ infiltrating cells was though stable when not moderately increased in oATP-treated Sgca mutants confirming the inhibition of Treg pro-apoptotic ATP induced signal upon purinergic blockade.

Impact on Patients and Future Developments

The translational impact of the study covers distinct aspects.

1. We indicate that innate and adaptive immunity are triggered in muscles from a mouse model of αSG deficiency. Differently from DMD, patients affected by LGMD2D, when also severe, are not treated with corticosteroids. The rationale of corticosteroid adoption in Dystrophinopathies is mainly based on the inflammatory reaction observed in the muscle biopsies. Indeed, steroid treatment, when started in ambulant boys, leads to a stabilization of respiratory function for about 2 years.

As concerns sarcoglycanopathies, the scientific literature reports few isolated and contradictory descriptions and a definitive study on mouse models of sarcoglycanopathies is missing (34, 35). Moreover, the unveiling of an inflammatory response in sarcoglycan deficiencies does not justify *per se* the clinical use of corticosteroids. The mechanisms of steroid action seem to be much more complicated. The clinical trial NCT00527228 (http://www.ClincalTrials.gov) conducted in patients with Dysferlinopathies, another LGMD characterized by an immune reaction, clearly showed deleterious effects of deflazacort, the steroid routinely used in DMD patients.

Therefore, a first complete and careful assessment of steroid safety and effectiveness in experimental models of sarcoglycan deficiency followed by appropriate clinical trial is mandatory before following this therapeutical route in these patients.

2. oATP was originally described as an irreversible P2X7R antagonist but was shown later to block also other P2XRs such as the P2X4 subtype, making this compound a good candidate for a first proof-a-principle of our hypothesis in Sgca mice, where we found an up regulation of both P2X7 and P2X4 receptors.
However, numerous other P2XR antagonists, more efficient and more specific, have been discovered in the past few years, especially anti-P2X7R. CE-224,535 has been tested in clinical trials for rheumatoid arthritis in patients with an inadequate response to methotrexate. The drug was not efficacious, compared with placebo, but demonstrated an acceptable safety and tolerability profile (NCT00628095). The purinergic P2X7 antagonist AZD9056 showed to have the potential to improve symptoms in patients with moderate-to-severe Crohn's disease combined with a beneficial risk profile. AZD9056 was well tolerated, and no serious adverse events were reported. The molecule GSK1482160 has already been explored as a possible tool to detect neuroinflammation, and a phase I clinical study in humans is currently undergoing (NCT00849134). Our study suggests the further analysis of these specific compounds in preclinical trials in Sgca or *mdx* mice (36-40).

3. The consequences of ATP inhibition on the stability and function of lymphocyte Treg are particularly intriguing since the potential clinical value of targeting this T cell pool in muscle diseases. Indeed, Treg exclusive immunosuppressive properties have already prompted a number of attempts to exploit them in cell therapy protocols both in murine models of autoimmunity and human graft versus host disease (NCT02088931, NCT02145325, NCT01210664, NCT02188719, NCT01624077, www.clinicaltrials.gov).

4. The adoption of CRISPR)/CRISPR-associated (Cas) protein has unprecedentedly increased the flexibility and versatility of gene editing. It changed perspectives and opened our vision towards effectively edited genes for therapy. However, cytotoxic and regulatory immune mechanisms can interfere with genetically edited muscle cells. The mechanisms are not known but may be of pivotal importance for the overall success of gene editing strategies in muscular dystrophies. Indeed, the clinical trials in progress for DMD are inexorably confirming that only a combined approach addressing genetic, inflammatory and metabolic aspects has the real potential to treat these disorders.

The observation that aSG deficient primary muscle cells display a higher sensitivity to DAMP signals underlies the direct involvement of muscle tissue in the immune-mediated damage. Purpose of our studies is to evaluate how the inflammatory niche contributes to endogenous repair and influence the fate of gene-edited cells. It is critical to unveil the mechanisms through which differentiated dystrophic cells signal to the innate and adaptive immune system and to dissect how these pathways are reversed and/or affected in gene edited cells.

References

1. Mercuri E, Muntoni F. Muscular dystrophies. Lancet. 2013, 381:845-60.
2. Fairclough RJ, Wood MJ, Davies KE. Therapy for Duchenne muscular dystrophy: renewed optimism from genetic approaches. Nat Rev Genet. 2013, 14:373-8.
3. Liewluck T, Milone M. Untangling the complexity of limb-girdle muscular dystrophies. Muscle Nerve. 2018, 58:167-177.
4. Kirschner J, Lochmuller H: Sarcoglycanopathies. Handb Clin Neurol 2011, 101:41-46.
5. Tasca G, Monforte M, Díaz-Manera J, Brisca G, Semplicini C, D'Amico A, Fattori F, Pichiecchio A, Berardinelli A, Maggi L, Maccagnano E, Løkken N, Marini-Bettolo C, Munell F, Sanchez A, Alshaikh N, Voermans NC, Dastgir J, Vlodavets D, Haberlová J, Magnano G, Walter MC, Quijano-Roy S, Carlier RY, van Engelen BGM, Vissing J, Straub V, Bönnemann CG, Mercuri E, Muntoni F, Pegoraro E, Bertini E, Udd B, Ricci E, Bruno C. MRI in sarcoglycanopathies: a large international cohort study. Neurol Neurosurg Psychiatry. 2018 Jan;89(1):72-77.
6. Bianchini E, Fanin M, Mamchaoui K, Betto R, Sandonà D. Unveiling the degradative route of the V247M α-sarcoglycan mutant responsible for LGMD-2D. Hum Mol Genet. 2014, 23:3746-58.
7. Carotti M, Marsolier J, Soardi M, Bianchini E, Gomiero C, Fecchio C, Henriques SF, Betto R, Sacchetto R, Richard I, Sandonà D. Repairing folding-defective α-sarcoglycan mutants by CFTR correctors, a potential therapy for limb-girdle muscular dystrophy 2D. Hum Mol Genet. 2018, 27:969-984.
8. Rodino-Klapac LR, Lee JS, Mulligan RC, Clark KR, Mendell JR: Lack of toxicity of alpha-sarcoglycan overexpression supports clinical gene transfer trial in LGMD2D. Neurology 2008, 71: 240-247
9. Mendell JR, Rodino-Klapac LR, Rosales XQ, Coley BD, Galloway G, Lewis S, Malik V, Shilling C, Byrne BJ, Conlon T, Campbell KJ, Bremer WG, Taylor LE, Flanigan KM, Gastier-Foster JM, Astbury C, Kota J, Sahenk Z, Walker CM, Clark KR. Sustained alpha-sarcoglycan gene expression after gene transfer in limb-girdle muscular dystrophy, type 2D. Ann. Neurol. 2010, 68:629-638.
10. Tedesco FS, Gerli MF, Perani L, Benedetti S, Ungaro F, Cassano M, Antonini S, Tagliafico E, Artusi V, Longa E, Tonlorenzi R, Ragazzi M, Calderazzi G, Hoshiya H, Cappellari O, Mora M, Schoser B, Schneiderat P, Oshimura M, Bottinelli R,

Sampaolesi M, Torrente Y, Broccoli V, Cossu G. Transplantation of genetically corrected human iPSC-derived progenitors in mice with limb-girdle muscular dystrophy. Sci Transl Med. 2012, 4:140ra89.

11. Sciorati C, Miglietta D, Buono R, Pisa V, Cattaneo D, Azzoni E, Brunelli S, Clementi E. A dual acting compound releasing nitric oxide (NO) and ibuprofen, NCX 320, shows significant therapeutic effects in a mouse model of muscular dystrophy. Pharmacol Res. 2011, 64:210-7.
12. Lei H, Schmidt-Bleek K, Dienelt A, Reinke P, Volk HD: Regulatory T cell-mediated anti-inflammatory effects promote successful tissue repair in both indirect and direct manners. Front Pharmacol 2015, 6:184.
13. Vetrone SA, Montecino-Rodriguez E, Kudryashova E, Kramerova I, Hoffman EP, Liu SD, Miceli MC, Spencer MJ. Osteopontin promotes fibrosis in dystrophic mouse muscle by modulating immune cell subsets and intramuscular TGF-beta. J Clin Invest. 2009, 119:1583-94.
14. Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, Jang Y, Sefik E, Tan TG, Wagers AJ, Benoist C, Mathis D: A special population of regulatory T cells potentiates muscle repair. Cell2013, 155:1282-1295.
15. Villalta SA, Rosenthal W, Martinez L, Kaur A, Sparwasser T, Tidball JG, Margeta M, Spencer MJ, Bluestone JA. Regulatory T cells suppress muscle inflammation and injury in muscular dystrophy. Sci Transl Med. 2014, 6:258ra142.
16. Schiaffino S, Pereira MG, Ciciliot S, Rovere-Querini P: Regulatory T cells and skeletal muscle regeneration. FEBS J 2017, 284:517-524.
17. Rubartelli A, Lotze MT: Inside, outside, upside down: damage−associated molecular−pattern molecules (DAMPs) and redox. Trends Immunol 2007, 28:429−436.
18. Morandi F, Horenstein AL, Rizzo R, Malavasi F. The Role of Extracellular Adenosine Generation in the Development of Autoimmune Diseases. Mediators Inflamm. 2018, 2018:7019398
19. Dosch M, Gerber J, Jebbawi F, Beldi G. Mechanisms of ATP Release by Inflammatory Cells. Int J Mol Sci. 2018, 19(4).
20. Di Virgilio F, Sarti AC, Grassi F. Modulation of innate and adaptive immunity by P2X ion channels. Curr Opin Immunol. 2018, 52:51-59.

21. Schenk U, Westendorf AM, Radaelli E, Casati A, Ferro M, Fumagalli M, Verderio C, Buer J, Scanziani E, Grassi F: Purinergic control of T cell activation by ATP released through pannexin−1 hemichannels. Sci Signal 2008, 1, ra6.
22. Vergani A, Fotino C, D'Addio F, Tezza S, Podetta M, Gatti F, Chin M, Bassi R, Molano RD, Corradi D, Gatti R, Ferrero ME, Secchi A, Grassi F, Ricordi C, Sayegh MH, Maffi P, Pileggi A, Fiorina P: Effect of the purinergic inhibitor oxidized ATP in a model of islet allograft rejection. Diabetes 2013, 62:1665-1675.
23. Kurashima Y, Amiya T, Nochi T, Fujisawa K, Haraguchi T, Iba H, Tsutsui H, Sato S, Nakajima S, Iijima H, Kubo M, Kunisawa J, Kiyono H: Extracellular ATP mediates mast cell-dependent intestinal inflammation through P2X7 purinoceptors. Nat Commun.2012, 3:1034.
24. Koo TY, Lee JG, Yan JJ, Jang J, Ju KD, Oh KH, Ahn C, Jang J: The P2X7 receptor antagonist, oxidized adenosine triphosphate, ameliorates renal ischemia-reperfusion injury by expansion of regulatory T cells. Kidney Int2017, S0085-2538:30097-30102.
25. Rawat R, Cohen TV, Ampong B, Francia D, Henriques-Pons A, Hoffman EP, Nagaraju K: Inflammasome up-regulation and activation in dysferlin-deficient skeletal muscle. Am J Pathol 2010, 176: 2891-2900.
26. Gazzerro E, Baldassari S, Assereto S, Fruscione F, Pistorio A, Panicucci C, Volpi S, Perruzza L, Fiorillo C, Minetti C, Traggiai E, Grassi F, Bruno C: Enhancement of Muscle T Regulatory Cells and Improvement of Muscular Dystrophic Process in mdx Mice by Blockade of Extracellular ATP/P2X Axis. Am J Pathol 2015, 185:3349-3360.
27. Sinadinos A, Young CN, Al-Khalidi R, Teti A, Kalinski P, Mohamad S, Floriot L, Henry T, Tozzi G, Jiang T, Wurtz O, Lefebvre A, Shugay M, Tong J, Vaudry D, Arkle S, doRego JC, Górecki DC: P2RX7 purinoceptor: a therapeutic target for ameliorating the symptoms of Duchenne muscular dystrophy. PLoS Med 2015, 12:e1001888.
28. Sandonà D, Gastaldello S, Martinello T, Betto R: Characterization of the ATP-hydrolyzing activity of alpha-sarcoglycan. Biochem J 2004, 381:105-112.
29. Duclos F, Straub V, Moore SA, Venzke DP, Hrstka RF, Crosbie RH, Durbeej M, Lebakken CS, Ettinger AJ, van der Meulen J, Holt KH, Lim LE, Sanes JR, Davidson BL, Faulkner JA, Williamson R, Campbell KP. Progressive muscular dystrophy in alpha-sarcoglycan-deficient mice. J Cell Biol. 1998, 142: 1461-71.

30. Feige P, Brun CE, Ritso M, Rudnicki MA. Orienting Muscle Stem Cells for Regeneration in Homeostasis, Aging, and Disease. Cell Stem Cell. 2018, 23:653-664.
31. Bruzzone S, Basile G, Chothi MP, Nobbio L, Usai C, Jacchetti E, Schenone A, Guse AH, Di Virgilio F, De Flora A, Zocchi E Diadenosine homodinucleotide products of ADP-ribosyl cyclases behave as modulators of the purinergic receptor P2X7. J Biol Chem. 2010, 285:21165-74.
32. Muratore M1, Srsen V, Waterfall M, Downes A, Pethig R. Biomarker-free dielectrophoretic sorting of differentiating myoblast multipotent progenitor cells and their membrane analysis by Raman spectroscopy. Biomicrofluidics. 2012, 6:34113.
33. Bianchi G, Vuerich M Pellegatti P, Marimpietri D, Emionite L, Marigo I, Bronte V, Di Virgilio F, Pistoia V, Raffaghello L. ATP/P2X7 axis modulates myeloid-derived suppressor cell functions in neuroblastoma microenvironment. Cell Death Dis. 2014, 5:e1135.
34. Albuquerque MA, Abath-Neto O, Maximino JR, Chadi G, Zanoteli E, Reed UC. Clinical aspects of patients with sarcoglycanopathies under steroids therapy. Arq Neuropsiquiatr. 2014, 72:768-72.
35. Bauer R, Macgowan GA, Blain A, Bushby K, Straub V. Steroid treatment causes deterioration of myocardial function in the {delta}-sarcoglycan-deficient mouse model for dilated cardiomyopathy. Cardiovasc Res. 2008, 79:652-61.
36. Caseley EA, Muench SP, Fishwick CW, Jiang LH: Structure-based identification and characterization of structurally novel human P2X7 receptor antagonists. Biochem Pharmacol 2016, 15:130-139.
37. Keystone EC, Wang MM, Layton M, Hollis S, Mcinnes IB: Clinical evaluation of the efficacy of the P2X7 purinergic receptor antagonist AZD9056 on the signs and symptoms of rheumatoid arthritis in patients with active disease despite treatment with methotrexate or sulphasalazine. Ann Rheum Dis 2011, 143578.
38. Stock TC, Bloom BJ, Wei N, Ishaq S, Park W, Wang X, Gupta P, Mebus CA: Efficacy and safety of CE-224,535, an antagonist of P2X7 receptor, in treatment of patients with rheumatoid arthritis inadequately controlled by methotrexate. J Rheumatol2012, 39:720–727.
39. Eser A, Colombel J-F, Rutgeerts P, Vermeire S, Vogelsang H, Braddock M, Persson T, Reinisch W: Safety and efficacy of an oral inhibitor of the purinergic receptor P2X7 in adult patients with moderately to severely active Crohn's disease: a

randomized placebo-controlled, double-blind, phase IIa study. Inflamm Bowel Dis 2015, 21:2247–2253.

40. Han J, Liu H, Liu C, Jin H, Perlmutter JS, Egan TM,Tu Z. Pharmacologic characterizations of a P2X7 receptor-specific radioligand, [11C]GSK1482160 for neuroinflammatory response. Nucl Med Commun. 2017, 38:372-382.

IV. Eidesstattliche Versicherung

„Ich, Elisabetta Gazzerro, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Rolle von dem Extrazellulären ATP in der Progression der Muskeldegeneration in Sarkoglykanopathien“ „Role of Extracellular ATP in the Progression of Muscle Damage in Sarcoglycanopathies“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen werden von mir verantwortet.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.
Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Berlin, 21.01.2018 Unterschrift

V. Ausführliche Anteilserklärung an der erfolgten Publikation

Frau Elisabetta Gazzerro hat den Artikel zusammen mit Herr Dr. Claudio Bruno gemeinsam verfasst.
Alleinige Verfasserin ist sie von den Abschnitten: „Materials and Methods and Results“. Frau Gazzerro hat die Abbildungen mittels Bildbearbeitungsprogrammen hauptverantwortlich zusammengestellt. Sie wurde dabei unterstützt von Frau Stefania Assereto und Frau Serena Baratto.
Beitrag im Einzelnen:

- Experimente mit Versuchstieren (Mäuse): Bei der Zuchtplanung und -kontrolle, Ausführung von dem Four-Limb-Hanging Test hat sie die Mitarbeiter Frau Simona Baldassari, Frau Stefania Assereto, Herr Davide De Battista koordiniert. Die Isolierung des Muskelgewebes hat sie persönlich mit den Kollegen ausgeführt.
- **Bild 1**: Für die Real-Time PCR Daten hat sie die statistische Auswertung vorgenommen die Ergebnisse zusammengestellt und graphisch dargestellt.
- **Bild 2**: Sie hat zusammen mit Frau Dr. Graziella Messina die Zellkultur von Myoblasten (Labormethode) etabliert. Die Daten aller nachgeführten Experimente hat Frau Gazzerro analysiert: Proteinaufbereitung und -analyse, biochemischer ATP-Messungen (Frau Prof. Santina Bruzzone) und Zytofluorimetrie zur Apoptosemessung (Frau Dr. Chiara Panicucci und Lizzia Raffaghello).
- **Bild 3**: Für die Real-Time PCR hat sie die statistische Auswertung der Daten und das Design der Graphiken durchgeführt.
- **Bild4**: Die immunhistochemische Fluoreszenzfärbung und die histologische Aufbereitung sowie jeweilige Mikroskopie hat Frau Gazzerro gemeinsam mit Herr Dr. Paolo Scudieri durchgeführt.
- **Bild 5**: Den Four-Limb-Hanging Test und die Messung der CK Werte (Blutwert) hat sie gemeinsam mit Frau Simona Baldassari und Frau Stefania Assereto durchgeführt.
- **Bild 6**: Bei der morphologische Analyse des Muskelgewebes hat sie die Gewebeschnitte zusammen mit Frau Stefania Assereto analysiert. Für die Bildbearbeitung und Analyse hat sie mit Herr Paolo Scudieri kollaboriert.

- **Bild 7**: Die immunhistochemische Fluoreszenzfärbung und Mikroskopie hat sie mit Herr Dr. Paolo Scudieri vorgenommen. Für die Immunoblot Analyse der ASC-1 Werte hat sie die Arbeit von Frau Serena Baratto koordiniert und die Daten alleine analysiert. Die Daten hat sie gemeinsam mit Frau Dr. Elisabetta Traggiai und Herr Professor Fabio Grassi diskutiert.
- **Bild 8**: Die immunhistochemische Fluoreszenzfärbung und Mikroskopie hat sie mit Herr Dr. Paolo Scudieri vorgenommen. Für die Experimente mit Real-Time PCR hat sie die statistische Auswertung der Daten und das Design der Graphiken durchgeführt.
- **Bild 9**: Bei den Experimenten mit Real-Time PCR hat sie die statistische Auswertung der Daten und das Design der Graphiken durchgeführt.
- **Bild 10**: Bei der Mikroskopie und Analyse (Masson Färbung) hat sie alle Experimente durchgeführt. Für die Immunoblot Analyse hat sie mit Frau Stefania Assereto zusammengearbeitet.

Unterschrift, Datum und Stempel der betreuenden Hochschullehrer/der betreuenden Hochschullehrerinnen

_____

Unterschrift des Doktoranden/der Doktorandin

_____

VI. Auszug aus der Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI
Selected Categories: **"PATHOLOGY"** Selected Category Scheme: WoS
Gesamtanzahl: 79 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	Annual Review of Pathology-Mechanisms of Disease	4,402	15.952	0.009920
2	ACTA NEUROPATHOLOGICA	18,783	15.872	0.041490
3	MODERN PATHOLOGY	13,649	6.655	0.022670
4	Seminars in Immunopathology	2,967	6.437	0.009290
5	JOURNAL OF PATHOLOGY	16,156	6.253	0.024060
6	BRAIN PATHOLOGY	4,952	6.187	0.007750
7	NEUROPATHOLOGY AND APPLIED NEUROBIOLOGY	3,654	6.059	0.006350
8	AMERICAN JOURNAL OF SURGICAL PATHOLOGY	20,873	5.878	0.023060
9	JOURNAL OF MOLECULAR DIAGNOSTICS	3,818	4.880	0.009420
10	CELLULAR ONCOLOGY	1,322	4.761	0.002020
11	Disease Models & Mechanisms	4,485	4.398	0.014760
12	LABORATORY INVESTIGATION	10,461	4.254	0.010460
13	AMERICAN JOURNAL OF PATHOLOGY	39,201	4.069	0.034310
14	CANCER CYTOPATHOLOGY	2,544	3.866	0.004380
15	ARCHIVES OF PATHOLOGY & LABORATORY MEDICINE	9,834	3.658	0.011600
16	JOURNAL OF NEUROPATHOLOGY AND EXPERIMENTAL NEUROLOGY	9,252	3.490	0.008680
17	EXPERT REVIEW OF MOLECULAR DIAGNOSTICS	2,554	3.326	0.005320
18	HISTOPATHOLOGY	9,839	3.267	0.013370
19	HUMAN PATHOLOGY	13,345	3.125	0.015480
20	PATHOLOGY	2,638	3.068	0.004460
21	DISEASE MARKERS	3,183	2.949	0.007020
22	VIRCHOWS ARCHIV	5,897	2.936	0.007480
23	JOURNAL OF CLINICAL PATHOLOGY	11,052	2.894	0.008940
24	CYTOMETRY PART B-CLINICAL CYTOMETRY	1,517	2.757	0.002470
25	SEMINARS IN DIAGNOSTIC PATHOLOGY	1,285	2.655	0.002050
26	EXPERIMENTAL AND MOLECULAR PATHOLOGY	3,734	2.566	0.005590
27	HLA	407	2.558	0.000480
28	ENDOCRINE PATHOLOGY	1,180	2.541	0.001710
29	Brain Tumor Pathology	639	2.535	0.001120

1

Selected JCR Year: 2017; Selected Categories: "PATHOLOGY"

VII. Publikation

The danger signal extracellular ATP is involved in the immuno-mediated damage of alpha-sarcoglycan deficient muscular dystrophy.Gazzerro E, Baratto S, Assereto S, Baldassari S, Panicucci C, Raffaghello L, Scudieri P, De Battista D, Fiorillo C, Volpi S, Chaabane L, Malnati M, Messina G, Bruzzone S, Traggiai E, Grassi F, Minetti C, Bruno C. Am J Pathol. 2018 Nov 15. pii: S0002-9440(17)31150-1.
https://doi.org/10.1016/j.ajpath.2018.10.008
PMID: 30448410

VIII. Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

IX. Wissenschaftlichen Veröffentlichungen

1. Murialdo G, Galimberti CA, Magri F, Sampaolo P, Copello F, Granelli M, Gazzerro E., Rollero A, Deagatone C, Mani R, Ferrari E, Polleri A, Tartara A. Menstrual cycle and ovary alterations in women with epilepsy on anti-epileptic therapy. J Endocrinol Invest. 1997; 20:519-526.

2. Rollero A, Murialdo G, Fonzi S, Garrone S, Granelli M, Gazzerro E, Barreca A, Polleri A. Relationship between cognitive function, growth hormone and insulin-like growth factor I plasma levels in aged subjects. Neuropsychobiology 1998; 38:73-79.

3. Gazzerro E, Gangji V, Canalis E Bone morphogenetic proteins induce the expression of noggin, which limits their activity in cultured rat osteoblasts. J Clin Invest. 1998; 12:2106-2114.

4. Gazzerro E, Rydziel S, Canalis E. Skeletal bone morphogenetic proteins suppress the expression of collagenase 3 by rat osteoblasts. Endocrinology 1999; 140:562-567.

5. Gazzerro E, Du Z, Devlin R, Rydziel S, Priest L, Economides AN, Canalis E. Noggin arrests stromal cell differentiation in vitro. Bone 2003; 32:111-119.

6. Canalis E, Economides AN, Gazzerro E. Bone morphogenetic proteins, their antagonists and the skeleton. Endocrine Reviews 2003; 24:218-235.

7. Gazzerro E and Canalis E. Glucocorticoid-induced osteoporosis EndoTrends 2004; 11:6-9.

8. Sciaudone M*, Gazzerro E. *, Priest L, Delany A, Canalis E. Notch1 impairs osteoblastic cell differentiation. Endocrinology 2003; 44:5631-9.

9. Cetani F, Pardi E, Viacava P, Pollina GD, Fanelli G, Picone A, Borsari S, Gazzerro E, Miccoli P, Berti P, Pinchera A, Marcocci C. A reappraisal of the Rb1 gene abnormalities in the diagnosis of parathyroid cancer. Clin Endocrinol. 2004; 60:99-106.

10. Palermo C, Manduca P, Gazzerro E, Foppiani L, Barreca A. Potentiating role of insulin-like growth factor binding protein (IGFBP)-2 on IGF-II stimulated alkaline phosphatase activity in differentiating osteoblasts. Am J Physiol Endocrinol Metab. 2004; 286:648-657.

11. Cetani F, Pardi E, Borsari S, Viacava P, Dipollina G, Cianferotti L, Ambrogini E, Gazzerro E, Colussi G, Berti P, Miccoli P, Pinchera A, Marcocci C. Genetic analyses of the

HRPT2 gene in primary hyperparathyroidism: germline and somatic mutations in familial and sporadic parathyroid tumors. J Clin Endocrinol Metab. 2004; 89:5583-91.

12. Gazzerro E, Pereira RCP, Jorgetti V, Olson S, Economides AN, Canalis E. Skeletal overexpression of gremlin impairs bone formation and causes osteopenia. Endocrinology 2005; 146:655-65.

13. Gazzerro E, Deregowski V, Vaira S, Canalis E. Twisted gastrulation inhibits bone morphogenetic protein action and prevents osteoblast cell differentiation in vitro. Endocrinology 2005; 146:3875-82.

14. Canalis E, Deregowski V, Pereira R, Gazzerro E Novel signals determining skeletal cell fate and function. J Endocrinol Invest. 2005; 28(8 Suppl):3-7.

15. Parisi MS, Gazzerro E, Rydziel S, Canalis E. Expression and regulation of CCN genes in murine osteoblasts. Bone. 2006; 38:671-7.

16. Deregowski V, Gazzerro E, Priest L, Rydziel S, Canalis E. Notch 1 overexpression inhibits osteoblastogenesis by suppressing Wnt/β-catenin but not bone morphogenetic protein signaling. J Biol Chemistry. 2006; 281:6203-10.

17. Deregowski V, Gazzerro E, Priest L, Rydziel S, Canalis E Role of the Ram domain and Ankyrin repeats on Notch signaling and activity. J Bone Miner Res. 2006; 21:1317-26.

18. Gazzerro E and Canalis E Skeletal actions of insulin-like growth factors. Expert Review in Endocrinology and Metabolism. 2006; 1: 47-56.

19. Gazzerro E, Deregowski V, Stadmeyer L, Gale NW, Economides AN, Canalis E. Twisted gastrulation, a bone morphogenetic protein agonist/antagonist, is not required for post-natal skeletal function. Bone. 2006; 39:1252-60.

20. Zara F, Biancheri R, Bruno C, Bordo L, Assereto S, Gazzerro E, Sotgia F, Wang XB, Gianotti S, Stringara S, Pedemonte M, Uziel G, Rossi A, Schenone A, Tortori-Donati P, van der Knaap MS, Lisanti MP, Minetti C. Deficiency of hyccin, a newly identified membrane protein, causes hypomyelination and congenital cataract. Nat Genet. 2006; 38:1111-3.

21. Gazzerro E, Canalis E. Bone morphogenetic proteins and their antagonists.Rev Endocr Metab Disord. 2006; 7:51-65.

22. Gazzerro E, Minetti C. Potential drug targets within bone morphogenetic protein signaling pathways. Curr Opin Pharmacol. 2007; 7:325-33.

23. Bonuccelli G, Sotgia F, Capozza F, Gazzerro E, Minetti C, Lisanti MP. Localized treatment with a novel FDA-approved proteasome inhibitor blocks the degradation of dystrophin and dystrophin-associated proteins in mdx mice. Cell Cycle. 2007; 6:1242-8.

24. Biancheri R, Zara F, Bruno C, Rossi A, Bordo L, Gazzerro E, Sotgia F, Pedemonte M, Scapolan S, Bado M, Uziel G, Bugiani M, Lamba LD, Costa V, Schenone A, Rozemuller AJ, Tortori-Donati P, Lisanti MP, van der Knaap MS, Minetti C. Phenotypic characterization of hypomyelination and congenital cataract. Ann Neurol. 2007; 62:121-7.

25. Gazzerro E, Smerdel-Ramoya A, Zanotti S, Stadmeyer L, Durant D, Economides AN, Canalis E. Conditional deletion of gremlin causes a transient increase in bone formation and bone mass. J Biol Chem. 2007; 282:31549-57.

26. Bruno C, Sotgia F, Gazzerro E, Minetti C, Lisanti MP. Caveolinopathies In: Pagon RA, Bird TD, Dolan CR, Stephens K, editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-.2007

27. Traverso Ma, Gazzerro Ea, Assereto S, Sotgia F, Biancheri R, Stringara S, Giberti L, Pedemonte M, Wang X, Scapolan S, Pasquini E, Donati MA, Zara F, Lisanti MP, Bruno C, Minetti C. Caveolin-3 T78M and T78K missense mutations lead to different phenotypes in vivo and in vitro. Lab Invest. 2008; 88:275-83.

28. Raz R, Stricker S, Gazzerro E, Clor JL, Witte F, Nistala H, Zabski S, Pereira RC, Stadmeyer L, Wang X, Gowen L, Sleeman MW, Yancopoulos GD, Canalis E, Mundlos S, Valenzuela DM, Economides AN. The mutation ROR2W749X, linked to human BDB, is a recessive mutation in the mouse, causing brachydactyly, mediating patterning of joints and modeling recessive Robinow syndrome. Development. 2008; 135:1713-23.

29. Traverso M, Bruno C, Broccolini A, Sotgia F, Donati MA, Assereto S, Gazzerro E, Lo Monaco M, Modoni A, D'Amico A, Gasperini S, Ricci E, Zara F, Lisanti M, Minetti C. Truncation of Caveolin-3 causes autosomal-recessive Rippling Muscle Disease. J Neurol Neurosurg Psychiatry 2008; 79:735-7.

30. Assereto S, Mastrototaro M, Stringara S, Gazzerro E, Broda P, Nicchia GP, Svelto M, Bruno C, Nigro V, Lisanti MP, Frigeri A, Minetti C. Aquaporin-4 expression is severely reduced in human sarcoglycanopathies and Dysferlinopathies. Cell Cycle. 2008; 7:2199-207.

31. Biancheri R, Zara F, Bruno C, Gazzerro E, Rossi A, Van der Knaap MS, Minetti C. Hypomyelination and Congenital Cataract. In: Pagon RA, Bird TD, Dolan CR, Stephens K, editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-.2008

32. Gazzerro E, Assereto S, Bonetto A, Sotgia F, Bonuccelli G, Cilli M, Bruno C, Lisanti MP, Minetti C. Therapeutic potential of pharmacological proteasome inhibition in Duchenne and Becker Muscular Dystrophies. Am J Pathol. 2010; 176:1863-77.

33. Gazzerro E, Sotgia F, Bruno C, Lisanti MP, Minetti C. Caveolinopathies: from caveolin-3 cell biology to human diseases. Eur J Hum Genet. 2010; 18:137-45.

34. Grandis M, Gulli R, Cassandrini D, Gazzerro E, Benedetti L, Narciso E, Nobbio L, Bruno C, Minetti C, Bellone E, Reni L, Mancardi GL, Mandich P, Schenone A. The spectrum of GNE mutations: allelic heterogeneity for a common phenotype. Neurol Sci. 2010; 31:377-80.

35. Gazzerro E, Bonetto A, Minetti C Caveolinopathies translational implications of caveolin-3 in skeletal and cardiac muscle disorders. Handb Clin Neurol. 2011; 101:135-42.

36. Biancheri R, Zara F, Rossi A, Mathot M, Nassogne MC, Yalcinkaya C, Erturk O, Tuysuz B, Di Rocco M, Gazzerro E, Bugiani M, van Spaendonk R, Sistermans EA, Minetti C, van der Knaap MS, Wolf NI. Hypomyelination and congenital cataract: broadening the clinical phenotype. Arch Neurol. 2011; 68:1191-4.

37. Striano P, Coppola A, Paravidino R, Malacarne M, Gimelli S, Robbiano A, Traverso M, Pezzella M, Belcastro V, Bianchi A, Elia M, Falace A, Gazzerro E, Ferlazzo E, Freri E, Galasso R, Gobbi G, Molinatto C, Cavani S, Zuffardi O, Striano S, Ferrero GB, Silengo M, Cavaliere ML, Benelli M, Magi A, Piccione M, Dagna Bricarelli F, Coviello DA, Fichera M, Minetti C, Zara F. Clinical significance of rare copy number variations in epilepsy: a case-control survey using microarray-based comparative genomic hybridization. Arch Neurol. 2012; 69:322-30.

38. Bello L, Piva L, Barp A, Taglia A, Picillo E, Vasco G, Pane M, Previtali SC, Torrente Y, Gazzerro E, Motta MC, Grieco GS, Napolitano S, Magri F, D'Amico A, Astrea G, Messina S, Sframeli M, Vita GL, Boffi P, Mongini T, Ferlini A, Gualandi F, Soraru' G, Ermani M, Vita G, Battini R, Bertini E, Comi GP, Berardinelli A, Minetti C, Bruno C, Mercuri E, Politano L, Angelini C, Hoffman EP, Pegoraro E. Importance of SPP1 genotype as a covariate in clinical trials in Duchenne muscular dystrophy. Neurology. 2012; 79:159-62.

39. Gazzerro E, Baldassari S, Giacomini C, Musante V, Fruscione F, La Padula V, Biancheri R, Scarfì S, Prada V, Sotgia F, Duncan ID, Zara F, Werner HB, Lisanti MP, Nobbio L, Corradi A, Minetti C. Hyccin, the molecule mutated in the leukodystrophy hypomyelination and congenital cataract (HCC), is a neuronal protein. PLoS One. 2012; 7(3):e32180

40. Traverso M, Yuregir OO, Mimouni-Bloch A, Rossi A, Aslan H, Gazzerro E, Baldassari S, Fruscione F, Minetti C, Zara F, Biancheri R. Hypomyelination and congenital cataract: identification of novel mutations in two unrelated families. Eur J Paediatr Neurol. 2013; 17:108-11.

41. Fiorillo C, Brisca G, Cassandrini D, Scapolan S, Astrea G, Valle M, Scuderi F, Trucco F, Natali A, Magnano G, Gazzerro E, Minetti C, Arca M, Santorelli FM, Bruno C. Subclinical myopathy in a child with neutral lipid storage disease and mutations in the PNPLA2 gene. Biochem Biophys Res Commun. 2013; 430:241-4.

42. Gazzerro E, Andreu AL, Bruno C. Neuromuscular disorders of glycogen metabolism. Curr Neurol Neurosci Rep. 2013; 13:333.

43. Traverso M, Assereto S, Gazzerro E, Savasta S, Abdalla EM, Rossi A, Baldassari S, Fruscione F, Ruffinazzi G, Fassad MR, El Beheiry A, Minetti C, Zara F, Biancheri R. Novel FAM126A mutations in hypomyelination and congenital cataract disease. Biochem Biophys Res Commun. 2013; 439:369-72.

44. Assereto S, Robbiano A, Di Rocco M, Rossi A, Cassandrini D, Panicucci C, Brigati G, Biancheri R, Bruno C, Minetti C, Trucks H, Sander T, Zara F, Gazzerro E. Functional characterization of the c.462delA mutation in the NDUFS4 subunit gene of mitochondrial complex I. Clin Genet. 2014; 86:99-101.

45. Vanni N, Fruscione F, Ferlazzo E, Striano P, Robbiano A, Traverso M, Sander T, Falace A, Gazzerro E, Bramanti P, Bielawski J, Fassio A, Minetti C, Genton P, Zara F. Impairment of ceramide synthesis causes a novel progressive myoclonus epilepsy. Ann Neurol. 2014; 76:206-12.

46. Gazzerro E, Baldassari S, Assereto S, Fruscione F, Pistorio A, Panicucci C, Volpi S, Perruzza L, Fiorillo C, Minetti C, Traggiai E, Grassi F, Bruno C. Enhancement of muscle T regulatory cells and improvement of muscular dystrophic process in mdx mice by blockade of extracellular ATP/P2X Axis. Am J Pathol. 2015; 185:3349-60.

47. Baskin J, Wu X, Christiano R, Oh M, Schauder C, Gazzerro E, Messa M, Baldassari S, Assereto S, Biancheri R, Zara F, Minetti C, Raimondi A, Simons M, Walther T, Reinisch K, De Camilli P The leukodystrophy protein FAM126A/Hyccin regulates PI4P synthesis at the plasma membrane. Nat Cell Biol. 2016; 18:132-8.

48. Assereto S, Piccirillo R, Baratto S, Scudieri P, Fiorillo C, Massacesi M, Traverso M, Galietta LJ, Bruno C, Minetti C, Zara F, Gazzerro E. The ubiquitin ligase tripartite-motif-protein 32 is induced in Duchenne muscular dystrophy. Lab Invest. 2016; 96:862-71

49. Gazzerro E, Striano P. Gap Junctions and Epileptogenesis: No Laughing Matter. EBioMedicine. 2016; 8:5-6

50. Marsolier J, Laforet P, Pegoraro E, Vissing J, Richard I; Sarcoglycanopathies Working Group. First International Workshop on Clinical trial readiness for sarcoglycanopathies 15-16 November 2016, Evry, France. Neuromuscul Disord. 2017; 27:683-692.

51 Al-Khalidi R, Panicucci C, Cox P, Chira N, Róg J, Young CNJ, McGeehan RE, Ambati K, Ambati J, Zabłocki K, Gazzerro E, Arkle S, Bruno C, Górecki D. Zidovudine ameliorates pathology in the mouse model of Duchenne muscular dystrophy via P2RX7 purinoceptor antagonism. Acta Neuropathol Commun. 2018; 6(1):27.

52. Traverso M, Assereto S, Baratto S, Iacomino M, Pedemonte M, Diana MC, Ferretti M, Broda P, Minetti C, Gazzerro E, Madia F, Bruno C, Zara F, Fiorillo C. Clinical and molecular consequences of exon 78 deletion in DMD gene. J Hum Genet. 2018 63(6):761-764.

53. Meraviglia V, Benzoni P, Landi S, Murano C, Langione M, Motta BM, Baratto S, Silipigni R, Di Segni M, Pramstaller PP, DiFrancesco D, Gazzerro E, Barbuti A, Rossini A. Generation of human induced pluripotent stem cells (EURACi001-A, EURACi002-A, EURACi003-A) from peripheral blood mononuclear cells of three patients carrying mutations in the CAV3 gene. Stem Cell Res. 2018 Mar; 27:25-29.

54. Iannotti FA, Pagano E, Moriello AS, Alvino FG, Sorrentino NC, D'Orsi L, Gazzerro E, Capasso R, De Leonibus E, De Petrocellis L, Di Marzo V. Effects of non-euphoric plant cannabinoids on muscle quality and performance of dystrophic mdx mice. Br J Pharmacol. 2018 Aug 3.

55. Iannotti FA, Pagano E, Guardiola O, Adinolfi S, Saccone V, Consalvi S, Piscitelli F, Gazzerro E, Busetto G, Carrella D, Capasso R, Puri PL, Minchiotti G, Di Marzo V. Genetic and pharmacological regulation of the endocannabinoid CB1 receptor in Duchenne muscular dystrophy. Nat Commun. 2018; 9(1):3950.

X. Danksagung

Es ist mir ein Anliegen und eine Freude, all jenen zu danken, die zum Entstehen der Forschungsarbeiten beigetragen haben.

Wenn auch ich dieses Projekt in Italien angefangen habe, wurde die letzten Experimente hieran ECRC programmiert und analysier. Ich habe meine Forschungen in der Arbeitsgruppe von Prof. Simone Spuler 2016 in Berlin begonnen. Ich möchte ihr für das mir entgegengebrachte Vertrauen herzlich danken. Neben ihrer Begleitung der Forschungsprojekte, ihren Anregungen und wertvollen Ratschlägen ging ihre Unterstützung weit über die übliche Betreuung einer Promotion hinaus. Immer wieder hat sie mir engagiert bei der komplizierten Überwindung bürokratischer Hürden zwischen Deutschland und Italien geholfen und ich verdanke ihr, jetzt in Berlin arbeiten zu können. Außerdem möchte ich allen ehemaligen und aktuellen Mitgliedern der Arbeitsgruppe Muskelerkrankungen für ihre vielfältige Unterstützung und kollegiale Atmosphäre danken. Ich danke meinem Mann, der mich in meiner Arbeit bestärkt hat und mir mit Rat und Tat zur Seite gestanden hat und meinen drei Kindern, die viel Geduld mit einer wissenschaftlichen Mutter haben mussten. Zuletzt danke ich meinen Eltern, die in aller Hinsicht die Grundsteine für meinen Weg gelegt haben.