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Workshop report

234th ENMC International Workshop: Chaperone dysfunction in muscle disease Naarden, The Netherlands, 8–10 December 2017

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1. Introduction

Twenty participants from Australia, Belgium, Denmark, Finland, France, Germany, Italy, Israel, The Netherlands, Sweden, UK and the USA met in Naarden, The Netherlands from December 8th–10th, 2017. This group included clinicians, clinical trialists, basic scientists, industry and patient representatives. Patients were represented by the Muscular Dystrophy Association and Alexander's Way (BAG3 associated myopathy). The goals were to discuss the role of protein chaperones in normal muscle function, muscle disease and future therapies. Protein chaperones are a class of proteins that participate in facilitating the proper folding and assembly of protein complexes. Thus, chaperones are essential for the development and maintenance of skeletal muscle. Specifically this large group of proteins ensures that other proteins (client proteins) maintain proper structure and function or if needed, facilitates their degradation via proteolytic pathways. Chaperone dysfunction is responsible for many rare hereditary myopathies; thus correcting chaperone function may be a therapeutic option.

The participants reported on various aspects of the involvement of chaperones in a large variety of muscle diseases and disease processes, ranging from primary defects in chaperone genes such as *DNAJB6, BAG3* and *HSPB8*, to the involvement of chaperones in the larger group of myofibrillar and rimmed vacuolar myopathies including sporadic inclusion body myositis (sIBM), and beyond. Because the activity of the chaperones can be increased by different drugs the main scope of the workshop was to identify opportunities to use the available current knowledge for direct therapies. One therapy, known to increase the activity of protein chaperones in cells and small animal models, arimoclolol, is already entering the second phase of clinical trials in patients with sIBM and familial amyotrophic lateral sclerosis (ALS). Chaperonopathy patients had their own representatives at the workshop and made highly appreciated contributions focusing on the difficulties in diagnosis and therapies for ultra-rare disorders. Other discussion related to utilizing large datasets and patient cohorts to study these diseases. A summary of topics and presentations are detailed below.

2. Chaperonopathies

Bjarne Udd and *Chris Weihl* briefly reviewed the current understanding of the different inherited neuromuscular disorders, which are directly caused by pathogenic mutations in genes encoding chaperones and co-chaperones. These can be collectively called chaperonopathies (Table 1). The first described entity was a myofibrillar myopathy caused by mutations in HSPB5 (CRYAB) [1], although most later identified chaperonopathies were CMT-types of neuropathies caused by HSPB1, HSPB8, CCTdelta/MKKS and DNAJB2 gene defects [2]. In the early days these were identified by linkage and positional cloning in larger families. A conceptual break-through came with the identification of DNAJB6 mutations to cause dominant Limb-girdle muscular dystrophy LGMD1 D with the clearly coherent pathology of aggregating sarcomeric proteins when the co-chaperone is not functional due to mutations [3,4]. Later both HSPB1 and HSPB8 were also reported to cause myopathy with aggregates in addition to neuropathy.

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Table 1
Chaperonopathies.

Chaperone family	Gene	Clinical syndrome	Inheritance	Reference
Small heat shock proteins	*CRYAB/HSPB5*	Myofibrillar myopathy, cardiomyopathy and cataracts	AD	[20]
	HSPB1	Distal motor neuropathy, CMT and distal myopathy with rimmed vacuoles	AD/AR	[21-23]
	HSPB8	Distal motor neuropathy, CMT and distal myopathy with rimmed vacuoles	AD	[24,25]
	HSPB3	Distal motor neuropathy, CMT and distal myopathy with rimmed vacuoles	AD	[26,27]
Co-chaperones	*DNAJB6*	LGMD1D and distal myopathy	AD	[28,29]
	DNAJB2	Distal motor neuropathy	AR	[30]
	DNAJB5	Distal atrophy	AD	[31]
	BAG3	Myofibrillar myopathy, cardiomyopathy and neuropathy	AD	[32,33]
ER resident co-chaperone	*SIL1*	Marinesco-Sjogren Syndrome, congenital myopathy with cerebellar ataxia	AR	[34,35]

3. Molecular and cellular functions of protein chaperones

Harm Kampinga first provided an overall introduction to the chaperone network that operates in cells to ensure proper protein handling from co-translational folding to protein complex remodeling, and protein degradation. Besides the functional differences between the chaperone machines (chaperonins, Hsp90s, Hsp70s), each of them have multiple functions that are all steered by co-chaperones. Some of these (co)chaperones have been suggested as putative targets in proteinopathies. Inversely, a subclass of these proteinopathies are due to mutations in chaperones (chaperonopathies). Most of the chaperonopathies are due to mutations in the co-chaperones and are often associated with protein aggregates that mainly affect slowly proliferating tissues. One of the most aggressive, early onset forms of chaperonopathies are due to mutations in the HSP70 co-chaperone BAG3 (BAG3P209L) [5]. In collaboration with the group of Jason Gestwicki (USA), the Kampinga group found that this may not only be due to the fact that this BAG3P209L lost its function to properly regulate the function of the HSP70 machine, but also by dominant negative effects on the function of other chaperones. One of these include DNAJB6, another, BAG3-independent co-regulator of the HSP70 machine. Interestingly, mutations in DNAJB6 are also associated with hereditary myopathies, but these are milder and later in onset. Finally, it was shown that genetic or pharmacological blocking the interaction of BAG3P209L with HSP70 can abrogate the dominant negative effects of the mutant. Whereas, this will not alleviate the loss-of-function of the BAG3P209L mutant, it does form a proof-of-concept for intervention with the progressive nature of the BAG3P209L-related myopathy.

Wolfgang Linke discussed chaperone regulation of the giant protein titin. Titin is considered the molecular blueprint and backbone of the muscle sarcomere, with a main function as a molecular spring and regulator of active contraction. The team of Prof. W.A. Linke has provided evidence that protein quality control of titin is a major factor in the assembly and maintenance of the sarcomeric structure. They showed that molecular chaperones bind to the elastic titin springs and exert a protective function [6]. Specifically, they demonstrated that HSP90 binds to a unique region in titin, the N2A segment, when the chaperone is methylated by cytosolic methyltransferase Smyd2. Methylated Hsp90 and Smyd2 in complex with N2A titin stabilize the sarcomeres in muscle cells, as shown by disruption of the sarcomeric Z-/I-band region upon knockdown of Smyd2/methyl-HSP90. Furthermore, the team showed that small heat shock proteins, such as αB-crystallin and Hsp27, translocate to the titin springs under (patho-)physiological stress conditions and protect them from aggregation and misfolding. Binding of specific HSPs/co-chaperones may support the integration of titin into the sarcomere, whereas in turn, HSP-binding to titin may be an early event in the targeted degradation and turnover of titin and the sarcomere. However, whether/how other components of the protein quality control machinery, notably the autophagy-lysosomal and ubiquitin-proteasome systems (UPS), affect titin proteostasis is unknown. With regard to protein chaperones, the group of W.A. Linke recently found that HSP90, αB-crystallin and Hsp27 were translocated to I-band titin in the muscles of patients with diverse myofibrillar myopathies or dystrophies [7]. While such binding was considered beneficial for sarcomere structure, it also had detrimental effects on the sarcomere and probably on whole muscle, as it increased passive myofiber stiffness. This raises concerns about unwanted side effects in the treatment with chaperones as a choice for improving sarcomere structure and function in myopathy patients.

Jörg Höhfeld presented his work on the functional characterization of the cochaperone BAG3, which is required for muscle maintenance [8]. BAG3 cooperates with members of the HSP70 chaperone family, small heat shock proteins, i.e., HSPB8, and chaperone associated ubiquitin ligases such as STUB1/CHIP. The BAG3 complex monitors the mechanical unfolding and damage of cytoskeleton proteins at the Z-disk of skeletal and cardiac muscle. Damaged forms are targeted for degradation through BAG3-mediated chaperone-assisted selective autophagy (CASA). A critical client of this degradation pathway in muscle is the actin-crosslinking protein filamin. In cooperation with Michael Hesse and Bernd Fleischmann, transgenic mice were generated, expressing the pathological variant BAG-P209L, which causes rapidly progressive childhood muscular dystrophy and cardiomyopathy. The animals recapitulate the human pathology and cardiomyocytes of these mice show progressive Z-disk disintegration and filamin aggregation, revealing the essential role of BAG3 in filamin and muscle homeostasis. Autophagosome formation during CASA depends on the cooperation of BAG3 with SYNPO2/myopodin, which links the BAG3 chaperone complex to a membrane fusion machinery essential for autophago-

some formation. Interaction between BAG3 and SYNPO2 is mediated by a WW domain within the cochaperone, which associates with a PPxY motif in SYNPO2. The WW domain also allows BAG3 to engage in the Hippo signalling pathway to stimulate the transcription of filamin and in mTOR signalling to coordinate protein synthesis and CASA activity under mechanical stress. Höhfeld suggested that the ability of the BAG3 cochaperone to balance transcription, translation and autophagy under mechanical stress, is essential for muscle maintenance, and should be taken into account when considering therapeutic approaches for BAG3-related myofibrillar myopathies.

Serena Carra reported on the role of deregulated phase separation as a pathomechanism involved in an increasing number of diseases, including neuromuscular and muscular diseases [9]. Dr. Carra discussed recent findings demonstrating that stress granules (SGs), a type of ribonucleoprotein (RNP) granules, can convert into amyloid-like aggregates with implications in neuromuscular diseases, such as Amyotrophic Lateral Sclerosis. In particular, Dr. Carra showed that SGs can sequester misfolded proteins, which promote an aberrant conversion of liquid SGs into solid aggregates. Importantly, Dr. Carra and colleagues identified a specific protein quality control (PQC) process that prevents the accumulation of misfolding-prone proteins in SGs and, by doing so, maintains the dynamic state of SGs. This quality control process is called granulostasis. Granulostasis relies on the concerted action of the HSPB8-BAG3-HSP70 complex. Additional players of granulostasis are p97/valosin containing protein (VCP) and other molecular chaperones (e.g., HSPB1). Importantly, mutations in the genes encoding for HSPB8, BAG3 and VCP have been associated with neuromuscular diseases, including myopathy, and deregulated function of these proteins at the level of PQC and SGs is suspected to contribute to cell dysfunction and death. Next, Dr. Carra reported on two small HSPs, HSPB2 and HSPB3, whose altered functions and mutations are linked to rare forms of myopathies. Dr. Carra showed that, in mammalian cells, HSPB2 forms nuclear compartments with liquid-like properties. In differentiating myoblasts, nuclear HSPB2 compartments sequester lamin A. Increasing the nuclear concentration of HSPB2 causes the formation of aberrant nuclear compartments that mislocalize lamin A and chromatin, with detrimental consequences for nuclear functionality and integrity. Of interest, HSPB2 compartmentalization is negatively regulated by HSPB3, but this ability is lost in two identified HSPB3 mutants that are associated with myopathy. Altogether, the work presented by Dr. Carra highlighted how deregulated phase separation of specific proteins can have detrimental effects on cell functionality, causing or promoting disease progression.

4. Chaperone dysfunction in disease

Vincent Timmerman presented work on chaperone involvement in Charcot–Marie–Tooth (CMT) neuropathies [10]. CMTs comprise a clinically diverse and genetically heterogeneous group of monogenic disorders affecting the peripheral nervous system. The most remarkable group of genes mutated in CMT are those coding for small heat shock proteins (HSPBs). Although regulated by all types of stress, HSPBs are constitutively expressed and responsible for quality control and protein folding. The HSPBs are not only molecular chaperones but also involved in many essential cellular processes such as apoptosis, autophagy, splicing and translation, cytoskeleton dynamics and neuronal survival. To understand the functional consequences of HSPB1 mutations they performed tandem-affinity purification and differential MS analysis (wild type versus mutant) and identified client proteins for mutant HSPB1. The wild type HSPB1 facilitates the formation of non-centrosomal microtubules and that mutant HSPB1 differentially interacts with tubulin. This anomalous binding leads to the stabilization of the microtubule network. Mutations in HSPB1 can also disrupt the neurofilament network and cause their aggregation. Transgenic mice for mutant HSPB1 (S135F and P182L) mimicking dHMN (CMT2F) confirmed microtubule stabilization and revealed a decreased acetylation of tubulin levels. HDAC6 inhibitors were shown to rescue axonal transport defects and increase motor performance of the transgenic mice. Through the differential MS analysis they also revealed that a specific HSPB1 mutant (P182L) causes loss of translational repression by binding to a poly-C binding protein (PCBP1) that may control the expression of hereditary neuropathy genes. Altogether specific mutations in HSPB1 can affect axonal transport via induced hyperphosphorylation of neurofilaments or lead to stabilization of the microtubule network. These data show that both mechanisms can be targeted by drugs in experimental models and may open opportunities for treatment strategies. Since the first description of the K141N missense mutation in HSPB8, a number of additional dHMN (CMT2L) patients and families have been reported. Interestingly, most mutations target the same lysine residue (K141E, K141 M, K141N, K141T) in the highly conserved α-crystallin domain of the HSPB8 protein. The spectrum of diseases caused by mutations in the HSPB8 gene was recently expanded to myofibrillar myopathy (MFM). HSPB8 is a chaperone that participates in clearing misfolded poly-Q containing proteins such as mutant huntingtin and ataxin-3 involved in respectively Huntington's disease and spino-cerebellar ataxia. HSPB8 directly interacts with the cochaperone BAG3 and their role in chaperone-assisted selective autophagy (CASA) and granulostasis is well described. To delineate the molecular deficits and functional consequences of HSPB8 mutations Timmerman and colleagues generated a knock-in (KI) model for the K141N mutation mimicking the dHMN phenotype. They observed that homozygous KI mice (HspB8K141N/K141N) develop a progressive axonopathy resulting in locomotor deficits. At the ultrastructural level, mice accumulate mutant HspB8 protein and display degenerative patterns similar to dHMN patients. Interestingly, these animals also develop a progressive MFM as observed in some rare patients with HSPB8 mutations. Additionally, the model allowed generation of an HspB8 knock-out (KO) using the same targeting vector. Strikingly, the homozygous HspB8-KO

animals (HspB8-/-) do not show any sign of axonopathy and display a much milder myopathy than the HspB8-KI animals. A therapeutic strategy may be feasible by modulating the expression of HspB8 and thus improve the dHMN and MFM phenotype. This strategy can be beneficial to treat mutations targeting the functional HSPB8-BAG3-HSP70 complex.

Jaakko Sarparanta presented on DNAJB6 – the cochaperone mutated in LGMD1D – and its connections with the chaperone-assisted selective autophagy (CASA) pathway. DNAJB6 has previously been found to associate with the CASA machinery, although the functional importance of this interaction has remained unknown. Moreover, zebrafish studies have suggested that the CASA cochaperone BAG3 may play a role in the pathomechanism of LGMD1 D [4]. LGMD1D-causing mutations have earlier been shown to slow down the turnover of DNAJB6. The Udd group has now focused on clarifying the role of CASA in LGMD1 D by studying whether the CASA proteins and mutant DNAJB6 modulate each other's turnover in a cell culture model. The first studies have indicated that overexpression of wild-type BAG3 may cause a modest decrease in the turnover of both wild-type and F89I mutant DNAJB6, and the relevance of this finding for LGMD1 D is under investigation. Interestingly, BAG3 harbouring the myopathy-associated P209 L mutation causes a dramatic block in DNAJB6 turnover, as well as redistribution of DNAJB6 to BAG3 aggregates. These results give additional support for a functional interaction between DNAJB6 and BAG3, and also suggest that DNAJB6 could be a downstream mediator of BAG3 mutations in BAG3 myopathy.

Robert Bryson-Richardson described the generation of zebrafish models for BAG3 myofibrillar myopathy [11]. These models expressing $BAG3^{P209L}$, or lacking the BAG3 protein, demonstrated that the dominant disease causing protein $BAG3^{P209L}$ was sufficient to induce aggregate formation but not loss of structural integrity or muscle weakness. Given previous work identifying that wildtype BAG3 protein and the binding partner FLNC is found in the aggregates formed upon $BAG3^{P209L}$ expression it was proposed to result from the loss of the BAG3 protein. Examination of BAG3 deficient zebrafish identified impaired swimming performance and loss of sarcomeric integrity at the Z-disk, supporting this hypothesis. Given the suggested role of aggregates as the initial trigger for muscle weakness, rapamycin treatment of the $BAG3^{P209L}$ expressing fish was evaluated and identified to reduce aggregate number suggesting a potential therapeutic approach.

Andreas Roos described his ongoing work on the ER resident co-chaperone, SIL1 [12]. SIL1 encodes for an ATP exchange factor (co-chaperone) for BiP, one major chaperone of the Endoplasmic Reticulum (ER) that controls import of nascent polypeptides into the lumen of the ER, protein folding, calcium homeostasis and activation as well as modulation of the unfolded protein response (UPR) and the ER-associated degradation pathway (ERAD). Thus, loss of functional SIL1 impacts these BiP-dependent processes. Recessive mutations in the *SIL1* gene have been linked to the clinical manifestation of Marinesco-Sjögren syndrome (MSS; MIM: 248800). MSS patients present with congenital or early onset cataracts, cerebellar ataxia, intellectual disability of varying degree and a progressive vacuolar myopathy. Additional clinical features that may manifest include somatic growth retardation, skeletal abnormalities and pyramidal tract signs. A genetrap mutation in the murine *Sil1* gene has been linked to the woozy mouse phenotype characterised by ataxia based on loss of neocerebellar Purkinje-cells and a vacuolar myopathy, thus representing a suitable phenocopy of the human disease. Pathomorphologically, diseased muscle fibres in human and mouse are characterized by proliferated ER, perturbed architecture of the nuclear envelope (including proliferation and abnormal localization of the nuclear lamina) and of mitochondria as well as by intermyofibrillar enrichment of electron-dense material corresponding to protein aggregates. Results of comprehensive biochemical investigations of woozy quadriceps muscles including proteomic profiling also revealed affection of the protein folding and clearance machinery and moreover suggest a particular vulnerability of mitochondrial proteins. This latter aspect particularly accords with the previous assumption that MSS is a mitochondrial disease. In the same context, during the ENMC workshop it has been suggested that loss of functional SIL1 might also have an impact on ER-mitochondrial organelle interaction and hence contribute to mitochondrial vulnerability. An established *in vitro* model of the disease showed similar pathomorphological features corresponding to ER-stress and activation of the protein clearance machinery. These electron microscopic findings correlate with obtained changes in the proteomic signature of the *in vitro* model: in addition to the protein processing machinery, mitochondrial proteins (especially mitochondrial membrane proteins) and cytoskeletal components are affected by SIL1-depletion. Apart from the fact that loss of SIL1 causes the manifestation of a neuromuscular phenotype in humans and in mice, there is growing evidence that this co-chaperone also functions as a modifying factor for neurological diseases: SIL1 is robustly expressed in ALS-disease-resistant slow motor neurons but not in ER stress-prone fast-fatigable motor neurons in humans and in mice and ER homeostasis is impaired in mouse motor neurons in response to loss of SIL1 function. In addition, loss of a single functional *Sil1* allele in SOD1-G93A mutant mice (ALS animal model) enhances ER stress and exacerbates ALS pathology. Remarkably, adeno-associated virus-mediated delivery of SIL1 to SOD1-G93A mutant motor neurons restores ER homeostasis, delays muscle denervation and prolongs the survival of the diseased animals. The notable beneficial effect of elevated SIL1 level on disease processes in animal and cell models of neurological diseases in addition to the identified molecular effects suggest SIL1 as a suitable target for the development of new therapeutic intervention concepts.

5. Therapeutic approaches for chaperonopathies

Linda Greensmith presented results from her group's recent studies in which the therapeutic potential of pharmaco-

logically targeting protein chaperones to ameliorate protein misfolding in muscle disorders was examined.

Muscle biopsies from sporadic inclusion body myopathy (sIBM) patients typically show several degenerative features, most notably the presence of ubiquitinated inclusion bodies and TDP-43 mislocalisation which lead to myofibre loss and atrophy causing severe muscle weakness in patients. Interestingly, these degenerative pathological features are also found in muscles of patients with multisystem proteinopathy caused by mutations in valosin containing protein (VCP), in which IBM also manifests.

Restoring protein homeostasis in affected cells may therefore may be a beneficial strategy to ameliorate pathology in these protein misfolding disorders. Our approach is to utilize the cell's endogenous chaperone system, and target the Heat Shock Response (HSR) in order to prevent protein misfolding. Heat shock proteins (HSPs) bind to misfolded proteins, to prevent protein–protein interaction and subsequent aggregation. Therefore, upregulation of this ubiquitous endogenous cytoprotective mechanism may be an effective approach to prevent protein misfolding and aggregation, particularly in disorders that manifest on a background of aging, such as sIBM, as the HSR is known to decline with age. Indeed, our previous findings have shown that augmentation of the HSR using a novel drug called Arimoclomol, attenuates disease in SOD1G93A models of ALS, as well as Spinal Bulbar Muscular Atrophy [13]. Arimoclomol is a co-inducer of the HSR that acts like a smart drug, augmenting the expression of HSPs only in cells under stress and in which the HSR is already activated, thereby avoiding off target side effects.

In an *in vitro* model of IBM, over-expression of full length human β-APP in primary muscle cells produces IBM-like pathology, including formation of protein aggregates. Treatment of these cells with Arimoclomol increases expression of HSP70 and HSP90, reduces protein aggregation and improves cell survival. We next tested the effects of Arimoclomol in vivo, in mutant VCP mice which closely model human IBM. Treatment of mVCP mice with Arimoclomol attenuates the degenerative muscle pathology including decreased protein aggregation and TDP-43 mislocalisation. Moreover, Arimoclomol significantly improved muscle function in mVCP mice, with an improvement in muscle force and a two-fold increase in HSP70 expression compared to that of untreated mVCP mice [14].

Based on these results, a randomized, double blind, placebo-controlled, proof-of-concept trial of Arimoclomol for the treatment of sIBM in human patients was undertaken as part of an international collaboration between clinical and basic scientists at University College London, UK, and Kansas University, USA.

Gordon Lynch described the therapeutic potential of heat shock protein (HSP) induction for Duchenne muscular dystrophy (DMD) and other muscle wasting disorders [15]. In a series of studies, overexpression of HSP72 was shown to ameliorate aspects of the dystrophic pathology in mildly affected *mdx* mice lacking dystrophin and in more severely affected *dko* mice lacking both dystrophin and utrophin. Treating young *dko* mice with BGP-15, a chaperone co-inducer of HSP72, reduced clinically relevant markers including kyphosis, serum creatine kinase and fibrosis, and improved specific force of isolated diaphragm muscle strips *in vitro* and tibialis anterior muscles in situ. Treatment improved maximal sarco(endo)plasmic reticulum Ca^{2+}-ATPase activity and enhanced survival in *dko* mice. As for many pharmacological treatments proposed for DMD, BGP-15's efficacy was dependent on the stage and severity of the pathology and effects were muscle specific. Treating *mdx* and *dko* mice with an established pathology had more limited efficacy, with no improvements in diaphragm or limb muscle function and no attenuation in the progression of kyphosis. In the heart, however, treating *dko* mice with BGP-15 either early or later in the pathology, was able to reduce fibrosis, indicating a potentially larger therapeutic window of opportunity for treating (cardiac) aspects of the dystrophic pathology with pharmacological induction of HSP72.

The prospect of HSP modulation to enhance muscle stem cell (MuSC) transplantation was also described. These included potential applications for HSP upregulation to: prime the host environment to receive transplanted cells; prime MuSCs before engraftment to improve their survival and proliferation after transplantation (through enhanced fusion, maturation and integration); and promote MuSC self-renewal to enhance the capacity to manage repeated cycles of muscle injury and repair/regeneration. With many muscle wasting conditions having a pathophysiology characterized by inflammation, atrophy and weakness, increasing HSP expression may well have therapeutic potential to address one or more contributing mechanisms.

Andrew Findlay presented work on the pathogenic mechanism and potential treatment of LGMD1 D. Mutations in DNAJB6, an HSP40 co-chaperone, cause LGMD1 D, an adult onset progressive myopathy with vacuolar and aggregate myopathology [3]. DNAJB6 mediates proper folding of client proteins by binding client, recruiting Hsp70, and stimulating Hsp70 activity to induce conformational changes in client. LGMD1 D mutations primarily reside within the 12 amino acid G/F domain, which is thought to be important for client protein handling. Several models have been generated to study DNAJB6 dysfunction in yeast, C2C12 cells, and mice. Previous studies have suggested that mutations in DNAJB6 alter protein aggregate handling leading myofibrillar protein aggregation. However, LGMD1 D mutations may also contribute to disease pathogenesis by over activation of DNAJB6's client, GSK3B, and downstream suppression of follistatin expression, a potent inhibitor of atrophy signaling pathways. Treatment of LGMD1 D mice with LiCl, a GSK3B inhibitor, increased skeletal muscle follistatin levels, improved skeletal muscle histopathology, and normalized grip strength. These findings indicate GSK3B inhibition or follistatin supplementation are attractive therapeutic strategies for LGMD1 D patients.

Per Harald Jonson reported on the function of DNAJB6 on preventing aggregation of aggregation-prone proteins in cell culture. So far all known disease-causing mutations in

DNAJB6 show a reduced capacity to prevent aggregation of polyQ-proteins. The disaccharide trehalose is reported to increase autophagy by an mTOR independent pathway and is also a commonly used food additive. New, unpublished results of the effect of trehalose on restoring DNAJB6s anti-aggregation properties were presented. As most oral trehalose is degraded before uptake trehalose is probably not a viable treatment strategy, but illustrates that a mild induction of autophagy is a potential strategy for restoring dysfunctional chaperones.

Lars Larsson presented on ventilation induced diaphragm muscle dysfunction (VIDD). Mechanical ventilation is directly involved in the progressive impairment of diaphragm muscle function during long-term ICU treatment, but the specific mechanisms underlying the muscle wasting and impaired muscle function associated with the ICU intervention have been poorly understood in the clinical setting. Our previous experimental results from both the porcine and rat experimental ICU (ExICU) models indicate that heat shock proteins (HSPs) play an important role in the protection of muscle from loss of function in response to the ICU intervention [16]. Chaperone co-inducers comprise an emerging class of pharmaceuticals. These agents are safe, well tolerated and have the ability to enhance stress-tolerance. Based upon our mechanistic studies and results demonstrating the protective role of HSPs during prolonged mechanical ventilation in the experimental models, a novel pharmacological intervention using a chaperone co-inducer has been evaluated in the rat ExICU model. Systemic administration of the chaperone co-inducer (BGP-15) in the ExICU model had a significant positive effect on diaphragm muscle structure and function by mitigating the loss in force generating capacity of diaphragm muscle fibers [17]. Thus, BGP-15 offers an efficient intervention strategy in reducing VIDD in mechanically ventilated ICU patients.

Robert Bryson-Richardson presented a screen of autophagy promoting drugs in the zebrafish model of BAG3 myofibrillar myopathy. Nine compounds capable of significantly reducing aggregates in the $BAG3^{P209L}$ model were presented, including 2 FDA approved small molecules. Further characterisation of BAG3 mutant zebrafish identified that autophagy was impaired suggesting that promoting autophagy could not only reduce aggregate number but help to overcome the deficiency in autophagy due to loss of BAG3. Testing of the FDA approved compounds in the zebrafish model demonstrated that in addition to reducing aggregate number, both were able to reduce the loss of sarcomeric integrity and one was able to restore swimming performance in the mutant animals. The compounds identified in the zebrafish model therefore being promising leads for the treatment of BAG3, and potentially other, myofibrillar myopathies.

6. Clinical trials modulating chaperone function

Mazen M. Dimachkie presented an overview of "Necessary Steps For A Clinical Trial – Lessons learned Phase 2/3 Study of Arimoclomol in IBM under FDA-IND # 76773 – RO1FD004809." Dr. Dimachkie is the PI on this study, Professor Michael Hanna is the co-PI and Drs. Richard J. Barohn and Pedro Machado are co-Is. The primary objectives of this study are to evaluate the therapeutic response and safety of Arimoclomol 1200 mg daily as compared to placebo in sporadic inclusion body myositis (IBM). Arimoclomol stabilizes heat shock factor 1 in stressed tissues and leads to increased expression of heat shock proteins (HSP) chaperones, particularly HSP70. This investigative team from the KUMC and the University College of London (UCL) – United Kingdom, had conducted and reported on a pilot study assessing the safety of Arimoclomol in IBM. Following funding by the FDA-Orphan Products Division (OPD) of the Phase 2/3 study, Orphazyme and the investigative team sought guidance from the FDA-regulatory about the study design. This led to protocol modifications in coordination with the FDA-OPD. Drug shipment across the Atlantic required the KUMC to act as Importer of Record, which was done successfully. The team updated the remote monitoring plan to an onsite monitoring. The team initially took steps to comply with FDA CFR Title 21 Part 11 Electronic Record compliance which evolved into adopting a different database platform that secures more robust database compliance such as data validation and audit trails. The team reformatted the protocol to be compliant with ICH E6. Another important component was reliance of the 10 additional US participating sites on the KUMC as the IRB of record, via the SMART IRB mechanism. The team secured clearance by the US Department of State to allow transfer FDA-OPD grant funds to the UCL. Approval processed via the MHRA and EMA were also initiated. An investigator meeting for all sites was planned for and held April, 2018 in Los Angeles. Leading international clinical trial requires energy, flexibility, strong communication and organizational skills, tenacity and most importantly excellent team collaboration. This phase 2/3 clinical trial embodies very well these principles.

Pedro Machado presented on a secondary biomarker endpoint in the Arimoclomol pilot study in IBM [14]. Specifically, no differences between the active and placebo groups were seen for changes in myosin-adjusted HSP70 expression in muscle. However, measurement of muscle HSP70 expression has several limitations, namely the fact that muscle HSP70 expression is extremely sensitive to multiple factors, including disease stage, physical activity, age, and gender. There are many physiological signals that can activate HSP expression and interactions between the immune system and HSP are complex and bivalent which also contributes to variability of HSP measurements in normal and diseased muscle. Furthermore, in the Arimoclomol trial, the fact that muscles on opposite sides of the body were biopsied at baseline and after treatment may have contributed to even more variability, given the heterogeneity between muscles in IBM (with different muscles being clinically and pathologically affected differently i.e., being at different disease stages). Therefore, using muscle HSP measurements as a longitudinal or therapeutic readout is in extremely challenging in IBM. There is an increasing need of tractable targets that can be used as trial outcome measures and quantitative MRI has shown promising results as a potential biomarker [18]. Fat fraction, trans-

verse relaxation time (T2), and magnetisation transfer ratio (MTR) are the most frequently used quantitative muscle imaging methods. In a prospective MRI quantitative study using the Queen Square MRI protocol, whole calf and thigh muscle fat fraction (measured using MRI Dixon fat water imaging) increased significantly after 1 year and correlated with the lower limb component of the IBM functional rating score (IBMFRS). Longitudinally, change in knee extension isometric torque correlated with change in the remaining muscle area (i.e. change in the non-fat quadriceps compartment area). This study demonstrated validity and responsiveness of MRI outcome measures (particularly fat fraction) in IBM, suggesting that quantitative MRI biomarkers might prove valuable in experimental trials.

7. Data networks to identify chaperone targets

Bjarne Udd reported on the possibilities to clarify which proteins are directly involved in the molecular pathways directed by chaperones in muscle. One possibility comes from the identification of which all muscle diseases are actually caused by primary gene defects in chaperone genes. Thus far some six chaperones are known disease genes, but in fact, beyond these known disease causing genes a very large number of chaperones are highly expressed in muscle, such as DNAJB4, DNAJB5, DNAJC8, DNAJC15, DNAJC19, HSPA1A, HSPA1B, HSPA2, HSPA4, HSPA8, HSPA9, HSPB2, HSPB6, all HSP60s (chaperonins), HSP90AA1, HSP90AB1, HSP90B1, and TRAP1. Based on our experience with SIL1, HSPB8 and DNAJB6 mutated myopathology, in which aggregations of sarcomeric proteins are early hallmarks and secondarily induced autophagy with rimmed vacuoles in the myofibers take place, all muscle diseases with protein aggregations and/or rimmed vacuolar pathology are good candidates for primary chaperone gene defects. Of course similar pathology can also be caused by other primary gene defects with secondary involvement of chaperones, as with gene defects in sarcomeric proteins such as MYOT or ZASP or with RNA-processing genes TIA1 or MATR3. In order to have a more complete understanding of the involvement of chaperones in myopathies and their target client proteins the study of families/patients with undetermined myofibrillar and/or rimmed vacuolar muscle pathology by genomic NGS or RNAseq could be rewarding. Exploring secondary effects in muscle of patients with established chaperone/co-chaperone defect by RNAseq and by proteomics should increase the list of client proteins for chaperones in muscle. Equally exploring secondary effects on chaperones in muscle of patients with primary aggregating other mutant muscle protein could complement the current view of the large interplay needed for protein homeostasis in muscle.

Chris Weihl described an approach to utilize existing proteomic data to explore and unify protein aggregation that occurs in skeletal muscle with other protein aggregate disorders such as Alzheimer's disease. The group of Rudy Kley has previously reported their work using laser microdissection to identify proteins that accumulate within the inclusion bodies of protein aggregate myopathies and inclusion body myositis [19]. Utilizing these datasets, Dr. Weihl explored the biophysical properties of these inclusion or vacuole enriched proteins and found that on average, they were more abundant and more aggregation prone when compared with the normal proteome. Further exploration of these proteins revealed that while they are not aggregated in normal appearing muscle, they are on average (as compared with other abundant proteins) present at higher levels. In addition, while these proteins are in many cases unique to protein aggregate myopathies and have little overlap with proteins from other aggregate disorders (i.e., Alzheimer's disease and ALS), this biophysical signature of "supersaturation" is present across all aggregate myopathies and neurodegenerative diseases.

8. Conclusions

(1) Skeletal muscle is a unique tissue that undergoes constant structural remodeling due stress and strain placed upon sarcomeric/structural proteins during normal use. This makes skeletal muscle particularly vulnerable to alterations in protein chaperone availability and function.
(2) Recognition of "chaperonopathies" as a distinct class of molecular disorders with common pathologies and pathogenic mechanisms is essential to rationale and future therapy development that may apply to multiple genetic diseases.
(3) The first in human clinical trial is underway using a chaperone inducing medicine, arimoclomol, in sporadic inclusion body myositis.
(4) The "chaperone in neuromuscular disease" working group will maintain and increase collaborations.

9. Participants – ENMC workshop study group

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