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Workshop report

246th ENMC International Workshop: Protein aggregate myopathies 24–26 May 2019, Hoofddorp, The Netherlands

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1. Welcome and introduction

Twenty participants including one patient representative from European countries, USA, Australia and Japan attended the 246th ENMC-sponsored workshop, the sixth one focusing on protein aggregate myopathies (PAM) [1–5]. Since the last ENMC Workshop on PAM in November 2007, a multitude of novel insights into clinical aspects, genetic bases and molecular pathogenesis of these diseases have been gained. The attending experts discussed recent discoveries of new disease entities, the morphological spectrum of protein aggregation and new pathophysiological and pharmacological treatment aspects derived from animal and cell models.

After a brief introduction and welcome from *Alexandra Breukel*, the attending ENMC representative, *Montse Olivé* (Barcelona, Spain) chaired the first session and gave an introduction on the nomenclature and classification of protein aggregate myopathies. PAM encompass a wide group of muscle disorders defined by the presence of protein aggregate in muscle cells. The term includes hereditary conditions such as myofibrillar myopathies, the largest group of protein aggregate myopathies, and many other disorders such as actin filament aggregate myopathy, myosin storage myopathy, core myopathies, nemaline myopathies, tubular aggregate myopathies, among others, and some myopathies with rimmed vacuoles. In addition, non-hereditary disorders such as inclusion body myositis are also considered within the group of PAM. In 2007, during the last workshop on PAM, discussions were mainly focused on myofibrillar myopathies related to mutations in desmin (*DES*), plectin (*PLEC*), αB-crystallin (*CRYAB*), myotilin (*MYOT*), LIM domain-binding protein 3 (*ZASP*), filamin-C (*FLNC*), and valosin-containing protein (*VCP*) genes. Since then, additional PAM-causing gene defects have been identified, e.g. in BAG family molecular chaperone regulator 3 (*BAG3*), four and a half LIM domains protein 1 (*FHL1*), DnaJ homolog subfamily B member 6 (*DNAJB6*), skeletal muscle alpha actin (*ACTA1*), titin (*TTN*), sequestosome-1 (*SQSTM1*), heat shock protein beta-8 (*HSPB8*), supervillin (*SVIL*), kelch-like protein 24 (*KLHL24*), kyphoscoliosis peptidase (KY), pyridine nucleotide-disulfide oxidoreductase domain-containing protein 1 (*PYROXD1*), and a digenic condition caused by mutations in E3 ubiquitin-protein ligase TRIM63 (*MuRF1*) and tripartite motif-containing protein 54 (*MuRF3*) [6–8]. In this context it is important to note that muscle biopsies from patients suffering from these conditions always show protein aggregates, however, not all of them can be classified as myofibrillar myopathies which denote sarcomeric destruction.

2. Terminology/nosology of protein aggregation

Hans H. Goebel (Berlin, Germany) spoke on the nosology of protein aggregation. Diseases marked by protein aggregation affect the nervous system

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(neuroproteinopathies/protein aggregate encephalopathies and neuropathies), striated muscle (myoproteinopathies/protein aggregate myopathies (PAM), and more rarely the liver (hepatopathies/protein aggregate hepatopathies). In myopathology, protein aggregation may be hereditary or acquired, extra- or intracellular, filamentous and/or granular, structured or unstructured, as a disease entity or a lesion. Among the PAM, we find myofibrillar myopathies, distal myopathies and congenital myopathies, as well as inclusion body myositis (sIBM). Lesions, characterized by aggregation or accumulation of proteins are cores, targetoids, inclusions and filamentous aggregates. Among these inclusions are desmin-positive cytoplasmic bodies containing electron-dense and filamentous components. Further characteristic intermediate filament-containing aggregates are Rosenthal fibers (mainly composed of glial fibrillar acidic protein), Lewy bodies (neurofilaments), or Mallory bodies (keratins). In desminopathies, granulofilamentous material prevails, and filaments, by immunoelectron microscopy, contain desmin, alpha-B-crystallin, and dystrophin, which is normally found in the sub-sarcolemmal region of the muscle fiber. Hence, intermediate filaments in desminopathy and other MFM perhaps too – lose, at least in part, their normal structural organization. A plethora of proteins has been identified in protein aggregates in skeletal muscle by immunohistochemistry and mass spectroscopy. Cores, tubular aggregates, ragged red fibers and crystalline bodies contain many proteins. In a few instances, drugs have been associated with protein aggregation, foremost emetin, a component of ipecac sirup which may induce ipecac myopathy and cardiomyopathy. Other drugs are elinafide, enfurvitide, griseofulvin, and, potentially, many more still unrecognized ones.

3. Myofibrillar myopathies – the spectrum

Duygu Selcen from the Mayo Clinic (Rochester, USA), defined myofibrillar myopathies as a group of disorders associated with myofibrillar degradation that begins in the Z-disk. The typical morphologic features include fibers containing pleomorphic amorphous, granular, or hyaline structures, best seen in modified gomori trichrome stained frozen sections. Vacuoles containing membranous material are also a frequent feature. Some of these abnormal structures are congophilic, however, congophilia is not found in all patients. Another important feature is an accumulation of multiple proteins in abnormal fiber regions including myotilin, desmin, αB-crystallin, and dystrophin. In a cohort of 82 MFM patients investigated at the Mayo Clinic, and 48 patients from France [9], the mean age of onset was 52 y and 42 y, respectively. The disease typically presented with slowly progressive distal and/or proximal weakness involving limbs as well as axial and facial weakness in some patients. Other rare symptoms at the time of diagnosis included ophthalmoparesis, dysphagia, dysphonia, muscle ache, stiffness, and wasting, paresthesia, head drop, joint contractures, palpitations, fatigue, and perioral fasciculations. Cardiomyopathy, peripheral neuropathy and respiratory dysfunction also occurred in a subset of patients. The EMG was typically myopathic and associated with abnormal electrical irritability, including myotonic discharges. Rarely patients had only neuropathic changes or a mixed pattern. Mutation analysis in different groups to date revealed mutations in a variety of Z-disk or Z-disk-associated proteins including desmin, αB-crystallin, myotilin, ZASP, filamin-C, and BAG3. In some patients, mutations in FHL1, DNAJB6, HSBP8, titin, actin and lamin A/C genes also resulted in MFM pathology. Current treatment options include physiotherapy and respiratory support for patients with respiratory failure. Pacemaker, implantable cardioverter defibrillator, and heart transplant must be considered in patients with cardiomyopathy.

4. DNAJB6-related myopathies

Bjarne Udd (Helsinki, Finland) reported on the DNAJB6-related myopathies and also gave a short overview of the many different mechanisms leading to increased abnormal expression of certain proteins in muscle tissue, either as accumulations of primarily normally structured proteins or aggregations of mutant, misfolded proteins. A third category of abnormal protein expression occurs in the incomplete autophagic processing observed as rimmed vacuolar muscle pathology with components of the autophagosomal machinery such as p62, LC3 and other proteins including TDP-43, which are abundantly found in the rimmed vacuoles. DNAJB6 is a co-chaperone belonging to the protein quality control system and responsible for the identification of misfolded proteins and bringing them to the main chaperones for refolding or degradation. As with the small heat shock proteins, J-domain co-chaperones also have an independent anti-aggregation function. It is therefore not surprising that defect mutant DNAJB6 leads to insufficient clearance of normally occurring misfolded proteins in the myofibrils, ultimately causing malfunction and disintegration of the sarcomeric structure. This also induces autophagic processing to clear the misfolded proteins. Since this is not sufficient, the result is a rimmed vacuolar dystrophic muscle pathology. Currently, 13 different mutations in the G/F-domain of DNAJB6 have been described, most often causing adult onset limb-girdle weakness, but some mutations result in a more distal leg weakness at onset [10,11]. Most recently mutations in the J-domain causing the same pathology have also been identified, but clinically these patients display a more distinct distal leg phenotype at onset [12]. Different approaches to manipulate the chaperonal system are currently under investigation including Lithium therapy.

5. HMERF/SVIL and KLHL24-related myopathies

Anders Oldfors (Gothenburg, Sweden) summarized the characteristics of hereditary myopathy with early respiratory failure (HMERF). There are now reports of a considerable number of families worldwide that are afflicted by HMERF

[13]. The disease typically presents at 20–30 years of age but with a great variability in age at onset and clinical severity. There is usually no cardiomyopathy. Most patients have reduced vital capacity and many need nocturnal ventilation assistance. The legs of affected patients typically show in MRI an early involvement of semitendinous and peroneal muscles. Muscle histopathology is characterized by cytoplasmic bodies, other protein aggregates and major myofibrillar alterations involving Z-bands and rimmed vacuoles. The accumulated proteins are not fully characterized but apparently do not to contain titin, the mutated protein. Twelve point mutations, all residing in fibronectin III domain119 of the *TTN* gene, located in the A-band region, have been identified as a primary cause of HMERF. During the workshop, a new myopathy, associated with lack of supervillin due to a homozygous truncating *SVIL* variant, was reported in two affected siblings [14]. This myopathy has pathological features of autophagic vacuolar myopathy as well as PAM features similar of myofibrillar myopathies. The skeletal muscle phenotype was clinically mild but in addition cardiac involvement was evident. Supervillin is an F-actin binding 250 kD sarcolemmal protein colocalizing with costameric dystrophin, suggested to constitute a high-affinity link between the actin cytoskeleton and the membrane. Supervillin is prevalent in skeletal muscle but is also present in smooth muscle and heart. Furthermore, biallelic truncating mutations in the kelch-like protein 24 (*KLHL24*) causing hypertrophic cardiomyopathy was reported during the workshop to be associated with a myopathy characterized by a cogwheel appearance of the muscle fibers in PAS and NADH reactions. In addition, desmin immunostaining revealed an accumulation of desmin intermediate filaments in intermyofibrillar strands [15] KLHL24 is a member of the kelch-like protein family, which acts as substrate-specific adaptors of Cullin E3 ubiquitin ligases. Recent reports on a dominant mutation in KLHL24 have demonstrated that KLHL24 interacts with intermediate filaments and that a specific dominant mutation causes skin blistering due to defective turnover of keratin 14 intermediate filaments [16].

6. MFM in Japan

Satoru Noguchi (Tokyo, Japan) reported on clinical and pathological features as well as the mutation spectrum of Japanese patients with myofibrillar myopathy. Initially, they screened a subset of the 19,081 undiagnosed cases in the muscle repository at the National Center of Neurology and Psychiatry by applying the criteria ‘presence of cytoplasmic inclusions/rimmed vacuoles in myofibers and myofibrillar disorganization’ and excluding the criterion ‘diagnosis of other diseases’. 297 cases from 288 families fulfilled both criteria. In a second step genetic variations were screened in these cases by target resequencing using a diagnostic panel for *DES, MYOT, LDB3, FLNC, BAG3, FHL1, DNAJB6, VCP, TTN, CRYAB* mutations. Putative disease-causing variants were identified in 34% of the cases (89 cases). Notably, the most frequently mutated gene was *TTN* (18 cases). In addition, several mutations in *VCP, DES and FHL1* genes were identified. *FLNC, DNAJB6, LDB3* and *MYOT* were mutated in (8, 7, 7 and 6 cases respectively), whereas a *BAG3* mutation was only found in a single case. Notably, in 21 cases novel mutations in 7 further genes were found. In the cases analyzed, the age of onset was variable, but the patients with mutations in V*CP, FLNC, DNAJB6, LDB3* and *MYOT* displayed relatively later onset as compared to those with a mutation in *TTN*, *DES*, *FHL1* and *BAG3* genes. The pattern of muscle weakness was also highly variable. Whereas patients harboring mutations in *TTN, DES* and BAG3 showed predominantly distal muscle weakness, patients with mutations in *VCP, FHL1 DNAJB6* and *LDB3* displayed proximal muscle weakness. The cases with *TTN* mutation suffered from respiratory failure, whereas those with mutations in *DES, FLNC, DNAJB6* and *MYOT* showed cardiomyopathy and arrhythmia, and those with mutations in *FHL1* had joint contractures.

7. Small heat shock protein (HSPB8) related neuropathy and myopathy

Vincent Timmerman (Antwerp, Belgium) reported that mutations in small heat shock proteins (HSPB1, HSPB3 and HSPB8 HSPBs) can cause axonal Charcot-Marie-Tooth neuropathy (CMT2), distal hereditary motor neuropathy (dHMN), rare cases of amyotrophic lateral sclerosis (ALS), and more recently myofibrillar myopathy. Most mutations target the highly conserved alpha-crystallin domain, but mutations can also affect the N- and C-terminal domains of the three small heat shock proteins (HSPBs). Studying the molecular mechanisms of mutations led to the hypothesis that HSPBs are more than chaperones. Through proteomics and immunoprecipitations they identified molecular partners differentially interacting with the mutant and wild type HSPB, pointing towards their different roles in health and disease [17]. Mutations in HSPBs can result in protein aggregates, and overexpression of wild type HSPBs can impede protein aggregation [18]. He further reported that mutations in HSPB1 can impair autophagy in cell models and in patient-derived induced pluripotent stem cells (iPSC) differentiated to motor neurons. The HSPB8 plays a protective role when upregulated by several forms of cellular stress like proteasome inhibition, autophagy modulation and oxidative stress. Together, this underscores the importance of HSPB8 in the maintenance of protein homeostasis, by being an essential player of the proteasome/autophagy routing system [19]. The Hspb8 knock-in and knock-out mouse models suggests that the neuropathy and myopathy phenotype can be ameliorated by reducing the expression of mutant Hspb8 [20]. Finally the role of HSPB8 in the chaperone assisted selective autophagy (CASA) complex was highlighted, and the link with BAG3 mutations causing CMT2 and MFM was discussed [21].

8. Plectin-related myopathies

Rolf Schröder (Erlangen, Germany) reported on human plectinopathies, which are caused by mutations in the plectin (*PLEC)* gene on chromosome 8q24. The human *PLEC* gene codes for a 4684 amino acids large protein in addition to various splice forms. Plectin isoforms exert essential roles as linker proteins in the structural and functional organization of filamentous cytoskeletal networks, thereby contributing to the fundamental biomechanical properties of mechanical stress-bearing tissues. The group of human plectinopathies thus far comprises five autosomal-recessive disorders, namely epidermolysis bullosa simplex with muscular dystrophy (EBS-MD), EBS-MD with myasthenic syndrome (EBS-MD-MyS), limb girdle muscular dystrophy type 2Q (LGMD2Q/LGMDR17), EBS with pyloric atresia (EBS-PA), skin-only EBS, and the autosomal-dominant variant EBS-Ogna. Irrespective of the individual PLEC mutations and their varying consequences on plectin protein expression, the skeletal muscle pathology in EBS-MD and EBS-MD-MyS is characterized by subsarcolemmal and sarcoplasmic desmin-positive protein aggregates, degenerative myofibrillar changes and mitochondrial abnormalities [22,23]. In analogy to the keratin pathology in skin, the key pathophysiological event in EBS-MD striated muscle cells seems to be the defective anchorage and spacing of preformed desmin intermediate filaments, which subsequently triggers the formation of desmin protein aggregates as well as the secondary mitochondrial pathology. To date no specific therapy exists for human plectinopathies. A pre-clinical treatment study with the chemical chaperone 4-phenylbutyrate (4-PBA) reported very promising results with an improvement of muscle strength and pathology in plectin knock-out mice, thus rendering the orphan drug 4-PBA as a potential therapy candidate for human EBS-MD patients [23,24]. However, since 4-PBA may negatively impact the stability and function of desmosomes in skin tissue, use of 4-PBA in human plectinopathy patients seems currently not justifiable [25].

Conrad Weihl (Saint Louis, MO, USA) presented an emerging family of PAMs known as "multi-system proteinopathies" or MSP. MSP's are autosomal dominantly inherited disorders affecting muscle, bone and the central nervous system. Patients may present with inclusion body myopathy, Paget's disease of the bone, amyotrophic lateral sclerosis or fronto-temporal dementia in isolation, or as a combination of phenotypes. Mutations in four genes have been associated with MSP and include valosin-containing protein (*VCP*), 'heterogeneous nuclear ribonucleoprotein A1′ (hnRNPA1, (*HNRNPA1*), 'heterogeneous nuclear ribonucleoproteins A2/B1′ (hnRNPA2B1, *HNRNPA2B1*) and sequestosome-1 (*SQSTM1*) [25,26]. Muscle weakness is the most common presenting feature. Muscle biopsies from affected patients display ubiquitin-positive and TDP-43 inclusions, suggesting a pathogenic mechanism that connects RNA binding proteins and protein homeostasis via autophagy. This connection is further bolstered by recent collaborative studies with Bjarne Udd's group that identified 9 patients with distal myopathy and rimmed vacuoles carrying digenic inheritance of a previously reported pathogenic SQSTM1 variant and a second rare variant in the RNA binding protein TIA1 [27]. Cell culture studies including studies with patient-derived fibroblasts demonstrated that TIA1 positive stress granules are cleared via an SQSTM1-dependent autophagic pathway and that SQSTM1 mutations affected this process. Future studies exploring the role of autophagy in stress granule clearance will be informative for this groups of PAMs.

9. Proteomic analysis in PAM

After a brief presentation of genetically unresolved cases by *Antoni Behin* (Paris, France), *Kristl Claeys* (Leuven, Belgium) and *Montse Olivé* (Barcelona, Spain), *Rudolf A. Kley* (Bochum, Germany) reported on proteomic analysis in PAM. Aggregate samples from abnormal muscle fibres containing protein aggregates and intraindividual control samples from aggregate-free fibres were collected by laser microdissection and analyzed by a highly sensitive label-free mass spectrometric approach for identification and relative quantification of proteins. In samples from 103 myofibrillar myopathy patients (filaminopathy ($n=7$), desminopathy ($n=11$), myotilinopathy ($n=17$), HMERF ($n=18$), ZASPopathy ($n=2$), FHL1-myopathy ($n=2$), alphaB-crystalliopathy ($n=1$), and 45 samples from patients with unknown causative mutation), a total of 3437 different proteins could be identified. From these, 294 proteins were significantly over-represented in aggregate samples compared to controls. Extensive immunofluorescence studies confirmed the proteomic data. In accordance with ultrastructural findings, a basic proteomic profile with a strong accumulation of proteins involved in mechanical stabilization and repair of Z-discs was found. Beyond that, more than 50 of the over-represented aggregate proteins are known to be involved in protein quality control and degradation. In addition to shared findings, specific patterns and biomarkers for different MFM subtypes were detected. In conclusion, proteomic analysis revealed important new insights into the composition of pathological protein aggregates in PAM and expanded our knowledge about proteins that seem to be involved in pathogenesis [28–30]. Moreover, detected specific biomarkers for different MFM subtypes can be helpful for differential diagnosis.

10. Role of kelch proteins and regulation of the ubiquitin proteasome system in protein aggregate myopathies

Vandana Gupta (Boston, USA) reported on protein-turnover defects and abnormal aggregation of sarcomeric proteins contributing to skeletal muscle dysfunction in KLHL41 deficiency. Nemaline myopathy (NM) is the most common form of congenital myopathy that results in hypotonia and muscle weakness. This disease is clinically and genetically highly heterogeneous. The defining diagnostic feature of all forms of NM, irrespective of genetic mutation,

is the presence of numerous red-staining rods with Gömöri trichrome stain, appearing at the ultrastructural level as rod-shaped electron-dense structures termed "nemaline bodies" containing aggregates of sarcoplasmic proteins. Three recently discovered NM-causing genes encode members of the Kelch family of proteins. Kelch proteins act as substrate-specific-adapters for CUL3 E3 ubiquitin ligase regulating protein turnover through the ubiquitin-proteasome machinery. Defects in thin filament formation and/or stability are key molecular processes that underlie the disease pathology in NM, however, the role of Kelch proteins in these processes in normal and disease conditions remains elusive. She also presented a role of NM-causing Kelch protein, KLHL41, in premyofibril-myofibril transition during skeletal muscle development through a regulation of the thin filament chaperone, nebulin related anchoring protein (NRAP). During myofibrillogenesis, KLHL41 promotes the degradation and removal of NRAP from sarcomeres through ubiquitination-mediated proteasomal degradation. KLHL41 deficiency results in abnormal accumulation of NRAP in muscle cells. Reducing NRAP levels in KLHL41 deficient zebrafish rescued the structural and functional defects associated with disease pathology. This work showed that defects in KLHL41-mediated turnover of sarcomeric proteins contribute to disease pathology in skeletal muscle [31]

11. Mouse and cell models for filamin C-related MFM

Dieter Fürst (Bonn, Germany) described filamin C (FLNc)-related myopathies and summarized their distinct pathomechanisms. To date more than 50 mutations have been reported, most often causing late onset myofibrillar myopathy and/or cardiomyopathy. The majority of these mutations results in protein misfolding and subsequent aggregation, whereas a second group of mutations leads to haploinsufficiency and loss of function due to altered protein or mRNA stability. In the third and smallest group altered protein properties may result in gain of function [32]. A crucial role for regulating FLNc protein homeostasis plays chaperone-assisted selective autophagy (CASA), based on the adapter protein SYNPO2/myopodin and the co-chaperone BAG3 in conjunction with its associated ubiquitination- and autophagy machineries [33]. Decisive for establishing the pathophysiology of FLNc protein aggregation-causing mutations was the generation of a knock-in mouse model mimicking the human p.W2710X nonsense mutation (in collaboration with R. Schröder. Thus the prime cause initiating the observed increasing muscle weakness is a problem in repairing myofibrillar lesions induced by increased mechanical stress (in particular eccentric contractions) [34,35]. Phosphoproteomics studies have revealed that FLNc is a signaling hub in the myofibrillar Z-disk and its interactions are regulated by stress-induced signaling pathways [36]. The resulting lesion pathology in filaminopathies is ideally revealed in longitudinal muscle sections, rather than the cross-sections used in standard histopathological diagnostics. Since the pathological findings are also reflected in a cell model, current investigations focus on efforts to manipulate the different FLNc homeostasis pathways.

12. Mouse and cell models for plectin-related MFM

Gerhard Wiche (Vienna, Austria) spoke on what we have learned from mice about plectin-related myopathies. Plectin is a large and widespread cytolinker protein that causes a broad spectrum of diseases involving the neuromuscular and vascular systems and the skin when missing or dysfunctional [37]. One of its outstanding features is an isoform diversity based on alternative N-terminal transcript splicing, leading to variants capable of recruiting and anchoring intermediate filaments to different strategic locations with consequences for cytoarchitecture, shape, polarization, mobility and signaling potential of cells [38]. To gain insights into plectin's diverse functions and disease mechanisms we generated and characterized a repertoire of ~20 different genetically altered mouse lines, which provided important insights into the pathomechanisms of plectinopathies, among them protein aggregate myopathies. Muscle-restricted conditional (MCK-Cre) and isoform-specific plectin KO mouse lines allowed us to dissect the various pathological aspects of late onset muscular dystrophy characteristic for EBS-MD patients (desmin network collapse; misalignment of myofibrils; sarcolemma detachment of the contractile apparatus; disruption of costameres; dislocation, aggregation, shape changes, and respiratory dysfunction of mitochondria; and changes in shape, mobility, and gene expression patterns of myonuclei) and correlate them with the dysfunction of specific plectin isoforms [39]. Moreover, we found that postsynaptic membrane infoldings and acetylcholine receptors clustering at neuromuscular synapses require the receptors' linkage to the postsynaptic desmin filament network via a specific plectin isoform. Conditional (Pax7-Cre) plectin KO mice mimicked EBS-MD patients with myasthenic syndrome, including their phenotypic locomotion behavior, and thus could serve as a model for the disease EBS-MD-MyS [40]. Screening assays performed with immortalized, differentiation-competent mouse KO myocytes revealed the chemical chaperone 4-phenylbutyrate to ameliorate protein aggregation ex vivo and in vivo, setting the stage for potential clinical trials involving EBS-MD patients [41].

13. Mouse model for LDB/ZASP-related MFM

Ami Mankodi (Bethesda, USA) spoke on Markesbery–Griggs Distal Myopathy. The disease, also called ZASPopathy is a late-onset, dominantly inherited distal myopathy reported in families of English, French and German descent [42–45]. Symptoms usually begin in the fourth decade of life with ankle weakness followed by involvement of wrist and finger extensor muscles. Proximal weakness occurs later in life. The ability to walk may be lost after 15–20 years of disease, progressing to complete incapacity after 30 years. Muscle imaging at onset of symptoms shows changes

in the posterior compartment of the legs; later, all lower leg muscles and proximal leg muscles become involved [43,44]. Muscle biopsies show prominent rimmed vacuoles and dark inclusions in trichrome stain. Immunostaining of muscle fibers reveals cytoplasmic accumulation of myotilin, desmin, ZASP (Z-disk alternatively spliced PDZ domain-containing protein, also termed LDB3), F-actin, and αB-crystallin [42,44]. Altogether these features in combination with ultrastructural findings are typical of a myofibrillar myopathy. The causative ZASP p.A165V mutation in the Markesbery–Griggs family was shown to be an ancient European founder mutation based on a relatively short common haplotype around the mutation in six unrelated families tested [44]. The other recurring ZASP mutation, A147T, causes an identical phenotype. These mutations are located within the actin-binding domain of ZASP and cause disruption of actin filaments in transfected C2C12 mouse muscle cells, and electroporated mouse muscle fibers [46]. Recombinant mutant ZASP protein has native structure and normal affinity for skeletal actin [47]. A heterozygous knockin of the p.A165V mutation in the mouse *Ldb3* gene causes progressive muscle weakness and pathological features of myofibrillar myopathy. Preliminary findings indicate a gain of function by this ZASP mutation and abnormal disposal of filamin C as an early molecular mechanism leading to myofibrillar degeneration in ZASPopathy. These observations raise the possibility that molecular pathways intersect between distinct myofibrillar myopathies. Identifying common targets for treatment will help in planning adequately powered clinical trials for such rare disorders with unmet medical need.

14. Zebrafish models/therapeutic approaches for MFM

Robert Bryson-Richardson (Melbourne, Australia) presented comprehensive analyses of BAG3 myofibrillar myopathy zebrafish models that demonstrate the protein aggregation, loss of muscle integrity, and reduced muscle function associated with the disease. Autophagy was found to be impaired in skeletal muscle of BAG3 mutant animals, a finding that was replicated in BAG3, LDB3, MYOT, and DES patient tissue. This finding, coupled with the previous demonstration that promoting autophagy could remove aggregates in models of BAG3 and FLNC myofibrillar myopathy [48,49], suggested that autophagy-promoting drugs could be beneficial for myofibrillar myopathy. Therefore, a screen of 71 autophagy-promoting drugs was performed. Nine compounds capable of removing aggregates were identified. Further analysis of two FDA-approved compounds identified metformin as being able to reduce both protein aggregates and fiber disintegration, and rescue swimming defects [50], providing a drug to reduce severity of BAG3, and potentially other forms, of myofibrillar myopathy. He also reported on the progress of an FDA library screen on a nebulin nemaline myopathy model [51]. Analysis of 750 compounds to date, in over 75,000 fish, identified multiple promising leads for further analysis upon completion of the drug screen. These two projects highlighting the use of zebrafish models for drug evaluation in aggregate myopathies.

15. Mitochondrial pathology in plectinopathies and desminopathies

Lilli Winter (Vienna, Austria) presented research dealing with the mitochondrial pathology of two different MFM mouse models corresponding to human plectin- and desmin-related MFM. Plectin, a 500-kDa cytolinker protein, directly interacts with a variety of cytoskeletal structures including the intermediate filament network, which is primarily composed of desmin in muscle. Muscle-restricted conditional plectin KO (MCK-Cre/cKO) revealed progressive degenerative alterations in muscle, pathological protein aggregation, misalignment of Z-disks, and changes in costameric cytoarchitecture [52,53], closely mimicking the phenotypes described for human plectinopathy patients [54]. Histochemical staining of muscle cryosections from MCK-Cre/cKO mice for SDH or COX displayed altered cristae structures, massive subsarcolemmal aggregation of mitochondria, and "rubbed-out" lesions with attenuated or even absent enzymatic activities. Likewise, R349P desmin knock-in mice, harboring the murine orthologue of the most frequently occurring human desmin missense mutation R350P, not only recapitulated the human MFM pathology by developing desmin-positive protein aggregation and skeletal muscle weakness [55], but also displayed mitochondrial abnormalities such as focal accumulation or depletion of mitochondria, as well as giant mitochondria [56]. Since muscle biopsies from MFM patients usually represent only late stages of the disease, MFM-mimicking mouse models like the ones introduced herein are required to decipher the sequential steps in the molecular pathogenesis of MFMs. As alterations in mitochondrial morphology were clearly noticeable already in samples obtained from young mice, at stages where no signs of muscle weakness were apparent, these data indicated that the mitochondrial alterations observed were an early characteristic of MFM rather than a late secondary effect.

16. Registries

Maggie Walter (Munich, Germany) presented the 'German Protein Aggregate Myopathies Patient Registry' (www.pam-register.de), where currently 68 patients with different subtypes are registered. Patient registries have already been proven to be useful tools to overcome fragmentation and to facilitate research in disease epidemiology, genotype-phenotype correlation, and natural history studies. They are also valuable for monitoring standards of care and greatly facilitate feasibility studies and recruitment of patients for clinical trials. Currently there are European and global efforts to set up patient registries for Duchenne Muscular Dystrophy (DMD) and Spinal Muscular Atrophy (SMA). Within the Network of Excellence TREAT-NMD, national registries for DMD and SMA collect data in a harmonized way and contribute them to a European meta-database. These databases

provide a useful model for how a PAM global registry should be organised. The workshop participants agreed on a concerted action towards setting up an international registry for PAM and volunteered to help setting up such a registry. The subsequent steps towards implementation were discussed and decided for a web-based patient self-report system along with professional reports. As a primary goal, it was decided to focus the PAM registries for the tasks of trial readiness, along with natural history and epidemiology.

17. Conclusion

The last decade has led to significant progress in our current understanding of the genetic and molecular basis of PAM, as well as genotype–phenotype and genotype–morphotype correlations within this clinically and genetically diverse group of these rare diseases. Beyond the identification of novel PAM-related gene defects, the rapid progress of next generation sequencing approaches now also provides a solid basis for efficient genetic testing, which will help improve diagnostics as well as counselling of patients suffering from the various subforms of PAM. Substantial progress in our current understanding of PAM has been gained by the generation and characterization of a great variety of animal and cell models that mirror central aspects of the disease pathophysiology. Beyond further pathophysiological studies, these disease models now provide the basis for pre-clinical therapy intervention studies using pharmacological and gene-editing approaches. Although no specific treatment is currently available for PAM, the deeper insight into the disease course and disease-specific complications (e.g. cardiac or respiratory disease manifestations) that was gained during the last decade already provides an improved clinical management of PAM patients.

Establishing a unifying classification of PAM, which would take into account all the clinical, pathological and genetic findings used in previous classification efforts, still is an unresolved and complicated issue. For instance, mutations in a specific gene (e.g. *FLNC*, *TTN*) may cause a broad spectrum of clinical phenotypes with different disease onsets, variable disease severity and distinct myopathological presentations. In contrast, various familial and sporadic clinical presentations (LGMD, distal myopathies, scapuloperoneal syndromes, and generalised myopathies) can be caused by a wide variety of different gene defects. Furthermore, seemingly identical myopathological presentations, as discussed in PAM, can again be attributed to a wide range of pathological gene alterations. This explains the complexity of the still unresolved classification issue in an exemplary fashion.

18. Future perspectives

The swift progress in genetic analysis approaches will open the opportunity to genetically clarify the still substantial number of PAM of unclear etiology. This will not only lead to better diagnostic approaches, but will also complete the picture of culprit genes in PAM and their related molecular functions. Deeper understanding of the individual and shared molecular disease mechanisms related to protein interaction networks and signaling pathways are mandatory to identify both new therapeutic targets and substances. The biggest challenge for future PAM-related research is to pave the way towards specific curative or truly ameliorating treatment options. The next decade will also show, if and to what extent PAM-related gene editing strategies can be exploited.

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