



Workshop report

215th ENMC International Workshop VCP-related multi-system proteinopathy (IBMPFD) 13–15 November 2015, Heemskerk, The Netherlands

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1. Introduction

The 215th ENMC Workshop on VCP-related multi-system proteinopathy (IBMPFD) took place in Heemskerk on the 13th–15th of November 2015. The workshop brought together a multidisciplinary group of 19 participants from 6 different countries (Germany, France, Japan, Finland, UK and USA) working on the clinical and basic aspects of VCP-related multi-system proteinopathy together with 2 patient representatives. In this report we summarize the state of the art and future directions related to the topics discussed during the meeting. The topics included characterization of phenotypes, phenotype–genotype correlations, pathophysiology, animal and cell models, therapeutic strategies and the need to create an international consortium and a registry for IBMPFD.

2. Background and aims

Inclusion body myopathy (IBM) with Paget disease of bone (PDB) and frontotemporal dementia (FTD) or IBMPFD is caused by mutations in the *valosin containing protein* (VCP) gene. Kimonis et al. first recognized VCP disease as a genetically distinct clinical syndrome in 2000 [1]. Clinically, the condition is heterogeneous even within families and with additional phenotypes being identified recently [2,3]. There is insufficient information about the incidence and prevalence, genotype–phenotype correlations, penetrance, natural history and causes of death in IBMPFD. Currently, there is no known specific treatment for IBMPFD, and even though there are some interesting leads from animal studies [4–6] there are no

established patient cohorts and outcome measures to test these compounds/approaches in appropriate clinical trials.

The aims of the workshop were 1. to achieve a better understanding of the epidemiology, phenotype and genetics of VCP related conditions; 2. understand genotype–phenotype correlations, intrafamilial variability and genetic modifiers of disease phenotype; 3. discuss currently available cellular and small animal models of the disease; 4. discuss the eventual creation of a global registry; 5. discuss existing preclinical data and how to translate that to human clinical studies; 6. agree on standards of care and therapeutic options for VCP disease; and 7. create an international consortium for study and treatment of VCP related diseases.

3. Session 1: introduction to VCP disease; clinical and molecular aspects

3.1. Clinical phenotypes: Virginia Kimonis

Dr Kimonis opened the meeting with an overview of the main clinical aspects of IBMPFD. Dr Kimonis first reported hereditary multisystem proteinopathy associated with the unusual combination of distal/proximal hereditary inclusion body myopathy (h-IBM), Paget disease of bone (PDB) and dementia (FTD) or IBMPFD in 2000 [1,2,7].

The critical region of the gene was identified by linkage analysis on chromosome 9p13.3-12 [8]. In 2004, Dr Giles Watts found six missense mutations in the gene encoding valosin-containing protein (VCP, a member of the AAA-ATPase superfamily) [9]. VCP is associated with many cellular activities, including cell cycle control, membrane fusion and the ubiquitin–proteasome degradation pathway. Dr Kimonis has studied the largest cohort of patients with VCP disease (n = 187 individuals in 37 families) [10]. Myopathy was present in 89%, PDB in 43%, and dementia in 30%. The phenotype has now expanded to include amyotrophic lateral sclerosis (ALS) in

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10%, Parkinson's disease in 4%, cardiomyopathy, Charcot–Marie–Tooth disease, and anal incompetence, among others [10–13]. VCP accounts for 2% of familial ALS [14]. Muscle fibres reveal rimmed vacuoles and cytoplasmic inclusions consisting of ubiquitin and TDP43 aggregates in VCP disease and also in ALS, and FTD suggesting a common molecular pathogenesis of these disorders [15,16].

Currently 41 different mutations have been reported (see Table 1) and undoubtedly more will be described (Al-Obeidi et al., manuscript in preparation). Genotype–phenotype analysis has been attempted; however, because of the intra-familial variation and small family size, establishing a genotype–phenotype correlation is difficult for most mutations

Table 1
VCP mutations described in the literature.

Mutation	Described phenotype	Transmission	Reference
p.I27V	IBM, PDB, FTD	AD	[24,25,69]
p.R93C	IBM, PDB, FTD	AD	[70]
p.R93H	HSP	Sporadic	[71]
p.R95C	IBM	Sporadic	[25]
p.R95G	IBM, PDB, FTD	AD	[9]
p.R95H	FTD	Unknown	[72]
p.G97E	IBM, PDB, FTD	AD	[73,74]
p.I114V	IBM, ALS	Sporadic	[75,76]
p.P137L	IBM, PDB, FTD	AD	[77]
p.I151V	ALS	Sporadic	[78]
p.R155H	IBM, PDB, FTD, ALS	AD	[9]
p.R155C	IBM, PDB, FTD, ALS	AD	[9,79]
p.R155P	IBM, PDB, FTD	AD	[9]
p.R155S	IBM, PDB, FTD	AD	[18]
p.R155L	IBM, PDB, FTD, ALS	AD	[80]
p.G156S	IBM, PDB, FTD	Sporadic	[81]
p.G156C	ALS	AD	[82]
p.G157R	IBM, PDB, FTD	AD	[83]
p.M158V	PDB, ALS	Sporadic	[84]
p.R159H	IBM, PDB, FTD, ALS	AD	[75,85,86]
p.R159C	IBM, PDB, FTD, ALS, Parkinson's disease, HSP	AD	[12,79,87,88]
p.R159G	FTD, ALS	AD	[14]
p.E185K	CMT2	AD	[89]
p.R191Q	IBM, PDB, FTD, ALS	AD	[9]
p.R191G	IBM, ALS	AD	[79]
p.L198W	IBM, PDB, FTD	AD	[7]
p.G202W	IBM, FTD	AD	[13]
p.I206F	IBM, PDB, FTD	AD	[90]
p.A232E	IBM, PDB	AD	[9]
p.T262A	IBM, PDB, FTD	AD	[91]
p.K386E	IBM	NR	[92]
p.N387H	IBM, FTD	AD	[7]
p.N387T	ALS	Sporadic	[93]
p.N387S	IBM, PDB, FTD	AD	[94]
p.A439S	IBM, PDB	AD	[18]
p.A439P	IBM, PDB, FTD	AD	[19]
p.A439G	IBM, FTD	AD	[13]
p.R487H	FTD, ALS	AD	[95]
p.D592N	ALS	AD	[14]
p.R662C	ALS	Sporadic	[93]
p.N750S	ALS	Sporadic	[96]

AD – autosomal dominant; AR – autosomal recessive; ALS – amyotrophic lateral sclerosis; FTD – frontotemporal dementia; IBM – inclusion body myopathy; PDB – Paget diseases of the bone; HSP – hereditary spastic paraparesis; CMT – Charcot–Marie–Tooth disease.

[10]. Modifier genes were studied and the ApoE4 was associated with a higher incidence of dementia [17].

The true incidence of VCP disease is probably higher than currently known. Patients suffering from this lethal disease have been misdiagnosed with a range of other diseases [3]. The complex manifestation of IBMPFD necessitates attention to family history, screening for PDB/FTD and consideration of molecular studies in individuals who do not have the classic syndrome.

3.2. The UK cohort: Teresinha Evangelista/David Hilton-Jones

Dr Evangelista gave an update on the previously published data regarding the UK cohort [13]. The identification of 42 patients from 21 families has allowed estimation of the prevalence of the disease for the UK in 0.66/100,000 population. Following this publication, 3 new patients were identified in 2015 harbouring pathogenic mutations, and three others were found to harbour variants of unknown significance. In our cohort there is an equal distribution among sexes, the onset is after the third decade of life with a peak around the 4th decade. Muscle weakness was the first symptom in all but one of the patients. The pattern of muscle weakness at onset was quite variable. However, combined shoulder and pelvic girdle weakness was most frequently observed. Consideration should be given to the relatively high frequency of distal weakness, in particular of the hands and arms, as the presenting symptom. The mean time to loss of ambulation was 13 ± 7 years after onset, and cognitive decline was becoming more prevalent with disease progression as 60% of the non-ambulant patients experienced some degree of cognitive decline. Other clinical manifestations were due to bone involvement, either as confirmed PDB, bone pain or high levels of alkaline phosphatase. Two out of 20 patients (10%) needed non-invasive ventilation (NIV) after more than 15 years of disease duration; 3 patients with the p.R155H mutation had mild cardiac dysfunction. Expanding the classical phenotype we have found the following atypical symptoms: 6 patients with camptocormia, 3 patients with Parkinson's disease; 9 patients with sphincter dysfunction and some other less frequent findings. Muscle MRI, although non-specific, frequently shows pockets of fat tissue within certain muscles, in particular the calf muscles but also the hamstrings and the quadriceps muscles. Also unspecific are the muscle biopsy findings; rimmed vacuoles were one of the most frequent changes found in our series together with a mixture of neurogenic and myopathic changes. Some biopsies have MHC1 up-regulation and a few have small inflammatory infiltrates.

The take home messages from our series are a high frequency of distal weakness affecting mostly the small hand muscles; the high frequency of scapular winging, the slow disease progression and the correlation of the loss of ambulation with the cognitive status; the phenotypic heterogeneity with common misdiagnosis such as limb girdle muscular dystrophy, FSHD, myofibrillar myopathy and distal myopathy such as GNE and Udd myopathy, and finally the occurrence of manifestations that were not classically

associated with VCP disease. All VCP mutations found in the different cohorts are summarized in [Table 1](#).

3.3. The French cohort: Bruno Eymard

Dr Eymard presented the French cohort that includes 28 patients, 14 males, from 17 families. Twenty-three patients are French, and 5 patients (2 families) originated from Spain (San Sebastian) [18]. In 7 families, 2 or more patients were genetically tested. An autosomal dominant inheritance was clearly evident in most families, with two patients having a negative family history. Age at onset ranged from 20 to 70 years with the following distribution: 20–30 years: 4 cases; 31–40: 7 cases; 41–50: 10 cases; 51–60: 3 cases and 61–70: 2 cases.

The first manifestation was a myopathy in 24/28 patients, PDB in 3/28 and in 1 case a distal motor neuropathy. Proximal weakness was the first manifestation in 21 patients with the following distribution: combined shoulder and pelvic girdle in 9 patients; shoulder girdle in 7 and pelvic girdle in 5 patients. Other phenotypes were less common: distal weakness with foot extensor involvement was the onset in 3 cases, scapuloperoneal pattern in 2 cases and in one there was proximal and distal upper limb involvement. In the lower limbs the pelvic muscles were the most affected, quadriceps and hamstrings being equally involved. Scapula fixator muscles were often affected with scapular winging being present in 22 patients. Axial involvement was very common, involving mainly the trunk flexors. CK (creatin kinase) values were normal or moderately elevated. Muscle biopsy was performed in 24 patients, revealing rimmed vacuoles in 17 cases. Dystrophic features were common with marked fibre size variation, internal nuclei, and fibrosis. PDB was present in 12 patients and was the first manifestation in 3 patients. Technetium-99-ECD proved to be more sensitive than bone X-ray to detect PDB. ALP level was increased in 50% of PBD patients. Mild cognitive impairment presenting as a dysexecutive syndrome, detected by neuropsychological tests, was seen in 7 patients. Severe progressive dementia was found in 4 patients. Disease was severe in most of patients and fatal in 9 patients due to weakness, respiratory distress, or dementia (often combined). Ten patients become wheel-chair users after a mean disease course of 9 years (range 5–24). Respiratory function was altered in 8 patients with a forced vital capacity in the range of 29%–70% of the predicted value. Non-invasive ventilation was necessary in 2 patients after 10–16 years. FSHD was the most frequent misdiagnosis when physicians were not aware the patient suffered from PDB. This was due to the scapular winging and the autosomal dominant transmission. After negative genetic test for FSHD, muscle biopsy was the next step and in most patients the rimmed vacuolar finding prompted molecular testing for VCP. Twelve different missense mutations were found in this cohort (see [Table 1](#)). One Algerian family with the mutation p.R155C was particularly interesting, due to the coexistence of one case with distal motor neuron involvement and all other with a myopathy. This illustrates the value of the electrophysiological examination in the characterization of these patients.

3.4. The Asian cohort: Satoru Noguchi

Although GNE myopathy is clearly more prevalent in Japan, Dr Noguchi reported the molecular, clinical and pathological features of Asian patients with VCP myopathy. His group has screened for VCP mutations in 174 unrelated undiagnosed patients with rimmed vacuoles in the muscle biopsy; 105 patients with a distal myopathy, 29 with a limb girdle phenotype and 40 with other phenotypic aspects. They identified VCP mutations in 7 patients (4.2%) [19].

In another study, 14 undiagnosed patients (13 Japanese and 1 Korean) from 13 families were screened and 9 mutations were identified, with three being novel.

The clinical features were varied with onset between the ages of 31 and 59 years. The initial symptoms and the pattern of muscle weakness showed high variability. In eight patients the muscle weakness was predominantly proximal and in 4 patients predominantly distal. Six patients showed scapular winging and four had weakness of the paraspinal muscles, facial muscle weakness was present in only one patient. Serum CK levels were normal or slightly increased. The EMG was neurogenic in all patients. Brain and bone involvement were rare, being found in only 2 and 3 patients, respectively. Rimmed vacuoles and cytoplasmic bodies were present in all muscle biopsies. With the ATPase staining (pH 10.6), group atrophy and fibre type grouping were seen in some patients, indicating neuropathic changes. On Cytochrome C oxidase staining, some fibres showed COX deficiency. VCP, TDP-43, polyubiquitin and HDAC were accumulated in nuclei and subsarcolemmal regions of the myofibres as reported previously [20].

3.5. The German cohort and proteomic analysis of sarcoplasmic protein aggregates: Rudolf Kley

Forty-two patients with pathogenic VCP mutations have been identified in Germany. Detailed clinical data were available in 24 patients from nine different families (5 women, 19 men). Except for three asymptomatic carriers, all patients presented with clinical signs of myopathy confirmed by muscle biopsy in nine patients. Paget disease was described in 8/24 patients, dementia in 9/24 patients, cardiomyopathy in 3/24 patients, and a peripheral neuropathy in 6/24 patients.

Protein aggregation in muscle fibres is a typical histological feature of VCP-associated myopathy. The authors applied a proteomic approach to decipher the composition of these aggregates in muscle samples from two patients. Protein aggregates and intraindividual control samples (from aggregate-free muscle fibres) were collected by laser microdissection and analysed by a label-free mass spectrometric approach for identification and relative quantification of proteins [21–23]. These analyses showed an over-representation of several proteins including Z-disc (-associated) proteins, chaperones, and proteins involved in proteasomal and autophagic protein degradation in aggregate samples (compared to control samples).

A comparison of proteomic findings in different protein aggregate myopathies revealed that the aggregate composition in VCP-associated myopathy was highly similar to that identified in 97 myofibrillar myopathy samples. The ten most

abundant over-represented aggregate proteins were identical and included filamin C, desmin, Xirp2, Xin, N-RAP, alphaB-crystallin, nestin, myotilin, and Hsp27. These findings suggest an overlap of pathomechanisms leading to sarcoplasmic protein aggregation. In contrast, the proteomic aggregate profiles in hereditary inclusion body myopathy caused by *GNE* mutations and in cap myopathy were not comparable to that found in VCP-associated myopathy.

3.6. Expanding VCP mutations to sporadic diseases (ALS, Paget's disease and sIBM): Chris Weihl

In a session entitled "Expanding VCP mutations to sporadic diseases" Dr Weihl discussed that in reviewing data from the EXAC database (<http://exac.broadinstitute.org/>) VCP is extremely intolerant to loss of function and missense variants. These data suggest that rare variants in VCP may be more clinically meaningful than for other genes. Indeed several known pathogenic mutations in VCP are present within the EXAC database albeit at very low frequencies. One exception is the I27V missense variant that has an allele frequency of 0.05% within the population. This variant has been described several times as being potentially pathogenic and has been identified in sporadic Parkinson's disease and sporadic inclusion body myositis [24,25]. These data suggest that variants within VCP may define risk of more common degenerative diseases.

4. Session 2: diagnosis, management and pathology of VCP disease

4.1. Clinic, neuropathology and management of frontotemporal dementias/VCP disease: Basil Ridha and Jonathan Rohrer

Frontotemporal dementia (FTD) is a heterogeneous group of disorders clinically, neuropathologically and genetically. What is common to all is the presence of a predominant focal cognitive deficit syndrome associated with focal brain degeneration affecting the frontal and/or temporal lobes [26].

The histopathology of these disorders is complex with several subtypes based on the abnormal deposition of certain proteins [27]. The commonest pathology is the one associated with the deposition of TDP-43 protein, which accounts for about 50% of cases. This is further subdivided into 4 subtypes: A, B, C and D depending on the level of abundance of dystrophic neurites, neuronal cytoplasmic inclusions and intranuclear inclusions, and the distribution of the pathology in the various cortical layers [28]. IBMPFD-associated pathology is exclusively type D, characterized by abundant neuronal intranuclear inclusions and short dystrophic neurites affecting all cortical layers. The second most common pathology is associated with deposition of tau protein, accounting for about 45% of cases. This is further subdivided depending on whether the deposited tau has predominantly 3 or 4 microtubule binding domains (3R or 4R), or both. 3R tauopathies include Pick's disease, where intracytoplasmic inclusions called Pick bodies are present, whilst 4R tauopathies include progressive supranuclear palsy (PSP) characterized by the presence of tufted astrocytes, and corticobasal degeneration (CBD)

characterized by the presence of astrocytic plaques. The third pathological subtype is the one associated with deposition of fused-in-sarcoma protein (FUS), which accounts for about 5% of cases. FUSopathies include atypical frontotemporal lobar degeneration with ubiquitin inclusions (aFTLD-U), neuronal intermediate filament inclusion disease (NIFID) and basophilic inclusion body disease (BIBD). Limited cases have none of these three pathologies, including patients with mutations in the CHMP2B gene. It is worthwhile noting that Alzheimer's disease pathology has been found in cases presenting clinically with any of the FTD syndromes.

Clinically FTD presents either as a syndrome predominantly affecting personality, behaviour and executive function (behavioural variant frontotemporal dementia, bvFTD), or as impaired language skills (primary progressive aphasia, PPA) [26,27,29]. The latter is subdivided further into at least three distinct clinical syndromes: nonfluent variant PPA where speech production is affected with grammatical errors and/or apraxia of speech, semantic variant PPA, where there is loss of word meaning at a conceptual level, and logopenic variant PPA, where there are word-finding pauses and impaired working memory. The latter is mostly associated with Alzheimer's disease pathology, although some cases have been reported with FTD pathological variants [30]. The FTD syndromes may overlap with other neurological conditions such as motor neuron disease or the atypical parkinsonian syndromes such as PSP or corticobasal syndrome. In patients with IBMPFD, most cases have bvFTD but a smaller number of cases have been described with PPA [31].

Management of patients with FTD is supportive in the absence of any disease modifying therapy [26,29]. It is important to provide early explanation and support to family members, who are often distressed by the change in personality and behaviour. Practical advice such as avoidance of confrontation and argument with patients with bvFTD, sticking to a daily routine, and getting patients to participate in activities that they enjoy such as listening to music, drawing or solving puzzles can be helpful. Removal of any potential source of harm from the patient's environment such as keeping sweets and chocolates away if they have a sweet tooth, or selling the car or removing car keys if the patient insists on driving, is advisable. Pharmacologic intervention is very limited. Only a few small studies have suggested a possible benefit of SSRIs in reducing the behavioural symptoms of bvFTD.

4.2. Paget disease of the bone: Stuart Ralston

Stuart Ralston gave presentations on the pathogenesis and management of Paget's disease of bone (PDB) and related disorders.

He reported that classical Paget's disease and other Paget's-disease like syndromes had a strong genetic component and that genome wide association studies coupled with linkage studies and positional cloning efforts had now identified nine genes and/or loci where there was robust evidence of predisposition to PDB [32]. These included *SQSTM1*, *CSF1*, *TNFRSF11A*, *TNFRSF11B*, *OPTN*, *RIN3*, *TM7SF4* and *VCP*. He mentioned that all of these genes were known or suspected to play a role in

osteoclast differentiation and function and that some (*SQSTM1*, *VCP* and *OPTN*) also play roles in autophagy. Finally, he said that additional loci had been discovered on chromosomes 7q33 and 15q24, where there was genome wide association with PDB but where the causal genes had not yet been identified. Whilst stressing the importance of genetic factors in PDB he mentioned that the disease had become less common and less severe in many countries over the past 50 years. The reasons for this were incompletely understood but possibilities would be changes in nutrition, a reduction in mechanical loading of the skeleton as the result of a more sedentary lifestyle and differences in exposure to infections.

With regard to the clinical features of PDB, he reported that in his experience, the PDB that occurred in association with mutations of the *VCP* gene was similar to classical PDB in terms of radiologic appearances and biochemical features – such as raised alkaline phosphatase (ALP) levels. However the age of onset was earlier, often occurring in the fourth decade. He went on to mention that the PDB he had observed in association with *VCP* mutations had usually been picked up as an incidental finding because of an elevated ALP level. He went on to review data on the clinical presentation of classical PDB, noting that in about 20% of patients the disease was asymptomatic and picked up as an incidental finding, whereas in the remainder, various symptoms were present, most commonly bone pain [33].

With regard to therapy of PDB he reported that bisphosphonates were highly effective at suppressing the elevated bone turnover that is characteristic of PDB [34]. He mentioned that bisphosphonates had been found to improve bone pain associated with PDB, but that there was no evidence as yet, that “intensive” therapy with bisphosphonates could alter the natural history of the disease [35]. Having said that, he noted that most clinical trials of bisphosphonate therapy had been conducted in patients with established disease where skeletal damage was already present. He mentioned that currently a trial was in progress (the ZIPP study ISRCTN 11616770), which was due to report in 2020 in which asymptomatic carriers of *SQSTM1* mutations had been randomized to receive placebo or zoledronic acid treatment. He said that this should hopefully give valuable information on the effects of very early bisphosphonate therapy on the progression of the disease and that perhaps similarly designed studies might of interest in other inherited bone diseases associated with increased bone resorption.

4.3. Neuromuscular pathology and muscle MRI in VCP disease: Bjarne Udd

Bjarne Udd reported on published and unpublished findings in muscle biopsy pathology and muscle MRI findings in VCP-myopathy.

The main finding on routine histopathology is that of rimmed vacuoles mainly in atrophic fibres. The rimmed vacuoles contain autophagosomal LC3 and aggregates of autophagy markers TDP-43 and p62 similarly to other rimmed vacuolar myopathies such as sporadic inclusion body myositis s-IBM, GNE-myopathy, Welander distal myopathy, MATR3

distal myopathy and others. In addition occasional ubiquitin positive VCP containing aggregates have been observed not directly associated with the rimmed vacuoles. Myofibres showing myofibrillar disorganization with larger accumulations of Z-disc or intermediate filament proteins such as myotilin and α B-crystallin are very rare, suggesting the histopathology is mainly categorized within the group of rimmed vacuolar myopathies. Some secondary caveolin-3 decrease has been shown in normal sized fibres.

Muscle MRI is very different from other muscular dystrophies that usually have highly differentiated and selective patterns of muscle involvement: some muscles are dystrophic with fatty degeneration and replacement, whereas others may be spared. In VCP-myopathy this is not the case as more or less all muscles are involved, but they show unusual patchy regions of lost muscle tissue replaced by fatty connective tissue. On the lower legs both predominant posterior and predominant anterior compartment muscle involvements can occur and exceptionally to the extent of anterior compartment lower leg involvement only as with distal myopathies. A major signal increase in fat suppression sequences has not been observed, in line with a usually very low increase of CK levels.

4.4. Genetic diseases with phenotypic overlap to VCP disease (*SQSTM1*, *HNRPN2B1* and *HNRNPA1*): Chris Weihl

Over the past several years, it has become clear that VCP is not the only gene associated with a syndrome of dementia, motor neuron disease, inclusion body myopathy and Paget’s disease of the bone. Mutations in two RNA binding proteins (*hnRNPA1* and *hnRNPA2B1*) were identified in non-VCP associated IBMPFD, suggesting that VCP disease or IBMPFD is an inadequate terminology [36]. The nomenclature “multisystem proteinopathy” or MSP has been suggested and utilized in several manuscripts [37]. This nomenclature would define MSP1 as mutations in *VCP*, MSP2 – *HnRNPA2B1* and MSP3 – *HnRNPA1*. Last year, MSP4 was identified as mutations in *SQSTM1* can cause IBM, PDB, ALS and FTD [38].

5. Session 3 – VCP function and dysfunction: molecular mechanisms and cellular function

5.1. Overview of VCP function and structure: Hemmo Meyer

Hemmo Meyer gave an overview of the structure and function of VCP. VCP is an essential hexameric AAA+-type ATPase that has emerged as a central element of a large variety of ubiquitin-mediated processes [39]. A major role of VCP is ensuring cellular protein homeostasis. It supports protein degradation in the two main degradation pathways. On one hand, it facilitates delivery of misfolded proteins from different compartments to the proteasome through its ability to untangle and/or partially unfold ubiquitinated client proteins. On the other hand, it supports degradation in the lysosome through both endolysosomal sorting and autophagy albeit the role of VCP herein is less well understood so far. In addition, VCP regulates regulatory degradation events that govern signal transduction and cell cycle regulation mechanisms crucial for

proliferation and genome stability. This raises the question as to why mutations cause a late onset degenerative disorder with no apparent genome instability phenotype, and which of the many functions of VCP is compromised in the disease.

Hemmo Meyer pointed out that an important feature of VCP is that it cooperates with diverse protein cofactors that function in different pathways. It was proposed that disease-associated mutations affect only a subset of cofactors with non-essential functions. For example, the current data suggest that the VCP-Ufd1-Npl4 complex, which mediates essential genomic functions linked to the proteasome, is not affected in the disease. In contrast, a complex with UBXD1 was recently identified that mediates endolysosomal sorting of caveolin, the major constituent of caveolae at the plasma membrane [40]. Disease-associated mutations specifically disrupt this complex and compromise proper endolysosomal sorting in cell models and patient tissue. Consistently, UBXD1 was found to bind VCP residues, which are hotspots for mutation, and regulates inter-domain communication within the VCP hexamer [41]. These data are consistent with a model in which defects of endosomal and lysosomal functions constitute a major contribution to the pathogenesis of the disease.

5.2. Autophagic dysfunction in VCP disease: Chris Weihl

VCP is necessary for several cellular processes; dysfunction in which of these processes underlies VCP mutant pathogenesis is unclear. Several lines of evidence suggest that VCP mutations that lead to disease affect autophagosome maturation to the lysosome. Transgenic mice and cell lines expressing VCP mutations accumulate autophagosomes that fail to degrade cytosolic contents. Augmenting autophagy with drugs like rapamycin fails to improve the autophagic flux leading to the accumulation of more non-degradative autophagic structures [42,43].

Work by Paul Taylor and Roy Parker has suggested that VCP may be more specifically involved in the autophagic degradation of RNA granules or “stress granules” [44]. Stress granules contain RNA binding proteins such as TDP-43, hnRNPA2B1, hnRNPA1 and TIA (all proteins associated with inclusion body myopathy pathology) and form under conditions of cellular stress as a means of arresting mRNA translation. However, how these RNA granules resolve after a cellular stress is unclear and what molecular machinery is required for this process is unknown. Using a yeast system, it was determined that VCP in addition to other autophagy proteins was necessary for stress granule dissolution and that this was also impaired in VCP mutant cells [44]. These studies connect VCP dysfunction with the RNA binding protein pathology seen in VCP disease.

5.3. Proteasome dysfunction in VCP disease: Christoph Clemen

Christoph S. Clemen reported on VCP studies in newly generated mouse, zebrafish and *Dictyostelium discoideum* models. To study the molecular pathogenesis of the IBMPFD disease, two novel VCP mouse models were generated. One targeting strategy resulted in a knock-in mouse model harbouring the

second most frequent human p.R155C VCP missense mutation. Although no homozygous animals could be generated, preliminary data from the analysis of young sedentary heterozygous animals showed no overt clinical or histopathological phenotype (unpublished results). The other targeting approach led to a VCP haploinsufficiency mouse model with significantly reduced VCP mRNA and protein levels. The analysis of the latter mouse model demonstrated a significantly reduced proteasome activity in tissue lysates of skeletal muscles, which could be markedly increased by the addition of recombinant VCP. Further work identified the proteasome inhibitor PSMF1/PI31 as a novel direct VCP interaction partner. Subsequent *in vitro* assays revealed that VCP and PSMF1/PI31 antagonistically regulate the proteasomal activity [45]. The knock-down of VCP in zebrafish resulted in a myopathic phenotype of fish larvae with a significant motility defect [46]. In the amoeba *D. discoideum* the UBX-domain containing protein UBXD9 was identified as a novel VCP interaction partner. Pull-down and surface plasmon resonance experiments using *D. discoideum* or human UBXD9 together with the respective wild-type or point mutant VCP demonstrated species-, mutation- and ATP-dependent differences in the binding affinities. Further, the *D. discoideum* and human UBXD9 proteins very efficiently disassembled wild-type and to a much lesser extent mutant VCP hexamers into monomers [47].

6. Session 4 – VCP function and dysfunction: pre-clinical studies and clinical studies of similar syndromes

6.1. Therapeutic modulation of VCP function using small molecules: Ray Deshaies

The presentation by Raymond J. Deshaies was divided into two parts. In the first half, he summarized his efforts to develop a competitive inhibitor of VCP ATPase activity. A high-throughput screen for ATPase inhibitors yielded DBEQ [48], which was optimized in a medicinal chemistry campaign to generate ML240 [49]. ML240 was licensed to a startup company, Cleave Biosciences, of which Dr. Deshaies was a founder. Cleave performed additional medicinal chemistry on ML240 to yield CB-5083 [50] which is now being evaluated in human phase 1 clinical trials as a potential treatment for cancer.

In the second part of his talk, Dr. Deshaies focused on the interaction of adaptor proteins with VCP. SILAC mass spectrometry experiments revealed that VCP complexes undergo extensive mixing during standard immunoprecipitation protocols, suggesting that the interaction of adaptors with VCP is extremely dynamic. To study this interaction in more detail, a FRET assay was devised to monitor binding of p47 to VCP. These proteins interact with high affinity in ATP but much lower affinity in ADP. Interestingly, the VCP-p.R155H mutant binds to p47 in a manner that is not regulated by nucleotide state. Dr. Deshaies suggested that the key effect of the p.R155H mutation may be to increase dynamics of VCP–adaptor complexes. Future studies will explore the effect of potential second-site suppressors of IBMPFD mutations, developed in collaboration with the laboratory of Brian Kuhlman, on the affinity and dynamics of the VCP–p47 interaction. Ultimately,

they hope to determine the 3-dimensional structure of full-length VCP-p.R155H and double mutants carrying second-site suppressors.

In concluding comments, Dr. Deshaies suggested that given the relatively low turnover number of VCP, it may be possible to develop compounds that enhance VCP ATPase activity, as potential therapies for IBMPFD or other neurodegenerative disorders that feature accumulation of aggregated proteins or stress granules.

6.2. Pre-clinical studies using mouse models: Virginia Kimonis

Human and mouse VCP proteins differ by only one amino acid residue at position 684. The targeted homozygous deletion of VCP by Cre-loxP technology was reported to result in early embryonic lethality [51]. In contrast heterozygous mice lacking one VCP allele and having one wild-type allele were apparently indistinguishable from their wild-type littermates [51]. Wehl et al. developed transgenic mice over-expressing the most common human VCP mutation (p.R155H) under the regulation of a muscle creatine kinase promoter. These mutant mice became progressively weaker in a dose-dependent manner. At 6 months of age they showed muscle pathology including coarse internal architecture, disorganized membrane morphology and vacuole-like clefts with reduced caveolin-3 expression at the sarcolemma [52]. Custer et al. [53] reported transgenic mice that overexpressed mutant forms of VCP. The mice had muscle weakness and pathology characteristic of inclusion body myopathy including rimmed vacuoles and TDP-43 pathology. Radiologic examination of the skeleton revealed focal lytic and sclerotic regions in the vertebrae and femur. Additionally the brain revealed widespread TDP-43 lesions and the mice also exhibited abnormalities in behavioural testing.

The knock-in mouse model of the common VCP p.R155H mutation generated by the Kimonis laboratory has features of human VCP-associated muscle, bone, spinal cord and brain pathologies [54]. The mice demonstrated progressive muscle weakness, and muscle sections showed increased numbers of autophagosomes, vacuolization of myofibrils and centrally located nuclei, in addition to TDP-43- and ubiquitin-positive inclusion bodies in quadriceps myofibrils and brain [55]. The mice also demonstrated spinal cord pathology with age dependent degeneration of ventral horn MNs, TDP-43 positive cytosolic inclusions, mitochondrial aggregation and progressive astrogliosis. Although the VCP^{R155H/+} heterozygous mice demonstrate a slow rate of progression the homozygous p.R155H mice with two mutant copies (VCP^{R155H/R155H}) only survive 2–3 weeks after birth, exhibit progressive weakness prior to their early demise as well as accelerated pathology and develop marked MN degeneration with TDP-43 pathology [55]. The mutation overexpressing VCP transgenic mice and knock-in mice thus replicated the human disease and represent useful models for trials of novel therapies for diseases with similar pathogenesis. Progressive uphill exercise in the knock-in VCP^{R155H/+} mice revealed significant improvement in muscle strength and performance by grip strength and Rotarod analyses when compared to the sedentary mice. Histologically, the uphill

exercised VCP^{R155H/+} mice displayed an improvement in muscle atrophy, and decreased expression levels of ubiquitin, P62/SQSTM1, LC3I/II, and TDP-43 autophagy markers, suggesting an alleviation of disease-induced myopathy phenotypes. In contrast, mice exercised to run downhill did not show any improvement [4]. Targeted excision of the p.R155H mutation in VCP mice using Cre-ERTM-VCP^{R155H/+} technology demonstrated improved muscle strength and quadriceps fibre architecture, reduced expression of autophagy markers, reduced brain neuropathology, decreased apoptosis, and improvement of the Paget-like bone changes [4]. This finding provides proof of principle that other allele silencing or downregulating technologies may be useful therapies in patients.

Autophagy is a major clearance pathway for the removal of mutant protein associated with many disorders. Autophagy is negatively regulated by the mammalian target of rapamycin (mTOR) and targeting the mTOR pathway ameliorates an increasing list of disorders. Ching et al. [43,56], studied the effect of mTORC1 inhibition with rapamycin and found that it hastened weakness, atrophy, vacuolation and accumulation of autophagic substrate p62, LC3II and ubiquitinated proteins in VCP mouse muscle. Nalbandian et al. [5], however, demonstrated improvement in muscle performance, quadriceps histological analysis, and rescue of ubiquitin, and TDP-43 pathology and defective autophagy as indicated by decreased protein expression levels of LC3-I/II, p62/SQSTM1, and optineurin. Differences in these reports may be explained by the different mouse models being used for the two studies. Further studies will clarify if VCP disease and related neurodegenerative multisystem proteinopathies can potentially be ameliorated by rapalogs.

Llewellyn et al. found that feeding pregnant heterozygous dams with a lipid-enriched diet (LED) results in a dramatic reversal of the lethal phenotype in homozygous offspring. These results were replicated with a diet supplemented with 3–6% soybean oil. A targeted lipidomic analysis of skeletal muscle and liver revealed elevations in tissue levels of non-esterified palmitic acid and ceramide, two lipotoxic substances which improved on the LED diet. The ability to reverse lethality, increase survival, and ameliorate myopathic deficits in homozygous animals suggests that lipid supplementation may be a promising therapeutic strategy for patients with VCP-associated neurodegenerative diseases [6,57].

6.3. Targeting protein dyshomeostasis in sporadic inclusion body myositis (s-IBM): Linda Greensmith and Michael G. Hanna

Linda Greensmith presented the findings of a translational study that has recently been completed as part of an international collaboration between clinical and basic scientists at University College London, UK, and Kansas University, USA. In this study, the therapeutic potential of targeting protein dyshomeostasis in models of the debilitating muscle disorder s-IBM was examined. Muscle biopsies from s-IBM patients typically show several pathological features broadly described as either inflammatory or degenerative. Many of the degenerative pathological features, including protein accumulation and

TDP-43 mislocalization, are also found in muscles of patients with VCP-disease.

Previous clinical trials in s-IBM have only tested agents directed at the inflammatory component of pathology and all were ineffective. Irrespective of whether s-IBM is primarily a degenerative or inflammatory disease, the degenerative changes observed in s-IBM muscle, including protein misfolding and aggregation, very likely have deleterious effects, and may therefore be a potential therapeutic target. Protein misfolding is normally controlled by endogenous chaperone proteins, which prevent aberrant protein–protein interactions and promote correct protein folding. Heat shock proteins (HSPs) are a family of ubiquitously expressed protein chaperones, which are upregulated following stress-induced activation of the heat shock response (HSR). Since the HSR declines with advanced age, up-regulation of the HSR in disorders in which there is evidence of protein mishandling, such as s-IBM, may be a potential therapeutic approach.

Arimoclomol is a novel pharmacologic agent that *co-induces* the HSR by prolonging the activation of Heat Shock Factor-1 (HSF-1), the main transcription factor that controls HSP expression, thus augmenting HSP levels [58]. Importantly, Arimoclomol only acts in cells under stress, in which HSF-1 is already activated, and does not induce the HSR in unstressed cells, thereby avoiding the side effects associated with widespread, non-targeted HSR activation. Our previous work has shown that Arimoclomol is effective at ameliorating disease in mouse models of motor neuron disease in which protein aggregation is a pathological characteristic [58,59].

In this study [60] Arimoclomol was found to ameliorate s-IBM-like pathology both *in vitro*, in primary rodent muscle cells induced to develop either the degenerative or the inflammatory features of s-IBM, as well as *in vivo*, in mice over-expressing mutant VCP (p97 in mouse) which have a phenotypic spectrum that includes inclusion body myopathy. In addition, in a randomized, double blind, placebo-controlled, proof-of-concept trial of Arimoclomol for the treatment of s-IBM in human patients, Arimoclomol was found to be safe and well tolerated. In conclusion, the results of this study show that Arimoclomol is effective at ameliorating s-IBM pathology in cellular and animal models, and has a safety profile that supports further investigation of Arimoclomol in an efficacy trial in s-IBM patients.

6.4. Sialic acid therapy in GNE myopathy: lessons from other HIBMs: Satoru Noguchi

Satoru Noguchi presented pre-clinical studies and clinical studies of sialic acid therapy in GNE myopathy, which has also been called hereditary inclusion body myopathy (hIBM), distal myopathy with rimmed vacuoles Nonaka-type, or quadriceps sparing myopathy. GNE myopathy is an autosomal-recessive disorder caused by *GNE* gene mutations. VCP and GNE myopathies share similar pathological features, as the presence of accumulated inclusions and rimmed vacuoles. A mouse model recapitulates the clinical, pathological and biochemical features of GNE myopathy patients. Sialic acid supplementation in the mouse model was efficient as a prophylactic

treatment to delay the onset of the disease [61] and also therapeutic treatment in symptomatic mice [62], showing partial recovery of motor performance, contractile force generation, muscle size, muscle pathology and sialic acid levels, indicating that GNE myopathy was due to cellular hyposialylation and would be treatable. Three clinical trials are currently ongoing [63]; Sialic acid (extended release) in a global phase 3 study by Ultragenyx, sialic acid in a phase 2/3 planned in Japan, and ManNAc on open-label phase 2 by NIH in the U.S.A. For the phase 2 study on sialic acid extended release (SA-ER), Ultragenyx reported positive data on the upper arm function compared to the placebo group. Muscle force in upper extremities showed dose-dependent efficacy and a larger effect in less advanced disease (e.g. individuals who had a 6MWT >200 m at baseline). The serum sialic acid level increased 2.6 fold in the high dose (6 gm) group. A Phase 3 global, randomized, double-blind, placebo-controlled clinical study of SA-ER in 80 patients was initiated in May 2015. Add-on therapies to sialic acid supplementation in preclinical studies on model mice were also discussed.

7. Session 5 – natural history and management of VCP disease: Tahseen Mozaffar

There is dearth of good quality natural history data on VCP related myopathy. The only study so far is an unpublished study by Kimonis et al. which has included 21 patients and for only a few of these patients has a second assessment been collected. Lack of funding, need for travel to a specific site and a large number of assessments, usually requiring two days, have hampered this study. There is a need for a substantial multicentre, multinational natural history study that would collect robust data from a carefully chosen and trimmed down (from the Kimonis study) set of outcome measures. In order to ensure that the data are of the highest quality, it is imperative that the number of parameters on which data are collected is reduced and the evaluators who are collecting these data are properly trained and certified. This would reduce some of the issues that some of the current registries, such as the Pompe Registry [64], are facing, where the data points that absolutely needed to be collected were not mandated and evaluators were not trained or certified. There are examples of neuromuscular specific natural history studies that are limiting themselves to carefully selected regional centres with a lot of attention paid to training and data collection, and therefore generating much better quality data. Examples include the ongoing natural history in GNE myopathy (Ultragenyx) (clinicaltrials.gov), which has only 5 international sites and sporadic Inclusion Body Myositis (Novartis), which has only 5 North American sites. However, these two are industry funded and thus any natural history study on VCP will have to have adequate funding to support such aims. Perhaps these can be run under the umbrella of TREAT-MND ALLIANCE, as exemplified by the Japanese GNE myopathy study [65]. Such a natural history study should address the current gaps, including natural history of the disease, variability in interfamilial as well as intrafamilial phenotypes, and provide phenotype–genotype correlations, information about disease modifiers and helpful biomarkers in the disease. We suggest

considering the following outcome measures in such a study: motor [66] and other toolboxes from the National Institutes of Health (NIH), fall diary, handheld dynamometry, pulmonary function tests, muscle MRI, and upper and lower extremity motor function tests. There is a need to create a vigorous biomarker biorepository. Finally since other neurological complications such as ALS, frontotemporal dementia and Parkinson's disease may occur in this myopathy, careful attention should be made to development of disease specific signs. A separate set of management guidelines for VCP disease, including routine multidisciplinary team visit (that includes visits to neurologists, physiotherapists, occupational therapists) and screening for cognitive impairment and Paget's disease, are being prepared and will be submitted separately for publication. Furthermore the presymptomatic carriers need to be followed on regular intervals and screening for emergence of disease defining features. Buy in and cooperative studies with larger dementia/FTD specific disease associations need to occur to promote our understanding of the development of FTD in this disease.

8. Session 6 – discussion on coordinated clinical research

8.1. Registry approach – Hanns Lochmüller

Hanns Lochmüller reported on the use of databases and patient registries in neuromuscular disorders which fulfil different objectives in clinical research, but also support care provision. Interestingly, several new medications have received marketing authorization by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) for rare disorders through prospective data obtained from patient registries, where a double-blind, placebo-controlled trial was impossible for practical reasons. For rare disorders such as VCP proteinopathy, international collaboration on patient registries is an essential prerequisite to assess the feasibility of clinical trials, plan and recruit into trials. This has recently been achieved for a number of rare neuromuscular disorders such as Duchenne Muscular Dystrophy with TREAT-NMD [67]. For very rare conditions, it is more appropriate to have a single registry with a global reach. Some examples have been established under the TREAT-NMD umbrella: Registries for FKRP-related muscular dystrophies, myotubular myopathy and GNE myopathy. First steps in setting up a registry are usually an agreement among the major stakeholders, e.g. patients, patient organizations, clinicians and scientists about the objectives of the registry, followed by the definition of a standardized core data set which is applied to all patients with the condition. Advanced software solutions have been developed that allow online reporting and consent by patients as well as curation and data provision by medical professionals. Currently, local databases on VCP proteinopathy patients exist in participating centres, but data sets are different and not standardized between centres, which hinders pooling and comparison of data across centres and countries. Workshop participants agreed that standardized patients registries on VCP proteinopathy would facilitate research and accelerate therapeutic developments. A registry could also link into international databases and

bioinformatics platforms such as RD-Connect for the identification of biomarkers and modifier genes [68].

8.2. Natural history studies – examples from other diseases – Teresinha Evangelista

In a session devoted to the discussion on coordinated clinical research, Teresinha Evangelista has shown two examples of ongoing International collaborations for the setup of Natural History (NH) studies and Disease specific Registries. The chosen examples were the GNE myopathy and the Dysferlinopathies NH studies. The former was supported by the industry and the latter set in place by the Jain Foundation. The GNE myopathy-Disease Monitoring program (GNEM-DMP) was established through a partnership between academia (TREAT-NMD) and industry (Ultragenyx). The GNEM-DMP involves a patient reported Registry and a Natural history study with data collected from clinical and physiotherapy assessments. The data originating from both sources form a united data set that allows a comprehensive understanding of the disease. This type of combined data sets will help in planning future clinical trials and can be used as baseline data. The GNE programme is intended to be in place for up to 15 years. The Jain Foundation together with Newcastle University and collaborators worldwide established the International Clinical Outcome Study for Dysferlinopathy (COS). This Natural History study involves medical and physiotherapy assessments and muscle MRI/MRS for 3 years. The study aims to define the natural history in a large unselected patient group, evaluate possible outcome measures for future trials, extend the existing registry activities co-ordinated by the Jain Foundation (the International Dysferlinopathy Registry) and establish and disseminate standards of diagnosis and care for dysferlinopathy. To date 193 patients (ambulant and non-ambulant) have been recruited worldwide. In conclusion, several well-developed models can be used to establish both a registry and a NH study. There is a need to think about how to fund these initiatives and other structures needed to underpin research including biobanks (concept of trial readiness).

8.3. Patient identification – Bruno Eymard

The associations myopathy + Paget disease of the bone (PDB) + dementia; myopathy + PDB or myopathy + dementia in a patient and/or in the family are a major clue for the diagnosis of VCP related diseases. Diagnosis is often more difficult if there is no family history and/or in the presence of an isolated myopathy. This is particularly true as PDB and dementia often occur later in the course of the disease. The occurrence of a myopathy and motor neuron disease in the same family is a good clue to test *VCP*. Phenotypic aspects that may help with the diagnosis are a FSHD like presentation with scapular winging, differing from "classical" FSHD1 by a more severe course of the disease, and a more severe involvement of the pelvic and quadriceps muscles. A negative molecular test for FSHD1 should prompt the clinician to look for a *VCP* mutation. In the FSHD-like patients, the muscle biopsy when performed may show an inclusion body myopathy pattern that

is a strong argument in favour of a VCP related disease, leading to VCP gene testing. Besides FSHD, several other myopathies may mimic VCP related disease; that is the case for some distal myopathies as the GNE myopathy where rimmed vacuoles may be present in the biopsy, however, show autosomal recessive transmission and sparing of the quadriceps muscles. The myofibrillar myopathies, mainly those associated with mutations in *ZASP*, *MYOT*, *DES*, may also be a cause of misdiagnosis; however the histopathology differs from VCP related disease through the presence of rubbed out fibres and significant accumulation of intermediate filaments (Desmin, Myotilin, Alpha-B crystallin). Inclusion body myositis differs from VCP related disease by the following characteristics: absence of family history, elective finger flexor involvement and quadriceps weakness which is much more severe than the pelvic weakness; a biopsy with inflammatory pattern and MHC overexpression. In cases of familial PDB or familial ALS, the VCP gene should also be considered and screened for the presence of mutations. In the absence of a VCP mutation in a patient with a VCP like phenotype (myopathy + Paget), other genes should also be tested: *SQSTM1*, *HNRPN2B1* and *HNRNPA1*.

9. Session 7 – plans for future collaborative efforts and summary of the discussions

Through the consortium it was established that there is a need to have a unique nomenclature for IBMPFD (OMIM number 167320) that would incorporate the different phenotypic aspects of this disease. It was proposed that it should be named VCP related disease and together with other genetic diseases with phenotypic overlap to VCP related disease (*SQSTM1*, *HNRPN2B1* and *HNRNPA1*) should be named as a group as Multisystem Proteinopathies (MP).

The general discussion revealed that there is a critical need for the discovery and validation of biomarkers for VCP related disease. The relatively slow course and the rarity of the disease make it imperative to have sensitive outcome measures for clinical trials. At present there are no functional scales validated for VCP related disease and it was seen with other conditions that quantitative tools for the measurement of muscle strength are not sensitive enough to show progression of disease in the short time period of a clinical trial. Muscle MRI has the potential to become a good biomarker of disease progression, but a considerable amount of work still needs to be done. As a first step B. Udd and T. Evangelista agreed to put together a review on muscle MRI reporting the data available through the Consortium.

There is a need to have a standardized way of collecting biological samples and making those available for research. The sample storage should make use of one of the established biobank facilities, where samples are standardized, quality-controlled, findable and accessible for research. An effort should be made to develop biomarkers that are clinical and predictive. Examples that need further development are the determination of TDP43 in platelets and LC3 in leucocyte. Do they correlate with disease progression? Also autophagy (P62)

or lysosomal biomarkers should be further investigated in the context of VCP related disease.

Mice models and cell lines are available through different laboratories (cell lines through Coriell, mice through Jackson Laboratory, iPS cells from Virginia Kimonis). It was perceived that there are no clear rules on how to standardize these mouse models, which model is more appropriate to use in preclinical trials or what outcomes should be used. It was decided that guidelines on these aspects should be developed.

A multi-disciplinary team that includes at least a neurologist, rheumatologist, physiotherapist and occupational therapist should be involved in the management and care of patients with VCP related disease. Patient should be screened for FTD when the genetic diagnosis is confirmed and thereafter if the patients experience changes in mood, behaviour or memory. At first evaluation a brain scan and neuropsychological tests should be done if possible in the context of a cognitive neurology clinic. For PDB the screening should consist of a bone scan and APH at baseline. T. Mozaffar will lead on preparing clinical guidelines.

10. Conclusion

Taking in consideration that the identified gaps were a lack of natural history data, wide disease variability with an expanding phenotype and scarce knowledge about the phenotype–genotype correlation, disease modifiers, and biomarkers, it was agreed that there is a need to collect and share resources on a standardized manner (patients' biomaterials, cell lines and animal models) and the clinicians have agreed that building a registry, collecting tissue samples and implementing a natural history study are a priority of this consortium.

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