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Workshop report

271st ENMC international workshop: Towards a unifying effort to fight Kennedy's disease. 20-22 October 2023, Hoofddorp, Netherlands

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ABSTRACT

The workshop held in the Netherlands from October 20–22, 2023, united 27 scientists from academia, healthcare, and industry representing 11 countries, alongside four patient and charity representatives. Focused on Kennedy's Disease (KD), also known as spinal and bulbar muscular atrophy (SBMA), the workshop aimed to consolidate knowledge, align on clinical trial designs, and promote participative medicine for effective treatments. Discussions emphasized KD's molecular mechanisms, highlighting its status as a neuromuscular disorder with motor neuron degeneration. Strategies for therapeutic intervention, including AR activity modulation and targeting post-translational modifications, were proposed. The need for diagnostic, prognostic, and target engagement biomarkers was stressed. Challenges in patient stratification and clinical trial recruitment were acknowledged, with the International KD/SBMA Registry praised for its role. The workshop concluded with a patient-focused session, underscoring challenges in KD diagnosis and the vital support provided by patient associations.

1. Introduction

Between October 20th-22nd 2023, a group of 25 clinicians and scientists, together with two pharma representatives and four patient advocates, from 11 Countries (Denmark, France, Germany, Israel, Italy, Japan, Portugal, Spain, Switzerland, UK, and US), met in Hoofddorp, The Netherlands, for the 271th European Neuromuscular Centre (ENMC) International Workshop: *Towards a unifying effort to fight Kennedy's Disease.* The focus of the meeting was the urgent need for the development of an effective disease-modifying therapy for patients suffering from KD.

This workshop was a follow-up of two previous ENMC meetings, the first held in 2015 [1], and the second in 2019 [2]. The 2023 ENMC workshop started with a welcome from the ENMC Workshop Coordinator (Wilma Hinloopen), followed by an overview of the topics to be discussed during the workshop by Maria Pennuto on the behalf of the workshop organizers, Linda Greensmith, Pierre-Francois Pradat and Gianni Sorarù. The workshop started with an introductory lecture from ***Kenneth Fischbeck***, who gave a broad overview of KD, starting from the clinical description, following with the discovery of the KD-causing mutation, and eventually describing to our current understanding of disease pathogenesis and emerging therapeutic approaches.

KD is a rare and progressive neuromuscular disorder characterized by the degeneration of specific MNs in the spinal cord and brainstem. Named after Dr. William R. Kennedy, who first described the condition in 1968 [3], KD predominantly affects males and is associated with an abnormal exonic expansion of the glutamine (Q)-encoding trinucleotide cytosine-adenine-guanine (CAG) tandem repeat in the androgen receptor (*AR*) gene [4]. This genetic abnormality leads to the synthesis of a mutant AR with an aberrantly elongated polyQ tract (polyQ-expanded AR). Clinically, KD is characterized by muscle weakness and atrophy, fasciculations, and bulbar dysfunction [5,6]. Whilst traditionally considered to be a form of MN disease, evidence emerging over the past few years suggests that KD is a multisystem disease in

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which degeneration and dysfunction of several peripheral organs is observed, including liver, heart, androgen-dependent tissues like gonads, and others, with skeletal muscle being a primary site of pathology and dysfunction, due to cell-autonomous and non-cell-autonomous processes.

KD uniquely exhibits androgen sensitivity, with symptoms often worsening in response to androgen exposure [7]. The AR is a member of the nuclear hormone receptor superfamily, alongside oestrogen, progesterone, glucocorticoid, and mineralocorticoid receptors. As a prototypical member of nuclear receptors, the AR is composed of three main domains, an amino-terminal domain (NTD), a DNA binding domain (DBD) formed by two zinc fingers, and a ligand-binding domain (LBD). Activated by male sex hormones, specifically testosterone and its more potent derivative dihydrotestosterone, the AR functions as a ligand-activated transcription factor. Upon ligand binding, a cascade of events ensues, contributing to the pathogenesis of KD through both toxic gain-of-function (GOF) and loss-of-function (LOF) mechanisms. These events encompass the dissociation of the AR from heat shock proteins (HSPs) in the cytosol, conformational changes associated with post-translational modifications, homodimerization, nuclear translocation, interaction with transcription co-factors (both co-activators and co-repressors of gene transcription), and the subsequent transactivation or repression of specific androgen-responsive genes. The mechanisms of transcriptional dysregulation in KD and how they contribute to the disease remains unknown. No treatment is currently available for KD and, to date, KD remains a condition of high unmet clinical need.

This workshop aimed to provide an in-depth exploration of the pathogenesis, clinical manifestations, diagnostic approaches, and therapeutic strategies associated with KD contributing to an up-to-date, comprehensive understanding of this complex and under-recognized neurodegenerative and neuromuscular disorder. The emerging picture of the clinical presentation of KD shows that it is a multisystem disease, which therefore calls for a multidisciplinary approach, not only in basic and preclinical research, but also in clinical intervention. Thus, this ENMC workshop had the following aims: i) to provide an update on recent pre-clinical findings that may have a clinical impact, including basic disease mechanisms, the role of skeletal muscle, preclinical validation for therapy development, therapeutics targeting the motor unit and gene therapy; ii) by combining preclinical findings with clinical experience, to assess new experimental and clinical biomarkers in KD, including preclinical biomarkers, clinical biomarkers, liquid biopsies; and iii) to discuss novel drug development, new clinical trials, tools for gene therapy and patient recruitment.

2. Overview of KD

KD is a progressive X-linked neuromuscular disease characterized by bulbar and extremity muscle weakness, atrophy, and fasciculations. Affected males may show signs of androgen insensitivity, such as breast enlargement and reduced fertility. KD is caused by expansion of a CAG repeat encoding a polyglutamine tract in the androgen receptor (AR). Although previous estimates of KD prevalence have been about 1/30,000, a recent study in a 74,000 population found about 3000 males with repeat expansion in the pathogenic range, indicating reduced penetrance or underdiagnosis of the disease [8]. There is evidence of both MN and muscle degeneration in KD. There are clinical features of denervation, with loss of MNs in the spinal cord and brainstem, and also increased serum creatine kinase and histological findings of muscle fibre degeneration. The AR polyglutamine expansion causes both loss of receptor function and a toxic gain of function that leads to MN and muscle degeneration. As with other expanded polyglutamine disease proteins, the mutant AR aggregates with increasing repeat length and forms nuclear inclusions. The toxicity of mutant AR is dependent on ligand (androgen) in cell culture and animal models. The ligand promotes nuclear localization, leading to aberrant interactions with other nuclear factors, altered histone acetylation, and transcriptional dysregulation, with adverse effects on signal transduction and mitochondrial function, resulting in motor neuron and muscle degeneration. The mutant AR may induce a vicious cycle of altered histone acetylation, decreased expression of metabolic genes, and decreased acetyl-CoA, leading to mitochondrial dysfunction and decreased ATP [9].

More than 20 different therapeutic approaches have been reported to be effective in mouse models of KD, either by blocking the pathogenic effects of the mutant protein or by enhancing cellular protective mechanisms such as molecular chaperones (heat shock proteins), proteolytic degradation (the ubiquitin proteasome system), and autophagy. Specific interventions that have worked in KD mice include reducing androgen levels (leuprorelin), altering post-translational modification of the AR protein to make it less toxic (IGF-1), selective AR modulation (ASC-JM17), and decreasing mutant AR transcript levels with miRNA and antisense oligonucleotides. Anti-androgen treatment blocks disease onset and prevents the development of motor deficits in KD mice, but it has had relatively modest benefit in clinical trials [10–12]. The AR modulator ASC-JM17 (AJ201) accelerates mutant androgen receptor degradation, activates cellular protective responses through Nrf1/Nrf2, and rescues the phenotype in transgenic flies and mice [13]. Selective modulation of the androgen receptor AF2 domain rescues degeneration in KD models. Co-regulator binding to the AF2 domain mediates AR toxicity in flies [14], and small molecules that modulate AF2 binding have beneficial effects in fly and mouse models of KD [15]. It has recently been reported that gene therapy targeting AR co-regulators or with AR isoform 2 rescues the KD phenotype in animals by modulating AR transcriptional activity [16,17]. AR-2, a truncated transcript more abundant than full length AR-1 in the brain and spinal cord, regulates AR transcriptional activity and mitigates AR toxicity in KD mice when delivered by AAV9 [17]. Interventional studies in KD patients are currently in progress or under consideration by AnnJi Pharma (small molecule AJ201), Nido Biosciences (small molecule AF2 modulator), Ionis Pharmaceuticals (oligonucleotide AR knock-down), and the University of Pennsylvania (AAV-mediated RNA inhibition).

3. Sessions of the meeting

Session 1: From basic disease mechanisms to preclinical approaches

The first session of the meeting commenced with an insightful discourse delving into recent mechanistic breakthroughs in the field of KD. The discussion was particularly illuminating, shedding light on the evolving landscape of our understanding of KD pathology. Key advancements were highlighted, emphasizing the intricate mechanisms underlying the disease. Of significant interest were the emerging preclinical approaches aimed at targeting peripheral tissues, with a particular focus on skeletal muscle. Over the past decade, skeletal muscle has been increasingly recognised as a pivotal tissue in the context of KD. This shift in perspective from KD being considered as primarily a neurodegenerative disease affecting MNs to one in which skeletal muscle plays a critical role has led to a deeper exploration of the interplay between disease mechanisms and peripheral tissues, paving the way for novel therapeutic avenues. The comprehensive discussion during this session not only highlighted recent advances in our understanding of the complexity of KD, but also set the stage for a robust

exploration of innovative preclinical interventions designed to address the disease at its roots.

Xavier Salvatella opened this session with a discussion of the effects of polyQ expansions on AR phase separation. The expanded polyQ tract starts at position 58 of the AR and is therefore part of the NTD. Like the activation domains (AD) of essentially all human transcription factors, the AR AD is intrinsically disordered, which has made it difficult to understand how it recruits the transcription machinery to transcribe the target genes. In the last few years, it has been proposed that ADs activate transcription partly due to their ability to co-phase with the intrinsically disordered domains of transcriptional co-activators and RNA polymerase II to form heterotypic biomolecular condensates known as transcription condensates. Salvatella's team has investigated whether this new paradigm applies to the AR AD. They obtained evidence that its ability to form homotypic and hetorotypic condensates is mediated by its aromatic character and is, indeed, key for the activities of AR as a transcription factor [18]. The fact that active AR is phase-separated from the nucleoplasm has implications for understanding the molecular basis of KD: in addition to studying the effects that the mutation has on the conformation of a single protein molecule [19], it is important to study how the mutation affects the properties of the condensates themselves. In his presentation Xavier Salvatella discussed the preliminary in vitro and *in cellulo* results obtained on the AR, which could contribute to explaining both the loss and gain of toxic function symptoms of KD.

Angelo Poletti presented data on the effects of modulation of the protein quality control (PCQ) system in KD. The toxicity of polyQ-expanded AR is at least in part associated with its tendency to acquire aberrant conformations and to aggregate into cytoplasmic and nuclear inclusions. This process is triggered by androgenic (and some antiandrogenic) AR ligands, possibly because the occupancy of the C-terminal hydrophobic binding pocket induces i) the dissociation of accessory proteins interacting with the AR NTD (e.g., HSP70), thus unmasking the polyQ tract, and ii) conformational changes required for AR activation, which might be prevented by the elongated polyQ tract. The PCQ system is the first line of defence against the accumulation of misfolded proteins, and PQC activation prevents protein aggregation and eventually their downstream aberrant effects on cell homeostasis. Poletti's group has identified a component of the PQC system, the small HSPB8, which in association with its co-chaperone BAG3 interacts with the heterocomplex HSP70/CHIP forming a complex capable to recognize misfolded substrates, including polyQ-expanded AR, thus enhancing its degradation via the chaperone-assisted selective autophagy (CASA) pathway [20]. They identified several compounds that can enhance HSPB8 expression. One of these compounds, trehalose, not only acts on HSPB8, but it also induces autophagy in a TFEB-dependent manner, and this results in a general improvement of polyQ-expanded AR clearance, thereby preventing its aggregation in cells [21]. In KD mice, trehalose treatment enhanced survival and ameliorated motor behaviour especially in rotarod tests [22]. In addition, trehalose reduced disease-associated apoptosis in muscle and ameliorated muscle pathology and mitochondrial deficits. Collectively, these data shows that trehalose, formulated for human use with specific pharmaceutic preparation aimed to enhance its stability and bioavailability, may be considered as a candidate for future therapeutic approaches for KD.

Carlo Rinaldi next spoke about AR transcriptional activity and mechanisms of KD pathogenesis. The overarching aim of Rinaldi's research programme is to advance the current understanding of the pathophysiology of KD, with the ultimate goal of developing an effective therapeutic approach for this yet incurable condition. The objective is to identify the molecular underpinnings of AR transcriptional activity in skeletal muscle and to understand how dysregulation of such functions leads to muscle atrophy and weakness in KD. Rinaldi's group has mapped the AR transcriptional landscape in skeletal muscle by performing chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) in healthy and KD skeletal muscle, testing the working hypothesis that polyQ-expanded AR alters chromatin regions of AR occupancy in KD (unpublished data). By integrating these results with the transcriptomic profiles of muscle biopsies from KD individuals and age- and sex-matched controls and single molecule FISH, they have identified target genes directly under the control of AR [23]. To investigate mobility and patterns of chromatin binding of AR molecules in real-time and at single molecule resolution, they engineered human myoblast lines to express wild type, mutant or NTD-lacking (NTL) AR tagged with Halo-Tag and subjected these cells to single-molecule tracking analysis. The duration of AR tracks predicted to be in the bound state and the frequency of these binding events in the tracks across the different conditions were calculated, showing that both polyQ-expanded AR and NTL AR display significantly longer residence times and overall reduced number of DNA binding events compared to wild type AR. Comparing wild type AR vs polyQ-expanded AR chromatin-bound regions, they observed that, while most genomic loci are shared, a few only occurs in KD and are directly associated with altered expression of target genes in muscle (unpublished data). Additionally, single-molecule tracking dynamics have revealed that polyQ-expanded AR is less efficient in searching and recognising its DNA targets, but when bound, tends to stay bound for longer times. Overall, these results support a mechanism of polyQ-expanded AR-driven transcriptional hijacking and represent an advancement in the molecular understanding of a driving mechanism of pathogenesis in KD.

Maria Pennuto presented her recent findings on the involvement of skeletal muscle in KD. It is now widely accepted that skeletal muscle serves as a primary site for toxicity in KD. Muscle contraction in the skeletal is initiated by the action potential in MNs through a process called excitation-contraction coupling (ECC). This process establishes a connection between the electrical events occurring in the sarcolemma and the generation of force during myofiber contraction at specialized structures known as triads. Triads consist of invaginations of the sarcolemma (T tubules) facing the sarcoplasmic reticulum (SR). Efficient and sustained muscle contraction relies on the presence of calcium (Ca^{2+}) and adenosine triphosphate (ATP). Ca^{2+} is stored in the SR lumen, while ATP is generated by mitochondria located proximal to the T tubules. The interplay between Ca^{2+} and ATP serves as a critical link between muscle contraction and energy production. Utilizing transgenic and knock-in KD mice, along with muscle biopsies derived from KD patients, Pennuto presented evidence that KD mice manifest early respiratory defects at the presymptomatic stage, coinciding with impaired expression of genes associated with ECC, altered contraction dynamics, and heightened fatigue [24,25]. After these early events, there is a stimulus-dependent accumulation of Ca^{2+} within mitochondria at the onset of the disease, accompanied by structural organization changes in the muscle triad. The dysregulation of ECC gene expression aligns with sexual maturity and an increase in androgen levels in the serum. In accordance with the androgen-dependent nature of these alterations, both surgical castration and androgen receptor silencing alleviate early and late pathological processes. These findings highlight that dysregulation of ECC and contraction dynamics, along with compromised mitochondrial respiration, represent early yet reversible events, ultimately leading to altered muscle force, calcium dyshomeostasis, and triad structure dismantling in KD skeletal muscle.

Moving from basic to preclinical approaches, ***Giuseppe Ronzitti*** introduced the topic of gene therapy approaches for rare diseases. The recent US Food and Drug Administration (FDA) approvals of Adeno-associated virus (AAV) gene therapies for rare diseases clearly support the impact of gene therapy based on AAV vectors on patients' lives. Despite these important successes, the clinical application of AAV gene transfer still presents limitations, particularly for neuromuscular disorders [26,27]. The high doses of AAV needed to transduce such a large target as skeletal muscle, as well as the challenges of targeting MNs within the central nervous system and in adult patients may be associated with unwanted and potentially severe toxicities. Thus, new AAV capsids with improved biodistribution are required to achieve safety and efficacy in clinical trials for neuromuscular diseases. A considerable portion of the human population is seropositive to AAV vectors, presenting neutralizing antibody levels that preclude AAV administration [28]. Another important limitation of AAV vectors is related to immune responses, both humoral and cellular, occurring after the administration of the vector. Transient immune suppressive treatment seems to be effective to reduce the impact of the cellular immune response, however, they are less efficient on the humoral counterpart and potentially also on the long-term innate and adaptive cellular response. Ronzitti summarised some of the current work underway at *Genethon* to develop gene therapy treatments for rare genetic diseases and to overcome the existing limitations of AAV gene therapy [27,29,30]. Recent data obtained in the context of an AAV gene therapy clinical trial for Crigler-Najjar syndrome clearly demonstrated the advantages of having clear endpoints to define efficacy and highlighted the therapeutic potential of AAV vectors for the treatment of rare diseases of genetic origin [31].

Linda Greensmith next presented early findings from a new collaborative study with Pietro Fratta's group, in which they aim to target skeletal muscle as a therapeutic approach in KD. Several gene therapy strategies have previously been examined that alter AR expression and have been shown to rescue disease phenotypes in KD mouse models. Thus, reducing AR expression with antisense oligonucleotides (ASOs) or altering AR activity by overexpression of alternative isoforms, have both been shown to ameliorate disease in KD mouse models. However, to date, these strategies have not been limited to specific targeting of the AR in skeletal muscle, and may therefore result in unacceptable off-target, loss-of-function (LOF) effects in non-neuromuscular tissue. The Greensmith/Fratta team is therefore developing a skeletal muscle-specific gene therapy approach for KD. This approach has two main objectives, firstly, to reduce AR expression and secondly, to do so specifically in skeletal muscle alone. They are using two gene silencing approaches to reduce the AR transcript, targeting identical sequences matching both mouse and human AR: 1) miRNA-embedded shRNA (shRNAmir): shRNAmir have the advantages of small hairpin RNAs (shRNA), which are highly efficacious and result in long-lasting silencing, but avoid the disadvantage of RNA polymerase III promoters used with shRNAs, which lack cell-specificity; shRNAmir permits the use of Pol-II promoters, which enable tissue-specific gene silencing; 2) CRISPR/Cas13: Cas13 is a CRISPR protein that targets and cuts RNA, rather than DNA, and can target a specific RNA sequence with the help of a guide RNA (gRNA) which can be designed to target the AR; this system can be used for efficient, multiplexable, and specific RNA knockdown or RNA sequence editing in mammalian cells, which makes Cas13 a potentially significant therapeutic approach for altering gene expression without altering genome sequence. Their results to date have shown that a novel shRNAmir was able to significantly reduce AR expression in vitro, using shRNA sequence designs targeting exon 1. Similarly, they tested different guide sequences and identified three novel Cas13d gRNA sequences that significantly reduce AR expression in vitro, at both the RNA and protein level. They have therefore identified two gene silencing approaches that can effectively reduce AR expression in vitro. Their second goal was to develop a strategy to *specifically* target skeletal muscle. AAV are widely used vectors that differ in their tropisms, making them a useful system for long-term transduction of specific tissues. Thus far, naturally occurring serotypes have been the most widely adopted in both pre-clinical and clinical trials. AAV9 is one of these naturally occurring serotypes which has been shown to have tropism not only for muscle, but also for other tissues. More recently, an engineered AAV, called MyoAAV, has been developed that preferentially targets skeletal muscle [32]. The Greensmith/Fratta group has tested the relative efficacy and specificity of AAV9 and MyoAAV as well as two different promoters, Cytomegalovirus (CMV) and the muscle specific myosin heavy chain kinase 7 (MHCK7) promoter. The results showed that the use of MyoAAV with the muscle specific MHCK7 promoter resulted in a high transgene expression (green fluorescent protein as a reporter) in skeletal muscle with reduced expression in the liver compared to AAV9/CMV promoter. They are currently examining the effects of reducing muscle AR expression long-term in vivo in wildtype and KD mice.

Manuela Basso presented findings using AR co-activator-targeting gene therapy to attenuate AR toxic gain-of-function (GOF) and ameliorate KD phenotype. Basso presented the updates of a collaborative project among members of the consortium dedicated to the preservation of the physiological functions of AR while inhibiting its detrimental effects caused by the polyglutamine expansion. AR is a transcription factor interacting with numerous protein modulators. Basso and colleagues identified two co-activators whose expression is aberrantly increased in the skeletal muscle of KD mouse models and patients' muscle biopsies. These coactivators, namely lysine-specific demethylase 1 (LSD1) and protein arginine methyl transferase 6 (PRMT6) [33], are increased by polyglutamine-expanded AR itself in a feedforward event [16]. Accordingly, their silencing in KD flies abrogated the alterations in the eye phenotype (classical readout of a neurological defect) or their specific inhibition with artificial microRNAs via adeno-associated (AAV) injection in a KD transgenic mouse model [24], resulting in an increased muscle strength and an amelioration of the deficits in locomotor activity [16]. Basso presented the ongoing activities focused on ameliorating the AAV delivery to the skeletal muscle, including the above described Genethon-developed AAV vectors with muscle tropism.

Emanuela Zuccaro next discussed her project aimed at tackling neuronal vulnerability in KD. Not all neurons are equally susceptible to the disease, so that within the same motor pool, distinct classes of alpha MNs (αMNs), responsible for muscle contraction, are differentially susceptible, with the fast-fatigable MNs (FF-MNs) degenerating first compared to fast fatigue-resistant (FFR) and slow fatigue-resistant (SFR) MNs [34,35]. Several groups have investigated the underlying mechanisms of disease-vulnerability or resistance of MNs. However, our understanding of the mechanisms that underlie this differential vulnerability beyond the observation of a preferential susceptibility of FF-MNs remains poor, hampering therapy development. Building on a novel strategy she has developed enabling the isolation of specific neuronal classes based on the expression of specific markers, rather than a need for gene reporter lines (patent submitted) [36], Zuccaro is now able to isolate and molecularly profile rare cell populations, such as FF-MNs, at the single cell level. Bioinformatic analysis of the transcriptomic profile of different MN classes in health and disease is aimed at revealing the molecular pathways driving selective vulnerability of FF-MNs. This knowledge is essential for the design of novel therapeutic strategies aimed at delaying, if not halting, disease progression. Preliminary data on a different

neurodegenerative disease, namely amyotrophic lateral sclerosis (ALS), have demonstrated the feasibility of the approach, which is being translated to KD.

Session 2: Biomarkers and therapeutic strategies

Session 2 was dedicated to the exploration of preclinical advances in the search for biomarkers for KD and emerging therapeutic strategies, including those under study in related disorders. It is widely recognised that the current KD landscape lacks robust indicators to effectively assess the preclinical efficacy of novel therapeutic interventions. The absence of reliable biomarkers and the challenges associated with their validation in preclinical models represent significant hurdles in the evaluation of the effectiveness of emerging medications in clinical trials. This issue becomes particularly significant in the context of KD, a chronic disease characterized by slow progression. The discussion during this session focused on not only highlighting the critical gap in our current diagnostic toolkit, but also fostering collaborative efforts to identify and validate reliable biomarkers. In addition, advances in preclinical methodologies tailored to assess the unique characteristics of KD were discussed, thereby addressing the urgency of enhancing our ability to gauge the efficacy of potential therapeutic interventions in KD. The insights gained during this session form part of the strategic groundwork required to accelerate progress of preclinical research and the subsequent translation of promising therapeutic strategies to clinical trials.

Illana Gozes presented data on overlapping disease biomarkers in neurodegeneration and novel drug development. Previous findings from Gozes and Pennuto showed that pituitary adenylate cyclase activating peptide (PACAP) reduces phosphorylation and toxicity of the polyglutamine-expanded AR in KD [37,38]. A critical target for PACAP regulation and muscle function is activity-dependent neuroprotective protein (ADNP), which in turn regulates sex steroid biosynthesis genes. ADNP deficiency/mutations lead to brain/muscle neurodegeneration, which are at least in part ameliorated by PACAP treatment, as well as by the ADNP fragment, drug candidate, NAP (also known as davunetide, or CP201). A major target for ADNP/NAP protection are microtubules/Tau, commonly disrupted in nerve-muscle diseases. Tau pathology is regulated by hyperphosphorylation, which in turn is controlled by testosterone [39]. Davunetide protection was therefore targeted in the pure tauopathy progressive supranuclear palsy (PSP), which is characterized by rapidly developing brain and muscle neurodegeneration [39]. The results indicated unexpected sex differences in PSP, with a significantly faster deterioration in women, suggesting a protective role of androgens. Further clinical evaluation revealed efficacy for davunetide in women with slowed female disease progression. Furthermore, treatment protected the bulbar and limb motor domains considered by the PSP rating scale, including speaking and swallowing difficulties caused by brain damage, and deterioration of fine motor skills. These results indicate a path for davunetide development for women suffering from PSP and beyond.

Nadia Pilati discussed how targeting ion channels can be pursued as a therapy to treat neurodegenerative disorders associated with hyperexcitability. Kv3 channels are a subgroup within the broader voltage-gated potassium channel family, characterized by their unique biophysical properties and distinct expression patterns in the nervous system. Kv3 potassium channels play a crucial role in the regulation of neuronal excitability by governing the repolarization phase of action potentials [40]. These channels contribute to the precision of spike timing and participate in the regulation of neurotransmitter release, thereby influencing synaptic transmission and network activity. The significance of Kv3 channels in neural function underscores their importance as potential therapeutic targets for neurological disorders associated with aberrant excitability, such as epilepsy and certain neurodegenerative conditions. Autifony is a company that has developed a series of small molecules that can positively modulate the Kv3 channels. These modulators can increase Kv3 currents and therefore increase neuronal firing. There is evidence to suggest that Kv3 channels are expressed and may exert functional roles in skeletal muscle. However, the specific expression pattern and roles of Kv3 channels in skeletal muscle is not as well documented as in neurons. The expression and role of Kv3 channels in the skeletal muscle in physiological and pathological conditions, such as KD, is therefore currently under study. Understanding the intricate roles of Kv3 channels in skeletal muscle might provide valuable insights and may pave the way for the development of targeted interventions to modulate neuromuscular dysfunction in KD.

Bilal Malik presented work from the Greensmith lab where they are studying the therapeutic potential of targeting skeletal muscle deficits, which recent findings from their lab and others have suggested may be a viable and accessible target for therapeutic intervention in KD [41]. They used an integrated transcriptomic analysis to identify disease pathways and genes [42], which may underly the muscle atrophy in tibialis anterior (TA) muscle of the AR100 mouse model of KD, which carry 100 pathogenic CAG repeats in the human *AR* gene [43,44]. Pathway analysis of differentially expressed genes highlighted multiple key pathways with involvement in: i) muscle regeneration, ii) muscle contraction and iii) muscle structure. Dysfunction in myogenesis was seen early in presymptomatic AR100 mice, with a significant decrease in markers of muscle regeneration, before symptoms such as muscle atrophy and loss of muscle force manifest. Importantly, dysregulation of galectin-1 and galectin-3 genes and proteins was also observed. Galectin-1 and galectin-3 promote myogenesis, muscular vascularisation and improve muscle function of WT and Duchenne Muscular Dystrophy mice [45,46]. Treatment of differentiating AR100Q C2C12 muscle cells with human recombinant galectin-1 increased myosin heavy chain protein levels and improved/enhanced nuclear fusion index as well as myotube area. These in vitro results suggest that galectin-1 may be beneficial in KD. To test possibility in *vivo*, AAV vectors were optimised to deliver transgenes to skeletal muscle. The recently developed MyoAAV [32] was tested and found to be more effective at targeting TA muscle than either AAV8 and AAV9. In vivo gene therapy experiments are currently underway to determine the effectiveness of galectin-1 in alleviating muscle dysfunction in AR100 mice.

Masahisa Katsuno presented findings from a recent study that aims to clarify the underlying mechanism of cold paresis since patients with KD often experience muscle weakness under cold exposure. The results showed that intramuscular levels of chloride voltage-gated channel 1 protein were decreased in both patients and mice, suggesting that excessive sodium current may cause motor symptoms including cold paresis [47]. Based on this evidence, they conducted a randomized controlled clinical trial to evaluate the efficacy and safety of mexiletine hydrochloride, a sodium channel blocker, in KD (MEXPRESS trial). In the MEXPRESS trial, tongue pressure and 10-s grip and release test under cold exposure were improved in the mexiletine group, although it did not restore cold exposure-induced prolongation of distal latency. Several studies have previously reported a potential benefit of exercise in KD, but the underlying mechanism has yet to be elucidated. Katsuno's group investigated the effect of running exercise in KD mice carrying human AR with 97 CAGs (AR97Q). Forced wheel-running during the early stage of the disease improved grip strength and rotarod performance and extended survival of AR97Q mice. Nuclear accumulation of polyQ-expanded AR in skeletal muscles and MNs was suppressed in the exercised mice compared to the sedentary mice due to activation

of 5′-adenosine monophosphate-activated protein kinase (AMPK) signalling [48].

Session 3: Translational Biomarkers for KD

The third session of the workshop centred around the critical and currently unmet need for reliable clinical biomarkers for KD, the lack of which hinders optimal clinical management and delays therapy development for KD. Recognizing the urgency of this requirement, the session featured a series of engaging presentations by leaders in the field of biomarker development for KD, with speakers sharing insights, novel methodologies and potential breakthroughs that could pave the way for enhanced diagnostic precision and therapeutic efficacy in KD. By focusing on translational biomarkers, this session sought to bridge the existing gap between basic research findings and their practical application in clinical settings. The aim was to catalyse collaborative efforts and innovative approaches that could lead to the identification and validation of biomarkers crucial for guiding both clinical management decisions and the development of effective therapeutic interventions for individuals affected by KD.

Markus Weber opened this session with a presentation of the phenotypic and neurophysiological changes that occur in KD. The most characteristic clinical feature when KD patients come to diagnosis is 'quivering of the chin', which occurs when patients speak or smile. By this time, muscle weakness is also present, typically in proximal muscles, more often and more prominent in legs than in the arms [7,49,50]. The pattern of weakness may be asymmetrical but usually affects both legs and/or arms. Motor symptoms, which precede weakness by many years, include fatigue, muscle cramps (above the knee) and fasciculations. This also holds true for non-motor symptoms, especially gynecomastia and postural tremor, which are other characteristic features of KD. As disease progresses bulbar symptoms leading to dysphagia and dysarthria also develop. Accordingly, the clinical findings at the time of diagnosis reveal a proximal pattern of weakness and atrophy, fasciculations, tongue atrophy with dysarthria and gynecomastia. Typically, the deep tendon reflexes are diminished or absent, which distinguishes KD from other MN diseases, such as ALS. In addition, vibration sense and light touch may be impaired also reflecting involvement of the sensory system. However, KD patients rarely complain about sensory loss, which might be explained by the fact that the disease evolves very slowly over years. The neurophysiological hallmark on EMG testing are very large motor unit potentials (MUPs), as observed in polio, which are unstable along with a reduced recruitment pattern, indicating slowly progressive MN loss allowing reinnervation through terminal sprouting. This feature has also been described in female carriers [51,52]. However, these prominent neurogenic changes contrast with muscle biopsy findings showing myopathic changes. This raises the question to what degree weakness and atrophy is the result of a neuropathic versus a myopathic process, which has implications for the primary target in drug development. In this context it is of note that the creatine kinase (CK) is prominently elevated (most often in the thousands), which is not seen in other motor neuropathies. Consistently, sensory nerve action potentials (SNAPs) are absent or reduced along with normal compound muscle action potentials (CMAPs) compatible with a motor-sensory neuronopathy. Involvement of small nerve fibres and autonomic fibres may also be present. It has become obvious that beyond the discussed core features, KD is in fact a multisystem disorder not only affecting the peripheral nervous system and muscle but also the brain as well as the hormonal and metabolic systems giving rise to a multifaceted syndrome [53,54].

Pietro Fratta next presented new data showing the value of skeletal muscle MRI and fat fraction evaluation as a biomarker in KD. His-group, along with the Vissing group, have previously shown how fat infiltration is increased in KD muscle and this changes and increases over an 18-month period [55–57]. New data now shows that this increase in fat infiltration can already be detected at 12 months from initial assessment, which is important measurable change that may be relevant for future clinical trials. Fratta also reviewed his group's findings that unlike in ALS, neurofilament light chain levels do not increase in KD patient plasma, whereas in contrast, miR206 is increased [58]. Lastly, data on KD and ALS patient skeletal muscle transcriptomics was presented, which reveals that there are numerous KD-specific changes occurring in KD skeletal muscle, including an alteration of immune pathways.

Patrick Weydt presentation focused on the potential of troponins as serum biomarkers in KD. Recent insights from ALS research have unexpectedly pinpointed the cardiac serum biomarker troponin T as useful in MN and neuromuscular diseases. Weydt's group has found that in serum of KD patients, troponin T is usually elevated, while another cardiac marker, troponin I, is usually normal. This is in line with recent findings reported by Sorarù and colleagues [59]. Further research is necessary to determine the source of these troponin T serum elevations in KD and other MN diseases, but these observations have immediate clinical implications. Because in conventional clinical practice serum troponin T is interpreted as a sign of myocardial injury, KD patients are sometimes misdiagnosed and undergo unnecessary and sometimes potentially harmful cardiac interventions aimed at identifying a suspected myocardial infarction. In the light of these findings together with those of the Sorarù group, it should be emphasized that in KD patients, troponin T elevations should be interpreted with caution and are usually not an indicator of cardiac problems. One way to differentiate cardiac from noncardiac troponin T elevations is the simultaneous measurement of troponin I, which is much more cardiac-specific.

Mamede de Carvalho followed up with a discussion about neurophysiological biomarkers for KD. The slowly progressive muscle weakness, with more severe involvement of proximal and bulbar innervated muscles, is mirrored on needle electromyography, which typically shows large motor unit potentials of long duration and polyphasic, with mild or moderate instability. Interference pattern on full contraction is markedly reduced in the most severely affected muscles, and motor unit potentials show an increased firing rate. Signs of active denervation are not usually observed, but large fasciculation potentials are common. Sensory action nerve potentials show reduced amplitude, or are absent, derived from an associated ganglionopathy. Needle EMG is not useful for following these changes overtime since the disease progression is very slow. The same holds true for the amplitude of the motor response on nerve stimulation, or its derived neurophysiological index, because the slowly progressive loss of axons is partially compensated by the reinnervation process. However, motor unit estimation (MUNE) could detect and quantify the slowly progressive loss of motor axons. Many MUNE methods have been investigated and applied. Multiple point nerve stimulation is a reliable technique that has been explored in ALS, which does not depend on patient cooperation but is time consuming [60]. The MUNIX technique has been standardized and is supported by several multicentre studies [61]. It depends on patient cooperation, but it is a rather quick test and is very well tolerated. Although it provides an index and does not intend to give a real estimation of the number of functional motor units, it is reliable and offers information about the mean size of the motor units (MUSIX). The CMAP scan technique, in which the target muscle is stimulated from 0 to maximal intensity (or vice-versa) to give a cloud of dots representing the different motor units, has been used for many years [62]. The number of dots gives information on the number of motor units and their separation indicates their mean size. More recently, MScanFit

MUNE has been proposed and investigated as a more sophisticated and reliable method to determine the number of motor units using a CMAP scan approach [63]. Multicentre international studies support their value and reliability in being applied as routine method in patients. It does not depend on patient cooperation, but some patients may not accept the electrical stimulation protocol. Each method therefore has limitations and potentialities. In KD we must consider that, whatever the method selected, the patients should be monitored for a long period of time, certainly above 1 year, to detect significant changes in motor unit number, which could be of statistical relevance in a potential clinical trial.

Matteo Zanovello discussed surprising recent data from the Fratta and Tucci labs which shows that the pathogenic *AR* CAG repeat expansion is underestimated in the population. Previous perspective prevalence surveys estimated KD prevalence at around 1:30,000 males, except for the Vasa region in Finland, with a prevalence of around 1:7000 males due to a founder effect. However, the prevalence of the *AR* CAG repeat expansion in the general population is unknown. Thanks to the development of new large biorepositories and bioinformatics tools, it is now possible to size repeat expansions from whole genome sequencing (WGS) data. Therefore, the group developed a pipeline that exploits Expansion Hunter (Illumina) and visual inspection and benchmarked it against PCR, the gold standard diagnosis. The allele distribution of the *AR* CAG repeats peaks at 21 repeats [8], as previously reported [64], with variations across different populations. By applying a pipeline to 74,277 unrelated individuals from four large WGS cohorts, they found the frequency of expanded alleles to be 1:3182 X chromosomes, around ten times more frequent than the reported disease prevalence. Disease prevalence modelling using the novel mutation frequency led to an estimate of the disease prevalence at 1:6887 males, four times higher than that reported. This discrepancy may be due to an underdiagnosis of KD, a reduced penetrance of the mutation, or the pleomorphic clinical manifestation of KD. Therefore, the team now aims to screen the largest biomedical dataset, the UK Biobank, for the repeat expansion and then use its phenotypic data to characterise the manifestation in carriers of the repeat.

Session 4: Clinical trials

This session placed a spotlight on ongoing and upcoming clinical trials designed to address the needs of patients living with KD. These trials, supported by several pharmaceutical companies, represent a collective and multicentre effort, bringing together neurologists, genetic counsellors, and shared resources to propel advancements in therapeutic interventions. The collaborative nature of these trials underscores the complexity of KD and the necessity for a unified approach to tackle this challenging genetic disorder. One notable aspect discussed during this session was the rapid evolution of multicentre trials and their increasing prominence in the field. The acceleration of such trials underscores the urgency and dedication invested in unravelling the complexities of KD. Importantly, this increase in clinical trial activity in KD prompts a critical consideration: the imperative need for a centralized patient registry specifically tailored for individuals affected by rare genetic diseases such as KD. Such a registry would serve as a comprehensive repository of patient data, facilitating seamless collaboration, data sharing, and a holistic understanding of the disease across various trial initiatives. In essence, Session 4 not only discussed the ongoing and prospective clinical trials for KD patients but also underscored the collaborative, multidisciplinary efforts required for their success. The overarching goal is to advance therapeutic possibilities, enhance patient care, and collectively contribute to the evolving landscape of KD research and treatment strategies.

Silvia Fenu started the session by discussing the difficulties in the recruitment of patients with rare diseases such as KD. This is a complex and challenging process that requires a comprehensive knowledge of the disease in order to design effective clinical trials. National and International collaboration is essential, as well as a comprehensive approach involving clinicians, researchers, and patient associations. In this context, patient registries for rare diseases are a valuable resource to better understand the disease, monitor its course, and facilitate the recruitment of patients into clinical trials [65]. The Italian Registry for KD has currently recruited 169 subjects, 138 of whom have performed a baseline visit, 115 a first follow up visit, 72 a second follow up visit, and 24 subjects a third one. The evaluation protocol includes: a minimal dataset of clinical and genetic information, the disease-specific SBMA-Functional Rating Scale (SBMA-FRS), the Adult Myopathy Assessment Tool (AMAT), self-administered questionnaires, International Prostatic Symptoms Score (IPSS), and International Index of Erectile Function (IIEF), blood tests and biological sample collection for a KD biorepository (DNA, serum, plasma, lymphocytes), EKG (including EKG specific for detecting Brugada syndrome), and spirometry, and in a subgroup of subjects a quantitative muscular MRI of lower limbs to measure fat fraction [2]. Collecting this data over several years allows accurate assessment of disease progression and of outcome measures' responsiveness: the SBMA-FRS showed the best responsiveness among all scales. Fenu and Pareyson are currently working on an international collaboration project for a retrospective study to merge the different databases from several specialised centres and establish a multinational database of clinical information on a large series of KD patients (including up to 700 patients and more) as a first step towards a common evaluation protocol to be shared internationally together with possibly a common registry.

Pierre-François Pradat's talk focused on the challenges of developing standardized care of KD patients. The management of KD is fraught with challenges due to its rarity. Addressing these challenges necessitates the establishment of a Standard of Care (SOC), particularly in the context of therapeutic trials. Standardization ensures uniformity and comparability in treatment approaches, allowing for the objective evaluation of different therapeutic interventions. Patient safety takes centre stage, especially concerning emerging treatments like gene therapy. Diverse treatment strategies may carry inherent risks, and KD often entails systemic complications, such as endocrine, cardiomyopathy, and metabolic disorders. Standardized care protocols serve as a protective barrier, mitigating potential hazards and safeguarding patient well-being during therapeutic trials. Ethical considerations underscore the critical need for SOC in therapeutic trials. Clinical trial participants deserve the highest possible care standards to ensure their well-being. Standardized care also streamlines data sharing across different studies, provides essential guidance for healthcare providers, and mandates the use of meaningful outcome measures. These measures are vital for obtaining robust research outcomes, crucial for advancing our understanding of KD and improving patient care. The French guidelines for KD prioritize a multidisciplinary approach, involving healthcare experts and patient representatives [66]. They comprehensively address various aspects of care, encompassing diagnosis, evaluation, screening for extra-neurological complications, symptomatic management, and effective dissemination of information. In conclusion, standardized care ensures treatment consistency, enhances patient safety, upholds ethical standards, and promotes rigorous research. Importantly, there is a need to generate certainty evidence for stronger recommendations and develop international guidelines with experts and patient representatives.

Giorgia Querin presented new trial designs to improve the study of innovative treatments in KD, discussing the potential utilization of new trial designs to accelerate research in finding

an effective treatment for KD. In recent years, significant progress in preclinical research has enabled the identification of molecular targets for innovative treatments, and various preclinical approaches have already undergone testing in mouse models of the disease [16,17,22,38,67–69]. In this context, there is a pressing need to efficiently organize clinical trials to evaluate these new therapeutic approaches. However, several challenges exist, particularly given that KD is a rare disease. Moreover, most clinical trials, especially gene therapy trials, require careful selection of eligible patients to mitigate the risk of significant side effects. Effective strategies should be implemented to enhance patient information and encourage participation in clinical trials. Additionally, supporting patient participation and retention should be facilitated through practical means, such as organizing transportation and limiting the number of on-site visits. Simultaneously, specific designs tailored to rare diseases can be proposed. Examples include the use of internal controls, adaptive designs incorporating different phases within a single protocol, or the utilization of master protocols allowing the recruitment of patients into different study arms over time, thereby reducing administrative and organizational burdens. Such designs have already proven successful in other rare neurological diseases like ALS, demonstrating their effectiveness in expediting the inclusion of a large number of potential treatments [70].

Kenneth Fischbeck presented a summary of recent clinical trials for KD. Randomized, double-blind, placebo-controlled trials of androgen reduction in KD patients have not shown a significant, clinically meaningful benefit [10–12]. A 7-year open-label extension study showed better outcome in Japanese patients taking leuprorelin compared to natural history controls [71], and this drug was approved for KD treatment in Japan in 2017. A study of insulin-like growth factor (IGF)−1 showed mitigation of mutant AR toxicity in cell culture and mouse models of KD [72]. IGF-1 was found to affect AR toxicity through post-translational modification by PI3-kinase-mediated activation of Akt. Native IGF-1 has a very short half-life. An IGF-1 mimetic developed by Novartis, BVS857, is much more stable, and it was well tolerated in a KD clinical trial [73], although with antibodies to endogenous IGF-1. A short-term, randomized, placebo-controlled trial showed a significant effect on the primary outcome measure, thigh muscle volume by quantitative MRI, but because of the immunological side-effects it was decided not to pursue it as a treatment for KD patients, whose IGF-1 levels are already low. Reducing levels of toxic AR mRNA and protein has been beneficial in KD pre-clinical studies. AAV9-mediated IV delivery of miR-298 targeting AR 3′UTR resulted in good distribution to muscle and spinal cord, reduction in mutant AR mRNA & protein levels, and mitigation of disease manifestations in KD mice [74], and toxic AR suppression with oligonucleotides was beneficial with either subcutaneous or intraventricular delivery [75,76]. Natural history studies are key to the design of clinical trials, by identifying measures that can show meaningful change in a given number of participants in a specified time frame. A KD natural history study in Denmark with 29 KD participants followed for 18 months showed significant changes in muscle strength (hand grip and knee extension), activities of daily living, 6-minute walk, stair climbing, and muscle fat content (see below) [55], suggesting these measures for determining efficacy in adequately powered interventional studies, perhaps in a larger number of participants in a shorter period of time. Another natural history study is currently underway at the National Institutes of Health in the USA, with a 2-year follow-up of clinical measures, total body MRI and ultrasound imaging, and biomarkers in plasma, CSF, and muscle. At least four studies are currently under consideration or underway: oligonucleotide-induced AR knock-down (Ionis), AAV-mediated RNAi (University of Pennsylvania), small molecule ASC-JM17/AJ201 (AnnJi), and a small molecule AF2 modulator (Nido Biosciences). The AnnJi drug is currently being evaluated in a phase 2 clinical trial at six sites in the US, with enrolment nearly complete and results expected in the next few months.

John Vissing's presentation focused on clinical, biological, and magnetic resonance imaging (MRI) outcome measures for trials in KD, emphasising the need for defining appropriate outcome measures in KD. Although unspecific to the disease, plasma creatinine concentrations correlate inversely with the muscle fat fraction and directly with the SBMA-FRS [56]. Muscle fat fraction assessed by MRI is likely to be a good surrogate biomarker for muscle function as it correlates with muscle functional measures, such as strength, 6 min walking test (6MWT) and SBMA-FRS [56]. However, care should be taken where to assess fat fraction as it may differ according to distal or proximal assessment. Muscle contractility in KD is another potential biomarker/endpoint as it is reduced in most muscle groups in KD and correlates with the fat fraction in the muscles [77]. A longitudinal study showed that progression in muscle fat fraction is a sensitive biomarker of change over 18 months, and interestingly, the changes were quite consistent irrespective of duration of disease, baseline composite muscle fat fraction, CAG repeats, and baseline walking distance [55]. This makes disease evolution predictable among patients at different disease stages, which is an important consideration for therapeutic trials. This finding was corroborated by assessing 6MWT and SBMA-FRS findings, which seemed to be the most consistent significant change over time with a stable progression line across five natural history studies in KD of which some are still unpublished [55,78].

Gianni Sorarù gave an overview of available symptomatic treatments for KD patients. In addition to motor dysfunction, KD patients exhibit various systemic symptoms. Shared evidence-based guidelines on the use of treatments for these symptoms, i.e. symptomatic therapies, have not yet been established. Sorarù's clinical team collected information on co-morbidities, symptoms, and drug treatments in the Padua (Italy) KD cohort, which includes 112 patients. Cramps, lower limb neuropathic pain, and urinary and sexual disturbances were among the most frequently reported symptoms. Anti-convulsants (e.g., gabapentin or pregabalin) were the medications of choice for cramps and pain. Patients with urinary symptoms were mostly treated with alpha-blockers or 5-alpha-reductase inhibitors, although with poor clinical benefit in some cases. Indeed, the pathogenetic mechanisms underlying urinary dysfunctions in KD remain a matter of debate. Only two patients with erectile dysfunction reported taking phosphodiesterase type 5 (PDE5) inhibitors, while many of them preferred not to take these drugs due to a fear of possible side effects. Two patients underwent surgery for gynecomastia, and in one case, histopathology confirmed adenocarcinoma. Notably, in most cases, gynecomastia was diagnosed through manual or visual inspection without additional investigations such as ultrasound examinations. Metabolic disorders represented another frequent issue. Hypercholesterolemia was treated with ezetimibe alone or in combination with low-dose statins to mitigate potential statin-related muscle toxicity. Some patients with glucose metabolism abnormalities meeting the criteria for type 2 diabetes mellitus missed the recommended endocrinological assessments and, as a result, were not receiving the appropriate therapy. All patients with alterations in bone mineralization, which in most cases consisted of osteopenia, were on vitamin D supplementation. Anxiety and/or depression successfully benefited from the use of Selective Serotonin Reuptake Inhibitors (SSRIs). Results from this survey point to the need for greater awareness of systemic symptoms by neurologists caring for KD patients [66]. A multidisciplinary approach to the disease, along with improvement in research

on mechanisms and therapy of non-neural symptoms in KD, is warranted.

Alexandra Maclean, as representative of Avenue Therapeutics, reported on a phase 1/2 study in KD patients using a novel Nrf2 activator (AJ201) to enhance degradation of polyQ-expanded AR (https://avenuetx.com/) [13].

Luca Zampedri discussed the need for more objective clinical outcome assessments in KD, with a focus on an important symptom, namely fatigue. The concerted international effort to collect a homogeneous set of data in KD has focused on identifying novel biomarkers (e.g., muscle MRIs), as well as clinical outcome assessments (COAs). The latter are classified in performance assessments, observer reported, clinicians reported, and patient reported outcome (PRO) assessments. Biomarkers and performance assessments such as the adult myopathy assessment tool, and the 6MWT are more sensitive at capturing the slow disease progression typical of KD. Although less sensitive, the SBMA-FRS, a mixed clinician reported and patient reported assessments, is especially useful for people unable to undergo MRIs and/or demanding performance assessments. Recourse to patient centred measures and PRO assessments is endorsed by a variety of health governing bodies, including the FDA to support claims in approved medical product labelling [79]. The subjective experience of fatigue collected via the fatigue severity scale (FSS) has several advantages over other PRO-Assessments. Most importantly, fatigue is one of the commonest non-motor symptoms reported by people living with KD [80]. In addition, when examined cross-sectionally, the average FSS score significantly correlates with certain muscle biomarkers (e.g. creatinine), as well as with the performance assessments listed above. It has however a major drawback, insofar that FSS does not capture progression when examined longitudinally. The concluding group discussion has centred on whether to select a more sensitive tool to capture the experience of fatigue and/or whether fatigue should be an important part of a multidimensional patient centred scale such as that being currently developed by the team at the National Institute of Health. A more frequent recourse to the use of digital health technologies could also reduce the need for patient and physician reported assessments.

3.1. From patient representatives

The patient group consisted of four patients from Italy, UK and the USA, all representing their respective national patient organisations (The Italian Kennedy's Disease Association (A.I.M.A.K), Kennedy's Disease UK (KDUK) and the Kennedy's Disease Association (KDA). The group started by stating how grateful they are for the focused research effort that is currently underway into KD and the efforts to identify a cure or a disease-modifying treatment. They felt that the KD patient community is fortunate to have such a committed group of researchers and feel that the relationships between KD patients and the researchers is a special one – something that is beneficial for all of us. The patients highlighted their personal experience of living with KD, and briefly summarised their symptoms and main problems associated with the disease such as general muscular weakness, cramps, tremors, sleep apnoea, speech and bulbar issues which affect their daily lives.

The patient group also believes that the most effective way to achieve the shared goal of finding a cure for this disease is to continue to foster the excellent international cooperation that exists within the KD research and patient community, a cooperation which is dependent on good communication between patients and researchers especially regarding possible treatments and cures.

The patient group highlighted four areas that might benefit from further work: i) Addressing the current lack of understanding and knowledge of KD by clinicians and, more specifically, non-specialist neurologists. The patient group is concerned that a treatment or cure will be of limited benefit if many of the people living with the illness have not been diagnosed. In cases where there is no known familial history of KD genetic testing for the disease is often too slow. The patient group felt that the disease is likely to be significantly under- and/or misdiagnosed. For example, one of the patient group took 23 years from first symptoms to be diagnosed, despite many neurologist appointments and tests; ii) The adoption of an international protocol, perhaps with country specific variations, that people living with KD can share with their neurologists and family doctors. It was acknowledged that the creation and distribution of the French Protocol is an excellent first step towards this and may help to resolve the problem of the lack of awareness of KD and the resulting late and misdiagnosis; iii) Addressing the difficulty that people living with KD have in accessing regional support. Treatment at present is largely focused on the major research centres that many patients struggle to access. As diagnosis levels ramp up, regional treatment protocols will need to be put in place; iv) Finding the right balance between research for a treatment or cure, and research into creating clinically proven treatment pathways. There is some concern that funding is almost entirely targeted on research into finding a cure, sometimes at the expense of developing treatments for patients that are living with the disease today. The patient group acknowledged that it is important that KD patients recognise that they also have a key role to play in finding a treatment for KD. This includes, for example, participating in patient registries and clinical trials. It was felt that researchers have an important role in encouraging such participation.

Finally, the group also highlighted the importance of the national Kennedy Disease charities and Patient Associations who provide vital support to people living KD and their families, in addition to providing support for research programmes and to research trainees. It was also acknowledged that the charities and associations value the strength of the existing relationship they have with the research community, and that it should continue to be nurtured.

4. Conclusions

The workshop drew to a close with a dedicated pledge to fortify existing collaborations, underscoring the significance of multidisciplinary approaches in addressing the complexities of KD. The roadmap for future international events involves strategic partnerships with patient associations, aiming to effectively communicate scientific discoveries to the KD community and actively engage them in the ongoing research endeavours.

Immediate objectives of the consortium encompass the reinforcement of international data collection efforts and collaboration with patients for the establishment of guidelines that facilitate the streamlined execution of clinical trials. Looking further ahead, aspirations revolve around a profound exploration of KD's pathology, aiming to significantly advance our understanding and, in turn, to pave the way for the development of targeted and effective treatments. The collective commitment at the conclusion of the workshop reflects a shared dedication to both immediate and enduring goals, envisioning a future marked by increased collaboration, enriched scientific insights, and improved prospects for individuals affected by KD.

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Declaration of competing interest

All authors, Maria Pennuto, Linda Greensmith, Gianni Soraru, and Pierre-Francois Pradat, have no conflict of interest to declare related to the manuscript entitled.

CRediT authorship contribution statement

M. Pennuto: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **P.F. Pradat:** Writing – original draft, Supervision, Funding acquisition, Conceptualization. **G. Sorarù:** Writing – original draft, Funding acquisition, Conceptualization. **L. Greensmith:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

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