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Workshop report

241st ENMC international workshop: Towards a European unifying lab for Kennedy's disease. 15–17th February, 2019 Hoofddorp, The Netherlands

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1. Introduction

The 241st ENMC meeting took place in the Netherlands on the weekend of 15–17th February, 2019, and was focused on Kennedy's Disease (KD), also known as Spinal and Bulbar Muscular Atrophy (SBMA). The meeting involved twenty-four scientists working in academia, hospitals and industry from 8 different countries (Denmark, France, Germany, Israel, Italy, Spain, United Kingdom, USA) along with three patients' representatives, including one who was also a representative of the USA patient group, The Kennedy's Disease Association (KDA). The goal of this workshop was to bring together leading clinical and basic scientists working in the field of KD to discuss the current understanding of basic disease mechanisms and to share and update the most recent developments in clinical evaluation of patients, with the objective of increasing the prospects of developing and testing new treatments that could effectively slow down disease progression in KD patients. The participants discussed recent developments in KD research and shared the most recent clinical observations. In this report, we summarize the presentations and discussions that took place during the workshop, which range from disease mechanisms, biomarker discovery, plans for an EU Registry for KD as well as an update on ongoing clinical trials. In addition, a dedicated session on the patient's perspective was presented.

Kenneth Fischbeck opened the meeting with an overview of the disease. Kennedy's Disease (KD) also referred to as SBMA 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. SBMA is caused by expansions of a CAG repeat in the androgen receptor (*AR*) gene. The disease typically presents in adult males with slowly progressive, relatively symmetrical, lower motor neuron weakness, perioral fasciculations, elevated creatine kinase, and decreased sensory potential amplitudes [1]. Common, but not invariable, manifestations are gynecomastia and a family history consistent with X-linked inheritance. Other non-neuromuscular features include Brugada syndrome [2], urinary retention [3], non-alcoholic fatty liver [4], and fatigue. Primary degeneration of both motor neurons and muscle occurs in SBMA; there are clinical features of denervation and loss of motor neurons in the spinal cord and brainstem, and also increased creatine kinase and histological evidence of muscle fiber degeneration. The disease phenotype can be partially rescued in mouse models with molecular correction in either muscle or the central nervous system [5–7].

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The expanded CAG repeat in the AR gene encodes a polyglutamine tract in the transactivation domain of the AR protein (polyQ-AR), a part of the protein that is separate from the DNA- and hormone binding-domains [8]. The polyglutamine expansion is known to cause a loss of normal AR function and also a gain of function that is toxic to motor neurons and muscle. The mutant proteins have been shown to be toxic in cell culture and animal models, where they form neuronal inclusions and have a tendency to aggregate with increasing repeat length. The toxicity is greater with localization to the nucleus, where the mutant protein has aberrant interactions with other nuclear factors, leading to altered histone acetylation and transcriptional dysregulation, with adverse effects on axonal transport, signal transduction, and mitochondrial function, resulting in neuronal dysfunction and death [9,10]. There is evidence for many paths to toxicity in animal models, and the challenge is to discern which paths are most important to the disease mechanism in patients.

2. Disease mechanisms: from AR structure to function in SBMA

Xavier Salvatella described the structural properties of the polyQ-AR associated with SBMA. Contrary to expectations the tract is not disordered but instead forms quite stable helices stabilized by an unusual type of hydrogen bond involving the glutamine side chain [11]. The size and the stability of these helices directly correlates with tract length, suggesting that changes in secondary structure upon polyQ tract elongation may contribute to onset of SBMA, possibly by altering that affinity that AR has for its binding partners [12]. Xavier Salvatella also showed how the molecular chaperones Hsp40 and Hsp70 interact with a specific motif in the activation domain of the AR and that this interaction contributes, on the one hand, to the efficient turnover of the inactive receptor and, on the other hand, to keeping the receptor soluble in the cytoplasm. Finally, he showed how it is possible to decrease the amount of monomeric and aggregated AR in a mouse model of SBMA by treating the mice with a small molecule that binds to Hsp70 and increases its affinity for intrinsically disordered substrates (unpublished data).

Angelo Poletti described how the testosterone-induced misfolded species of the polyQ-AR impacts on degradative systems. Normally, polyQ-AR is processed by the proteasome, but after binding to testosterone polyQ-AR tends to aggregate and by losing its monomeric structure it cannot be recognized for proteasomal degradation. However, aggregates sequester potentially neurotoxic misfolded polyQ-AR species into a specific subcellular compartment, thus making polyQ-AR less harmful to cells. Aggregates could be removed by autophagy, but polyQ-AR blocks the autophagic flux limiting its own clearance. To enhance polyQ-AR degradation Poletti's group has examined the possibility of modulating its routing to the two degradative systems, taking advantage of chaperones and co-chaperones function. They found that polyQ-AR can be efficiently removed from neuronal and muscle cells if the activity of a peculiar form of autophagy is enhanced. Overexpression of a small heat shock protein B8 (HSPB8) when proteasome activity is impaired, greatly reduces testosterone-induced aggregation of polyQ-AR and facilitates its autophagic clearance. HSPB8 acts in conjunction with BAG3/HSPA8 and in association with the E3-ubiquitin ligase CHIP, generating a complex responsible for Chaperone-Assisted selective autophagy (CASA). The CASA complex recognizes misfolded proteins, which are then ubiquitinated by CHIP for SQSTM1/p62 recognition and dynein-mediated transport to microtubule organization center (MTOC) where autophagosomes are generated and lysosomes collected in normal neurons. Notably blockage of dynein transport does not result in polyQ-AR accumulation, as expected, but rather in an increased degradation via the proteasome prior to its aggregation. We found that this alternative degradation is facilitated by the presence of another HSPA8 co-chaperone, BAG1, which is induced when retrograde transport is blocked, and associates with HSPA8/CHIP in an opposite way to HSPB8/BAG3, leading to misfolded protein degradation via the proteasome. Thus, polyQ-AR degradation can be differentially obtained via proteasome and autophagy and this fine equilibrium is finely tuned by the relative expression of the HSPA8/CHIP1 partners: (i) HSPB8/BAG3 to mediate polyQ-AR autophagic clearance, and (ii) BAG1 to direct polyQ-AR proteasomal clearance. Based on these findings, Poletti proposed that factors capable of maintaining the correct ratio of autophagic and proteasome-mediated degradation of polyQ-AR may prevent its accumulation and its consequent deleterious effects in SBMA.

Maria Polanco described how the neurotoxicity of polyQ-AR can be modified by phosphorylation at specific sites, thus providing the rationale for the development of specific treatments for SBMA. Cyclin-2-dependent kinase (CDK2) phosphorylates polyQ-AR specifically at serine 96, increasing the stability and toxicity of polyQ-AR; and this phosphorylation is negatively regulated by the adenylate cyclase (AC) / protein kinase A (PKA) pathway [70]. Illana Gozes described various pituitary adenylyl cyclase activating polypeptide (PACAP) derivatives developed as activators of the AC/PKA pathway, and discussed how the native PACAP is involved in stress responses and regulates activity-dependent neuroprotective protein (ADNP). In order to develop a potential therapy, Illana Gozes synthesized a chemical analogue of the activating polypeptide of pituitary AC (PACAP), a potent activator of the AC / PKA pathway. Maria Polanco tested these analogs in a mouse model of SBMA. Chronic intranasal administration of this analog to SBMA mice reduced phosphorylation of polyQ-AR at serine 96, promoted its degradation and improved the SBMA outcome. These results show that non-invasive strategies based on the use of PACAP analogues may be a therapeutic option in SBMA.

3. Disease mechanisms: peripheral polyQ-expanded AR mechanisms of mutant AR toxicity

Although traditionally considered to be a motor neuron disease, recent findings strongly suggest that in addition to motor neurons, other tissues are also affected in SBMA. Indeed, SBMA is now considered a neuromuscular disorder rather than a pure motor neuron disease, since skeletal muscle may be a primary and early site of pathology. In this session, scientists presented findings that indicate that skeletal muscle represents a good target for therapeutic intervention as not only is it affected very early in the disease, as it may be more accessible to treatment than motor neurons which reside within the central nervous system.

Carlo Rinaldi opened this session and began by describing how in SBMA and indeed all genetic conditions caused by mutations in ubiquitously expressed genes, the clinical picture is the result of a complex interplay between differentially affected tissues, which struggle to cooperate to maintain homeostasis. Carlo Rinaldi discussed the current understanding of the extra motor-neuron involvement in SBMA, with a particular focus on the primary muscle involvement, sensory neuropathy, and autonomic dysfunction, which are emerging as clinically highly impactful in patients' quality of life and disease progression. Their thorough investigations are proving critical not only because they might provide important insights into common mechanisms of pathogenesis, but also because they may offer opportunities for single or combinatorial treatment strategies in the near future. In particular, he discussed ongoing research in his lab to disentangle the molecular mechanisms of muscle atrophy in SBMA and the effects of muscle-specific therapeutic targeting on motor neuron homeostasis. Additionally, studying the peripheral abnormalities in SBMA may offer the opportunity for direct functional assessments and repetitive samplings, therefore representing potentially exploitable biomarkers to track disease progression and/ or response to therapy, a critical need now that therapeutic opportunities are starting to reach the clinical trial phase. The second part of the talk was to introduce a new study aimed at identifying blood-based epigenetic biomarker to monitor the progression of muscle atrophy in SBMA. Epigenetic biomarkers are one of the latest and most promising development in the field of biomarkers today and present highly attractive opportunities for clinical use. Moreover, epigenetic biomarker development involves the generation of omics data, which can be mined and analysed to objectively investigate the underlying pathways relevant for disease pathogenesis. By employing the state-of-the-art of epigenetic biomarker discovery in collaboration with a highly successful commercial partner, in the outstanding setting of the recently established Oxford Neuromuscular Translational Research Centre and in synergy with the largest dedicated SBMA clinic in UK, it is expected that this project will have a major impact on SBMA and other neuromuscular diseases. Lastly, this work will establish a framework of biomarker generation that could be useful for other human conditions.

In their presentations, Maria Pennuto and Linda Greensmith presented compelling and complementary evidence to show that motor neuron dysfunction and loss in KD is associated with deficits and degeneration of skeletal muscle. Dr Pennuto showed genetic and pharmacological evidence that supports the concept that muscle atrophy is not only secondary to motor neuron degeneration. Rather, polyQ-AR has been shown to cause primary toxicity in muscle that in turn leads to motor neuron loss [13]. However, the mechanism through which polyQ-AR causes muscle atrophy remains to be elucidated. Maria Pennuto and Linda Greensmith reported evidence obtained in two different animal models of disease and in patient-derived muscle biopsies. Signs of neurogenic and myopathic atrophy can be detected in the skeletal muscle of transgenic mice overexpressing polyglutamine-expanded AR and knock-in SBMA mice [14,15]. Myopathic processes and muscle atrophy precede spinal cord pathology in knock-in mice [15]. Muscles in transgenic and knock-in mice are intrinsically weak [16]. Fast-twitch muscles are mainly affected and display a glycolytic-to-oxidative fiber-type switching regardless of denervation with alteration in lipid homeostasis [17]. Early muscle pathology is present also in patients. Elevated levels of serum creatine kinase, myofiber degeneration and structures similar to central cores occur, even before clinical symptoms [18,19]. Glycolytic-to-oxidative myofiber-type switch, myofiber atrophy, and primary myogenic defects, such as necrotic myofiber and centrally located nuclei, are hallmarks of the disease as well [20–23]. Importantly, genetic and pharmacologic silencing of polyQ-expanded AR expression in skeletal muscle prevented disease manifestations in mouse [5,6]. Moreover, genetic and pharmacologic approaches to stimulate muscle anabolic pathways and inhibit catabolic pathways, and at the same time reduce polyQ-expanded AR toxicity ameliorated disease manifestations in mouse [6,14,24].

Bilal Malik presented results from his study that aims to establish the genes and pathways that may be altered and may underlie motor neuron dysfunction in SBMA. Transcriptomic profiling of cultured primary embryonic motor neurons from SBMA mice showed that transcriptional dysregulation occurs early in development in SBMA motor neurons. *Chmp7* was found to be downregulated in SBMA motor neurons, and was also altered *in vivo* in spinal cord of SBMA mice before symptom onset, and crucially it was upregulated in motor neurons derived from SBMA patient stem cells (iPSC-MNs). Furthermore, genes were enriched in SBMA motor neurons in several important pathways including p53, DNA repair, WNT and mitochondrial function. Functional analysis of SBMA embryonic motor neurons revealed dysfunctional mitochondria along with DNA damage, which may result from mitochondrial dysfunction and/or DNA repair gene dysregulation. These results highlight the interplay of multiple pathways in SBMA and suggest that this early coordinated dysregulation, especially *Chmp7* dysfunction, may play a critical role in the development of disease. Bilal

Malik is currently extending these findings and is undertaking a transcriptomic analysis of hindlimb muscle from SBMA mice as well as from muscle biopsies of SBMA patients. By analysing gene expression in muscle of two different models of SBMA (human and rodent), this team hopes to gain a better understanding of the mechanisms of muscle dysfunction, as well as provide targets for therapeutic intervention.

The results presented in this session clearly confirm that SBMA should now be considered as a multisystem disorder and a greater understanding of the non-neuronal tissues affected in SBMA is a priority area for research. Future studies will therefore focus on unravelling the mechanisms of mutant AR toxicity in peripheral tissues to increase our understanding of tissue-specific functions of the AR.

4. Preclinical approaches to reduce polyQ-expanded AR toxicity

The discussion on potential novel preclinical approaches for SBMA focused on three main strategies to modulate AR activity by (i) employing AR pharmacological antagonists; (ii) silencing AR coactivators; and (iii) enhancing AR degradation via induction of lysophagy, a lysosome specific form of autophagy.

The first strategy was presented by Aria Baniahmad**,** who showed that new AR antagonists based on single benzene rings reduce polyglutamine-expanded AR aggregation. These novel AR antagonists target specifically the AR and the polyglutamine-expanded AR but act in a distinct manner, not yet uncovered, to inhibit AR activity compared to the therapeutically used antiandrogens (unpublished data; [25]). Manuela Basso illustrated the possibility to modulate the aberrant AR transcriptional activity in SBMA by decreasing the expression level of AR co-activators. She showed preliminary evidence that silencing protein arginine methyl transferase 6 (PRMT6) in vivo is beneficial in a transgenic mouse model of SBMA. PRMT6 is a co-activator of AR that potentiates the transcriptional activity of polyQ expanded AR by methylation of arginines present at the Akt-consensus site (RXRXXS) [26]. Of note, arginine methylation correlates with increased cellular toxicity while serine phosphorylation confers protection, highlighting the importance of modulating AR post-translational modifications (PTMs) in therapy. Along the same lines, Andrea Caricasole presented the pipeline of investigation of PTMs in huntingtin, another protein with polyQ expansion that leads to Huntington's disease (HD). The talk highlighted the need to develop robust tools (reference proteins, antibodies) and assays (immunoassays and MS approaches) to detect and quantify PTMs in polyQ proteins and the challenges related to this approach. The inhibition of key factors contributing to cell toxicity can be achieved with different strategies, among which the use of adeno-associated viruses. Giuseppe Ronzitti presented the development of a liver-based gene therapy approach for Pompe disease. The conclusions of this study indicate the safety and the efficacy of the treatment [27] and support the clinical translation of this approach. AR aggregates are present in muscle and motor neurons of SBMA patients. Paola Rusmini presented her recent work [28] in which she showed that trehalose, a disaccharide present in plants, fungi and some bacteria, activates lysophagy and enhances the clearance of aggregated AR.

5. Towards a European registry for SBMA

The next presentation and discussion centered around the necessity to put in place an EU registry of SBMA patients, similar to that which has been implemented in the US for SBMA or for other rare diseases such as Huntington's Disease in Europe.

Davide Pareyson reported the experience of the Italian SBMA Registry, which has been developed to (i) collect data useful for epidemiological and natural history studies, (ii) test outcome measures, (iii) facilitate recruitment in clinical trials, (iv) develop biomarkers, and (v) build a biorepository. Davide Pareyson presented the format of the Registry which is active at the https//:www.registronmd.it website [29] The minimal dataset to be collected has been agreed during a previous ENMC workshop [30]. The Registry is already predisposed to become an international registry as all items are in English [31]; only the section filled by the patients requires translation. It is a dual registry where the patient registers herself/himself, chooses a reference centre among three in Italy (Milan, Padua, Rome, with 22 other supporting centres), where the attending clinician collects the information and administers the clinical measures including the SBMA-FRS (SBMA-Functional Rating Scale) [32]; AMAT (Adult Myopathy Assessment Tool) [33]; 6MWT (6-minute walking test); self-administered questionnaires comprise the IIEF (International Index of Erectile Function) and the IPSS (International Prostatic Symptoms Score). Visits are repeated every year. All data are encrypted. Quantitative lower limb muscle MRI (qMRI) employing 3-point Dixon sequences [34] is performed in a subset of patients. Biological samples (serum, plasma, lymphocytes for immortalization, and in selected cases fibroblasts and myoblasts) are collected from consenting patients.

Davide Pareyson next presented the data thus far collected on the 143 patients who registered and the 112 for whom the data were entered. Nineteen patients have performed the first follow up visit. The workshop discussed the importance of developing an international Registry starting from the Italian one (funded by Telethon Foundation-Italy). The possibility of joining directly the IT platform in Italy or to developed parallel interacting and interoperable Registries was discussed. Workshop participants considered and debated issues related to different national regulations, ethical requirements, compliance with the novel GDPR, and sustainability. Such points have been considered when developing the Italian Registry which is compliant with all requirements and can be an example to follow. All participants (including patients' representatives) agreed on the importance of developing an International Registry in a joint common effort.

The clinicians also agreed on the need for sharing data, particularly regarding outcome measures. Knowledge of the responsiveness of the different outcome measures is fundamental in the planning of clinical trial power and number of patients to be recruited. They agreed to build a clinical team coordinated by Davide Pareyson to share and pool the data on the outcome measures (AMAT, SBMA-FRS, 6MWT). They also agreed that it would be important to pool data on the qMRI.

The Workshop participants also proposed that a European Biobank of tissues from animal models, as well as a SBMA Patient biobank would be extremely valuable resources for the SBMA research and clinical communities. Moreover, resources generated within individual laboratories investigating SBMA and which form part of this KD Consortium (e.g. plasmids, cell lines) should be made available to the research centres that form part of the SBMA network. The scientists believe that making resources promptly accessible to this group could guarantee a more rapid and efficient progress of research on SBMA. However, to establish the animal and patient tissue biobanks it is clear that appropriate funding will need to be sought from European funding agencies.

6. Biomarkers and outcome measures for SBMA

Apart from functional measures of disease status (e.g. walking distance, swallowing, grip strength), there are currently no reliable biomarkers for SBMA. The Workshop participants recognized the urgent need for more accurate, reproducible biomarkers of disease progression in order to enable patient stratification and for the evaluation of disease progression, both of which are essential for effective clinical trials.

Pierre-Francois Pradat presented results of neuroimaging studies which confirmed the multisystem nature of SBMA-associated pathology, and demonstrated that neurodegeneration is not limited to lower motor neurons but also involves the corticospinal tracts (CST) and widespread cerebral regions. Quantitative brain imaging studies revealed white matter alterations in the corticospinal tracts (CST), limbic system [35,36], brainstem and cerebellum [38]. Voxel-based morphometry (VBM) of SBMA cohorts revealed gray matter atrophy in the frontal lobes and in the brainstem [35–38]. Frontal hypometabolism has been detected by positron-emission-tomography (PET) [39]. Neuropsychological studies have detected subtle frontal dysfunction in small study populations [40,41], which were not confirmed in larger cohorts [42,43]. With recent technological advances, spinal imaging now offers unique opportunities to appraise lower motor neuron degeneration. It has been applied successfully to other motor neuron diseases such as ALS [44–46], and SMA [47,48], to characterize gray and white matter pathology. There is an ongoing monocentric longitudinal study to evaluate spinal cord imaging in SBMA patients and test whether it is a promising candidate biomarker (NCT02885870).

John Vissing next presented data on the disease phenotype in a cohort of 40 patients affected by SBMA and natural history data on 29 SBMA patients followed for 1 year. Phenotypic data were based on muscle MRI, functional tests and questionnaires. Muscle MRI showed that fat replacement of muscle was most pronounced in posterior muscle groups of thigh and calves and in the tongue [49]. This pattern together with relatively preserved sartorius, gracilis and tibialis anterior muscles suggest a diagnosis of SBMA. Muscle fat content correlated with muscle strength, SBMA functional rating scale score, and 6-minute walk test distance. It was also shown that muscle contractility is impaired in SBMA patients vs healthy subjects, even when correcting for fat content, and fat seemed to have an adverse effect on contractility more than could be explained by the replacement of muscle alone [50].

One-year follow-up showed a significant increase in muscle fat by on average 2% in all studied muscle groups [51]. Only a few strength measures (knee extension and handgrip) decreased and functional rating scores did not change, while the 6MWT dropped significantly from 362 to 336 m. The findings suggest that Dixon MRI of muscle is a suitable candidate as an outcome measure for natural history or treatment studies in SBMA, along with the 6-minute walk test and handgrip strength [52].

Pietro Fratta presented an update from the UK National Kennedy's Disease Clinic, focussing on disease biomarkers. Since the first KD ENMC Meeting in 2015, a dedicated clinic was started in London and now more than 60 KD patients are seen and followed up yearly. There has been focus on developing biomarkers for disease progression, as these are essential for effective clinical trials. Through the collection of plasma and serum at each visit, the UK group was able to measure molecules that indicate the damage occurring in the muscles and neurons of patients. Results highlighted how neuronal damage appears to be occurring at very low levels, whilst there is prominent active muscle damage, and markers such as creatinine show promise for use in clinical trials [53–55]. Further work analysing miRNAs in blood also confirmed a high degree of myopathic alterations. In parallel to blood analysis, the UK group also investigated muscle MRI as a tool to assess disease, and, when comparing KD patients with ALS patients and controls, they identified very specific patterns of muscle involvements that correlate with disease severity [56]. Overall, they suggest that a combined use of blood molecules and imaging studies, along with refined functional rating scales (discussed by Luca Zampedri) should be used in clinical trials.

Luca Zampedri, a specialist research nurse from UCL in London, next described the functional assessments the UCL team is employing in the dedicated National KD Clinic at Queen Square. People with SBMA attending the clinic undergo a battery of tests consisting of functional rating scales, 6MWT, AMAT, as well as myometries. Functional assessments collected from 128 people in three European services are currently being reviewed with Rash Analysis, by comparing each measures difficulty with the cohort's ability to fulfil the required tasks. Preliminary data have shown the

need to re-score some measures as to better capture the high functional variability found amongst KD sufferers.

In addition to participating in research, the majority of patients find it useful to obtain tangible measurements of their physical fitness. However, some participants have reported feeling extremely fatigued for days following testing, a factor not necessarily dependent on disease progression. Moreover, the potential negative psychological impact of functional assessments in a condition should not be overlooked, and motivation remains important in the patients' willingness and ability to engage with testing. Certain tests such as AMAT and 6MWT, which are designed to capture both muscular strength and endurance, can be particularly demanding. It has been suggested that the 2 min walking test (2MWT) might be a valid alternative to the 6MWT in a variety of neuromuscular conditions including SBMA [52]. A shorter, less demanding assessment might be completed by those experiencing advanced lower limb impairment and at the same time reduce post-testing fatigability. Systematic recording of intermediate times is required to evaluate the validity of the 2MWT in our cohorts.

Beyond the use of bulbar scales there exist few minimally invasive methods to capture bulbar function. Tongue pressure measurements have been suggested to be a reliable biomarker in SBMA [57,58]. In the UK cohort, tongue pressure measurements have been shown to be lower in people with SBMA with normal bulbar functional score when compared to healthy controls. However, tongue pressure did not capture disease progression, at least over 12 and 24 months.

As part of the process of revising the assessments used in SBMA, the possibility of merging the existing scales into one has been suggested in order to eliminate repeated measurements. However, whilst accounting for both ceiling and floor effects and for participants' tolerability, an important objective should be the preservation of historical data for comparison.

7. Clinical trials for SBMA

Kenneth Fischbeck next presented an overview of recent clinical trials in SBMA. Over the past 16 years a number of treatments have been found to be effective in mouse models of SBMA; the challenge has been to convert these findings into effective treatment in patients. Most promising in this regard are interventions close to the mutant AR protein, including androgen reduction, selective AR modulation, altering AR PTMs, and decreasing AR expression. The toxicity of the mutant AR has been shown to be dependent on androgens in transgenic flies and mice, and anti-androgen treatment (leuprorelin) blocks the disease onset and prevents the motor deficit in mice [59]. Three randomized, placebo-controlled trials have addressed whether such treatment works in patients, and none showed significant effects on the primary outcome measures of swallowing and muscle strength and function, although each had indications of secondary benefit [60–62]. A 7-year open-label follow-up study showed that patients did better with the androgen-reducing agent leuprorelin, and this drug has now been approved for SBMA treatment in Japan [63].

Other therapeutic targets close to the genetic defect include selective AR modulation (SARMs), PTM of the disease gene product, and disease gene expression. ASC-JM17 is an AR modulator that accelerates the mutant protein degradation, activates cellular protective responses through Nrf1/Nrf2, and rescues the phenotype in transgenic flies and mice [64]. Overexpression of insulin-like growth factor 1 (IGF-1) blocks AR toxicity through phosphorylation by Akt, and IGF-1 treatment improves muscle pathology and behavior in SBMA mice [65]. SBMA patients have low serum IGF-1, allowing room for therapeutic benefit. A recent placebo-controlled clinical trial of an IGF-1 mimetic showed a significant positive effect on the primary outcome measure (change in thigh muscle volume) [66]. The drug was well tolerated, but induced antibodies against endogenous IGF-1, which precluded further development. AAV-mediated intravenous delivery of a microRNA targeting the 3′UTR of the AR mRNA resulted in reduction in mutant AR protein levels and mitigation of disease manifestations in SBMA mice [67]. Finally, an antisense oligonucleotide targeting AR reduces mutant mRNA and protein levels and mitigates disease manifestations with peripheral or central administration in mice [6,7]; this too may be worth pursuing as a candidate therapeutic for SBMA.

Gianni Soraru' discussed findings which suggest that beta2-agonist stimulation of muscle may represent a therapeutic avenue for SBMA and describe plans to undertake a clinical trial of clenbuterol in SBMA patients. Treatment of SBMA knock-in mice with clenbuterol started at disease onset ameliorated motor function and extended survival [68]. Moreover, beta2-agonists have been found to be effective in improving motor function without relevant adverse events in a small cohort of SBMA patients [69]. To establish safety and efficacy of clenbuterol as a cure for SBMA, Gianni Soraru's team are conducting a multicenter, phase II, randomized, double-blind, parallel-group, single dose, placebo-controlled trial. The effectiveness of clenbuterol will be assessed with a number of outcome measures, including measures of the motor function, such as the 6MWT and the AMAT [33]; established rating scale such as the SBMA-FRS [32]; the forced vital capacity, a measure of respiratory muscle function; and quality of life by the ALSAQ–40 Questionnaire.

The tolerability of clenbuterol will be assessed by listing and counting all adverse events occurring during the trial. Ninety eligible subjects will be randomized to receive either clenbuterol or placebo tablets for 12 months. Adverse events, serious adverse events, suspected/unexpected adverse events, drug-related events will be recorded, along with duration, attribution to treatment, outcome and actions. To monitor potential side effects on cardiovascular system, a full cardiac protocol will be employed including echocardiogram, standard and 24 h Holter EKG. Given the concern about a potential muscle damage raised by finding increased CK levels in SBMA patients treated with clenbuterol [69], the

trial will also evaluate any muscle involvement by the means of the muscle MRI findings of thighs (i.e. presence of edema, degree of atrophy and/or fat substitution).

8. Patients perspective

The patient representatives, Marco Bertolotti and Gianni Fabris discussed the importance of collaboration between patient associations, clinicians and basic scientists for the advancement of SBMA. The role of patient associations in the dissemination of information was highlighted, for example about the importance and need for national registries as a means to update the SBMA patient community in international scientific efforts in SBMA research.

Edward Meyertholen from the USA was present during the Workshop as a representative of the patients' association – The Kennedy's Disease Association (KDA), supporting relationships and exchanges between patients and researchers from all over the world. Edward Meyertholen gave a brief outline of the KDA, which was founded in 2000 by Terry and Susanne Waite. The KDA is a patient-centric organization developed to meet the needs of those with KD, which has concentrated its efforts on education and research; it has been very successful to achieving these ends.

With regard to education, the KDA maintains a website with information about KD, and it is one of the best sites for newly diagnosed patients to get information regarding the signs and symptoms of KD. In addition to its website, the KDA sends out a monthly newsletter and it sponsors an annual conference that brings together researchers with patients, carriers and their families. During the Annual Conference information is presented that offers advice to KD patients and their families on how to live with KD. In addition, the KDA Conference has leading researchers present their recent scientific and clinical findings. Feedback from people attending KDA conferences consistently rates interaction with the researchers as one of the highlights of the conference. Nonetheless, there remains a wide gap between the researchers and patients not only in scientific knowledge but also in expectations. Researchers should be cognizant of these gaps when their research is publicized.

With regard to research, the KDA has develop a strong and growing grant program. The KDA awarded its first grant in 2003 and since then, has given almost 1 million dollars in funding. While a small group, the KDA has been successful at fundraising to support this program and more than 80% of its budget goes to funding grants. This program has grown from awarding a single grant of $20,000 in 2003 to having funded four grants at $50,000 each in 2018. It is expected that the funding levels will increase again in 2019. Generally, the program has been directed toward supporting young researchers (post docs, assistant professors). Grants have been awarded to over 20 researchers across the world. Feedback from the grantees has indicated that these grants have been instrumental in helping them advance their careers as well as maintaining their interest in researching KD.

9. Next steps

In order to increase scientific and clinical collaborations among groups working in different countries, it was agreed that an International Conference on SBMA should be organized. The Workshop participants highlighted the importance of researchers collaborating with patients' associations in the organization of the meeting, in order to reinforce the communication of scientific and clinical progress to SBMA patients and families, and to providing the community with the possibility of directly collaborating in the research process.

10. ENMC workshop participants - KD Consortium

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