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260th ENMC International Workshop: Congenital myasthenic syndromes 11-13 March 2022, Naarden, The Netherlands

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1. Introduction

The 260th ENMC workshop on congenital myasthenic syndromes (CMS) was held in Hoofddorp in March 2022 in a hybrid format. It was attended by clinicians, researchers, industry experts, and patient representatives from Germany, the UK, Italy, Senegal, Spain, the Netherlands, France, the US, and Canada. The last ENMC workshop on CMS was held in 2011 in Naarden and focused on diagnostic difficulties, clinical phenotypes, animal models, novel genes, and clinical management. Since then, great strides have been made in the discovery of novel genes and disease mechanisms, in addition, progress has been made in elucidating the natural history of CMS. These advances provide an opportunity for future treatment development, and thus this workshop was strongly focused on translation from basic science to providing the clinical infrastructure required for clinical trials.

The aims of the meeting were to; (1) Review insights from preclinical studies and decide upon strategies that could be used to develop new treatments in CMS, (2) Share available data on natural history in adult and paediatric CMS, (3) Establish clinical and non-clinical outcome measures (OMs) that could be adopted to provide consistency across different centres and clinical trials. The workshop was structured around eight sessions on classification of CMS, new phenotypes, next generation sequencing, animal models and therapies, and non-clinical biomarkers.

The workshop was attended by two patient representatives who opened the presentations. Marguerite Friconneau highlighted the important role patients play in research and how it can be difficult for scientists to reach them for valuable data collection. The role that patient organisations can play in this endeavour through using newsletters and helping to spread information about databases, natural history, questionnaires, trials results, and guidelines, was stressed.

This was followed by Ignacio Escuder, Secretary of ASMIC (Asociación de Síndrome Miasténico Congénito), a young organisation created in 2015 and headquartered in Barcelona (Spain). The context in which ASMIC was founded was presented along with information about the diverse types of patient members. Patients' concerns and expectations were described

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which have helped to generate the key goals of the organisation. These goals are:

- Improving the quality of life of CMS patients and their relatives by ensuring they can develop their lives within society from a position of equal opportunity.
- Informing and raising awareness within society and public institutions to guarantee their integration in all areas of life.
- Helping the opening of new research avenues and the progress of existing ones, with the objective of finding new drugs to improve symptoms and embrace new genetic therapies.
- Becoming a meeting point for all affected people and their families, constituting a hub for discussion, and exchanging knowledge about breakthrough medical advances.

It was highlighted that for patients, diagnosis, particularly in the first months of life remains the main challenge. Emotional and psychological aspects are important in the following years and improving quality of life is paramount for the adult patients in general. The presentation included a video developed in-house by ASMIC to communicate to others what living with CMS means (https://vimeo/com/632733829. The video should be a useful resource for other patients and their family members to explain the condition.

Session 1. CMS new classification and natural history.

1.1. New classification and nomenclature

The first session of the workshop concentrated on the classification of CMS and natural history data. Jacqueline Palace reported that there are several complexities in producing a CMS classification system. This includes the multiple pathophysiological mechanisms, the multi-system involvement in some genetic subtypes, and the lack of a known clear mechanism in others. Coding and classification systems were reviewed as outlined previously [1] with an emphasis on shortcomings. The ICD coding system used mainly to categorise health care usage does not divide into numbered categories beyond CMS. The MeSH subject headings used mainly for searching for publications simply mentions a list of terms that may be used. OMIM lists genetic diseases without any hierarchical categorisation and not all CMS genotypes are included. Orphanet (orpha.net) has a better categorisation system dividing patients into pre-synaptic, post-synaptic, synaptic, and glycosylation subgroups. The classification system suggested by Thompson et al. used the four subgroups as in Orphanet as level 1 categories, then further subdivided these up to level 3 or 4. It was felt overall this may be the best system currently. However, it was noted that not all genotypes fit neatly into one category e.g., AGRN is classified as post-synaptic to link it with the *MuSK, DOK7, LRP4* clustering pathway despite having pre-synaptic and synaptic actions. Classification according to treatment response or involvement of other systems was also discussed, as was removing the level 1 categories suggested in Thompson et al., other than for the glycosylation subgroup.

1.2. Natural history in CMS

Ulrike Schara-Schmidt then gave a presentation on natural history data in paediatric patients with CMS. This presentation drew from two papers describing a total of 101 patients from 78 families including different CMS subtypes: acetylcholine receptor (AChR) deficiency/*CHRNE, CHRNB1, CHRND*, AChE deficiency, GFPT1 deficiency, DOK 7 deficiency, slow and fast channel CMS, Rapsyn deficiency, CHAT deficiency, and MUSK deficiency [2,3]. The clinical hallmarks of CMS in paediatric patients are muscle weakness, hypotonia and exercise intolerance, additionally ptosis with or without ophthalmoparesis, respiratory and bulbar symptoms, contractures, and scoliosis. In rare cases dysmorphic features due to *RAPSN* mutations can occur. Marked creatine kinase elevation as an overlap with congenital muscular dystrophies in CMS due to *GMPPB* mutations was described, and less so in the case of *GFPT1* and *DPAGT1* mutations. Current data suggest a better treatment response and less severe progression in patients with mutations in genes directly associated with AChR deficiency. Recently mutations in genes coding for pre-synaptic proteins have been described. These cause more complex phenotypes with mental impairment and/or central nervous system (CNS) abnormalities, for example in *SNAP25B, UNC13, MYO9A, SLC18A3, SLC5A7, DPAGT1, SYT2, and VAMP1* mutations. The involvement of other organs was also highlighted, for example *LAMB2* (kidney), *PLEC* (skin), *SLC18A3, ALG14* (cerebral atrophy) and *PREPL* (growth hormone deficiency) mutations. Early genetic diagnosis and appropriate therapy are important for prognosis, but not all patients will recover completely. Treatment resistant muscle weakness, scoliosis and contractures, ptosis, strabismus, and ophthalmoparesis are observed during long-term follow up. Additionally worsening of symptoms can occur during infections and pregnancy, though often with recovery. Early genetic diagnosis and appropriate therapy are important for prognosis, but not all patients will recover completely. Especially other affected organs cannot be adequately addressed. Therefore, development of new treatment options is necessary.

Lorenzo Maggi described the literature on CMS natural history in adults. While this data is relatively poor, most patients are or will be adults in the future. Most of the studies are retrospective and focused on single genes and major events, such as loss of walking ability, loss or acquisition of motor milestones, need of assisted ventilation or bulbar involvement, occurring mainly in the paediatric population [4,5]. It has not yet been elucidated whether patients presenting in paediatrics may further progress in adulthood. Available data suggest substantial disease stability during adulthood or, less frequently, slow progression. Conversely, CMS patients presenting in adulthood probably display a less severe disease than those with paediatric onset. Further open issues related to CMS in adults include high clinical and molecular heterogeneity, lack of specific outcome measures and shared standard of care. In the Italian cohort, 14/116 (12.1%) patients presented as adults (>18 years), mostly due to mutations in *DOK7* and *CHRNA1*, with the latter always causing a slow-channel syndrome. All these patients maintained independent ambulation and showed stable/improved disease course during the follow up.

Session 2. New phenotypes in CMS.

1.3. New phenotypes in CMS

Pedro Rodríguez Cruz summarised subtypes of CMS that have been recently identified. Next-generation sequencing has helped to identify novel CMS genes and their associated phenotypes. Presynaptic CMS has greatly expanded with the discovery of CMS genes involved in axonal transport (*MYO9*), synthesis and recycling of acetylcholine (*SLC5A7, SLC18A3, PREPL*) and vesicle exocytosis (*SYT2, VAMP1, SNAP25B, UNC13A*). In general, these are severe early-onset CMS presenting with episodic apnoea and central manifestations derived from the expression of the mutated protein beyond the neuromuscular junction (NMJ). At the synaptic level, the spectrum was broadened with the discovery of *COL13A1, LAMB2,* and *LAMA5* as CMS genes. Patients harbouring *COL13A1* mutations suffer from severe manifestations early in life: breathing and feeding difficulties and greater axial than appendicular weakness [6]. Interestingly, disease severity appears to improve over time, which could be related to the

predominant role of *COL13A1* in endplate maturation. Novel CMS forms where the mutated protein is ubiquitously expressed have been reported, specifically in the early stages of N-glycosylation (*GFPT1, DPAGT1, ALG2, ALG14, GMPPB*). The associated phenotype combines a myasthenic-myopathy syndrome with prominent proximal weakness and sparing of facial and ocular muscles. Finally, *TOR1AIP1*, a gene previously associated with limb-girdle muscular dystrophy encoding a nuclear envelope protein, has been associated with CMS providing further evidence for the existence of mixed myasthenic-myopathy phenotypes [7].

1.4. Presynaptic CMS

Duygu Selcen presented an overview of presynaptic CMS, with *CHAT*-CMS being the most common form, accounting for about 5% of CMS [8]. The clinical clues are recurrent episodes of apnoea/respiratory distress in early life with or without other myasthenic symptoms. Electromyography (EMG) may not show decremental response at rest but will do after subtetanic stimulation for 5–10 min, followed by slow recovery over 10 to 15 min. A presynaptic CMS secondary to mutations in *SLC5A7* was reported in 17 patients from 13 unrelated families [9]. The presentation is usually at birth or in infancy with apnoea, ophthalmoparesis, ptosis, bulbar weakness, and some with profound global developmental delay. A few present antenatally with polyhydramnios and arthrogryposis, minimal response to stimuli with no spontaneous activity or respiratory drive. EMG shows a decremental response to low-frequency stimulation. Most patients respond to pyridostigmine and some benefit from albuterol/salbutamol. Dominant mutations in this gene cause distal motor neuropathy with vocal cord paresis. No specific hotspot or genotype-phenotype correlation is noted. Mutations in *SLC18A3* [9], responsible for transporting ACh into presynaptic vesicles, were reported in ten patients with early onset symptoms including foetal akinesia, apnoea episodes, ptosis, ophthalmoplegia, fatigable weakness, severe hypotonia, distal arthrogryposis, and profound global developmental delay in two, and learning disability in one. EMG shows decrement in four and is normal in one patient. Patients respond partially to pyridostigmine, 3.4-DAP and ephedrine. Mutations in *SNARE* complex proteins namely *SNAP25* and *VAMP1* result in CMS with onset of symptoms at birth or early infancy [9]. Mutations in Synaptotagmin 2, that interacts with SNARE proteins, result in autosomal dominant LEMS-like phenotype as well as recessive loss of function mutations resulting in severe phenotype of early onset severe hypotonia, generalised muscle weakness, areflexia with moderate bulbar deficits, fatigable ptosis, multiple anoxic episodes, and bradycardia [9]. Some of these patients have a partial response to pyridostigmine, 3,4-DAP and salbutamol. Recessive mutations in another SNARE complex protein, *Munc13*, results in severe myasthenia with apnoeic spells, LEMS features in the EMG, and encephalopathy with abnormal EEG [9]. There is wide variation in clinical presentation of presynaptic CMS with some proteins causing a severe CNS phenotype as well as peripheral nervous system (PNS) involvement and with variable or partial response to therapy.

1.5. Multi-system CMS

David Beeson explained that while mutations that underlie CMS were first identified in genes encoding proteins that are localised to and have a specific function at the NMJ, this is not true for many of the more recently recognised genes. It is now apparent that there is a broad spectrum of gene mutations where a myopathy/dystrophy and NMJ function can overlap (reviewed in [10,11]). The extent to which classical myopathies or dystrophies can disrupt neuromuscular transmission is contentious, but there are multiple reports where pyridostigmine has been found to be of clinical benefit in treating symptoms consistent with a NMJ transmission disorder in particular individuals for a range of congenital myopathies, typically in *RYR1, MTM1, BIN1, DNM2, TPM2* and *TPM3*. In a few cases EMG has suggested defective transmission. These findings warrant more detailed studies to define underlying mechanisms and to determine which patients would get clinical benefit from appropriate treatment.

Conversely, a subset of 'myasthenia' patients with a 'limb girdle' pattern of muscle weakness was described around fifty years ago [12]. Many of these turned out to have *DOK7* mutations, but the variability in presentation, affected muscle pattern, and EMG in some patients consistent with a myopathy, led to many cases being initially classified as myopathies. A greater overlap between myasthenia and myopathy occurs in forms of CMS due to mutations in the early steps of the N-linked glycosylation pathway (*DPAGT1, ALG2, ALG14, GFPT1* and *GMPPB*). Mutations in these genes can lead to a severe multisystem congenital disorder of glycosylation (CDG), but surprisingly milder cases can have clinical features largely restricted to an NMJ disorder. Myopathy is more marked in patients with *GMPPB* mutations, possibly because these variants are likely to affect the O-linked as well as the N-linked glycosylation pathway. In fact, relatively few of the total number of *GMPPB* cases have a clear NMJ defect. It should be noted that for many of the glycosylation pathway cases EMG needs to be performed on an affected weak muscle in order to detect the NMJ abnormality [13]. A newly identified location for abnormal function causing a CMS is in the nuclear envelope. *TOR1AIP1* encodes the protein lamin-associated protein1 (LAP1) located on the inner nuclear membrane. Mutations can give rise to CMS if they are located exclusively in the muscle isoform *LAP1B* [7]. LAP1 is thought to interact with other nuclear envelope proteins, such as emerin, but dystrophy-causing mutations in other nuclear membrane proteins have not been found to affect the NMJ. Although symptomatic treatment for these CMS subtypes can be very effective, it does not treat the associated myopathic muscle weakness which may gradually progress.

Carsten Bönnemann reported on a recently characterised recessive CMS gene, *SYT2*, coding for a calcium sensing presynaptic vesicle protein. Pathogenic dominantly acting variants in *SYT2* had been initially reported as causing a syndrome of motor neuropathy with NMJ abnormalities. The recently described recessive *SYT2* syndrome presents as a severe congenital syndrome with myasthenic features and also features suggestive of a terminal axonopathy, as well as potential mild CNS involvement [14]. There were indications in several patients of a clinical response to pyridostigmine and 3,4-DAP, making this an important diagnosis to establish. *SYT2* also joins the group of CMS syndromes with CNS involvement that include presynaptic forms and those associated with protein glycosylation.

Sithara Ramdas reviewed the increasing reports of multi-system involvement in CMS. The genes involved include *SLC5A7, SYT2, SLC5A7, SLC25A1*, Plectin, *LAMB2, PREPL, COL13A1* and glycosylation genes. *SLC5A7* encodes for the sodium dependant high affinity choline transporter. The CMS phenotype is autosomal recessive and can be of neonatal onset with severe respiratory involvement, global developmental delay, epilepsy, and poor response to treatment to a milder spectrum of episodic apnoea. There is a good response to pyridostigmine and improvement in motor function depending on severity [15]. *SLC5A7* is also described to have a dominant Charcot-Marie-Tooth (CMT) phenotype with non-progressive distal weakness in the 1st/2nd decade with or without vocal cord palsy [16]. *SYT2* is another pre-synaptic protein with both recessive and dominant mutations causing CMS. The recessive phenotype was reported in seven cases of antenatal or neonatal onset CMS phenotype, with additional features in two patients of

thin corpus callosum on MRI, two with learning difficulties, one with congenital heart disease with aortic stenosis and prolonged QT. The dominant phenotype is foot deformities, with phenotypes ranging from a dominant NMJ syndrome resembling Lambert-Eaton myasthenic syndrome to mixed manifestations of distal hereditary motor neuropathy and presynaptic NMJ dysfunction [17]. Cases of NMJ transmission defect with a mutation in *SLC25A1*, a mitochondrial citrate carrier, are reported with non-progressive fatigable weakness with mild learning difficulties. In addition, *SLC25A1* is known to cause a severe early onset epileptic encephalopathy with D-2 and L-2 hydroxyglutaric aciduria [18]. Pathogenic *LAMB2* mutations present with classical renal and ocular dysfunction of Pierson syndrome with NMJ defect manifesting in a patient who survived due to renal transplant [15]. Plectinopathy can present with CMS with and without epidermolysis bullosa [15]. *COL13A1* CMS patients have dysmorphic features and significant skeletal abnormalities [9]. *PREPL* mutations can be with or without cystinuria but can have other features including dysmorphic features, growth hormone deficiency and hypergonadotropic hypogonadism [15]. Disorders of glycosylation can cause CMS and are well recognised to cause CMS, congenital muscular dystrophy and CDG with multi-system involvement with epilepsy, developmental delay, movement disorder, optic atrophy, neuropathy [9]. Current CMS treatments are focused on NMJ abnormalities and there is a need to recognise the multisystem involvement with some CMS genes which pose challenges to management and development of future therapies.

Session 3. Next-generation sequencing: future and issues.

1.6. Shared platforms

The following day began with Daniel Natera-de Benito describing how the RD-Connect Genome-Phenome Analysis Platform (GPAP) (https://platform.rd-connect.eu/) works and how this online system facilitates the collation, analysis, interpretation, and sharing of standardised genome-phenome data of individuals with CMS and other rare diseases within a collaborative environment [19]. Authorised clinicians and researchers submit pseudonymised phenotypic profiles encoded using the Human Phenotype Ontology (HPO), and raw genomic data which is processed through a standardised pipeline. After an optional embargo period, the data is shared with other platform users, with the objective that similar cases in the system and queries from peers may help diagnosis. Additionally, the platform enables bidirectional discovery of similar cases in other databases from the Matchmaker Exchange Network [20]. To facilitate genome-phenome analysis and interpretation by clinical researchers, the RD-Connect GPAP provides a powerful user-friendly interface and leverages tens of information sources. GPAP is hosted by the data centre of the Centro Nacional de Análisis Genómico (CNAG-CRG, Barcelona, Spain) and currently has more than 600 users from over 100 research groups in more than 22 countries. It is a helpful clinical and research resource and has already aided in the molecular diagnosis of hundreds of patients with rare diseases, including CMS.

Session 4. Trial design in CMS- perspectives and concerns.

1.7. Patient registries

Hanns Lochmüller described the data collection efforts of the EURO-NMD patient registry (https://erne-euro-nmd.eu/) for NMDs and specifically for CMS. EURO-NMD is a European Reference Network (ERNE) for rare NMDs that brings together around 80 highly specialised clinical centres, often university hospitals, providing top-level care to patients with NMDs [21]. The main objective of the EURO-NMD registry is to improve healthcare for patients with NMD, particularly diagnosis and therapy. The data items collected for CMS were therefore chosen to inform key performance indicators such as “time to diagnosis”, “percentage of patients receiving a molecular diagnosis in 12 months”, “time to remission or improvement under therapy” and “percentage of patients reaching remission under therapy”. However, the breadth of data items collected, and the number of patients expected to be enroled means that the registry will also be able to provide insights into research questions including natural history, epidemiology, and response to treatment, as well as to establish cohorts for future clinical trials in CMS. The EURO-NMD registry is governed by the EURO-NMD coordination at the Institut de Myologie in Paris and housed at the University of Freiburg Medical Centre. European sites participating in the ENMC workshop were invited to participate as test sites or early adopters before the registry will be rolled out to all EURO-NMD sites from 2023. Workshop participants from sites outside the EU, including the UK and Canada, discussed ways they could align their own data collection and data models to facilitate exchange of data, joint analysis, and trial readiness. The Canadian Neuromuscular Disease Registry [22] has recently launched a deep dataset for CMS that collects many of the same data items, thus facilitating international data comparison (https://cndr.org/).

1.8. Lessons from clinical trials

Francesco Muntoni divided his presentation into learning from natural history and real word data, and considerations focused on specific aspects of clinical trial design. Regarding natural history studies, in myopathies these have been instrumental in developing outcome measures that have been studied first in the real-world setting, before being adopted in clinical trials. Specific examples are functional scales devised for ambulant boys with Duchenne muscular dystrophy (DMD) (The North Star Ambulatory scale), and the Performance of Upper Limb Scale for the non-ambulant individuals. The wide utilisation of scales like these have allowed researchers to build information on the rate of progression of DMD, the role of different standards of care on progression, and the role that specific genotypes have in determining disease trajectories. Other important concepts regarding functional scales are both the minimal detectable difference (i.e., the minimal “true” difference in reading, not affected by variability of performance) and the minimal clinically important difference (MCID), which is an essential concept both for clinical trial powering, and eventually for registration.

In DMD a lot of effort is currently being placed in better understanding the drivers of the different trajectories observed, with the identification of multiple gene modifiers responsible for different biological aspects of DMD pathogenesis, from regeneration to strength of muscle contraction, to fibrotic response to the inflammatory environment, and to provide accurate estimation of the extent of their role in DMD disease course. Parallel efforts are refining the understanding of the prognostic factors and how taking them into account can facilitate study design, by having an appropriate “weight” for these factors that may allow the recruitment of a broader patient population instead of concentrating on an extremely narrow patient population that, while reducing variance, also make recruitment problematic.

Finally, the increasing role that digital capture of activity is playing in many clinical studies was discussed; the importance of surrogate endpoints, and the requirement to link them to clinically meaningful outcomes for affected individuals. It was remarked that in myopathies and in spinal muscular atrophy (SMA), the journey of learning in clinical trial design has been going on for

over a decade with very helpful feedback by regulators in several meetings co-hosted by advocacy groups and academics, with the essential contribution of regulators from EMA and FDA [23,24].

Session 5. Perspective natural history studies in CMS-preliminary data.

1.9. Clinical tools for evaluating CMS patients

Sithara Ramdas compared SMA trial success and the challenges of DMD. The important criteria for clinical trial success were considered, including a good understanding of the incidence/prevalence, prospective natural history data, phenotype/genotype correlation, validated/good outcome measures, disease biomarkers and measures of disease burden. The concept of MCID was discussed and its crucial role in trial design. The challenges in CMS include more than 30 causative genes, a lack of natural history studies, variable disease burden with different genes and lack of CMS specific validated outcome measures. Several current validated neuromuscular clinical assessments like Quantitative Myasthenia Gravis scale (QMG), Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND), WHO motor milestones, Revised Hammersmith functional scale (RHS), Hammersmith infant neurological examination (HINE), Motor Function Measure (20/32), Performance of upper limb (PUL, Lung function tests etc.) were discussed briefly. The pros and cons of few specific outcome measures like QMG were discussed further, for example, most CMS patients (compared to MG) are stable once on treatment and improvements are not accurately picked up on a QMG score, and total QMG may not capture significant change (individual raw scores are more valuable). The importance of measuring fatigability in CMS needs to be considered and timed assessments would be of value. These can include the 6-Minute Walk Time (6MWT), repeated sit to stand in one-minute, timed 10 m walk/run, and timed rise to stand. Activity monitors are another useful outcome measure in many clinical trials. They have added value in CMS as they can capture fatigability which is variable hour to hour and day to day compared to a one-point clinical assessment of best motor ability, they capture functional change better, are not patient dependant and can capture changes over short periods. Patient reported outcome measures (PROMs) utility was discussed. Biomarkers that could be considered include EMG but there are practical challenges, especially in children. The Oxford group has recently initiated a 2-year prospective natural history study.

1.10. Natural history studies

Lorenzo Maggi talked about the Italian experience with a 2-year study in adult CMS patients. The natural history of CMS has not been clarified yet, particularly in adults and available data are mainly based on retrospective studies. They longitudinally assessed 40 genetically-defined CMS patients over a 2-year period with the Myasthenia Gravis–specific Activities of Daily Living scale (MG-ADL) [23], Myasthenia Gravis-composite scale (MGC) [24], timed tests (time to get up-from the floor and chair), and upper and lower limb fatigability (Mingazzini I and II) tests. The most frequently mutated genes in this study were *CHRNE* (n = 17), *DOK7* (n = 5), *GFPT1* (n = 4), *CHAT* (n = 3) and *COLQ* (n = 3). Median age at baseline was 39 years (range 10–71). Median MG-ADL score at baseline was 5 (range 0–13) and median MGC score 9 (range 1–27), without any significant correlation between age at baseline and disease duration. They observed a good concordance between MG-ADL and MGC scores at baseline and at the 2-year visit (T2). MG-ADL (p-value <0.0001) and, to a lesser extent, MGC (p-value=0.0037) significantly improved across the 2-year period, mainly due to treatment modifications. Conversely, no significant change in upper and lower limb fatigability and timed tests was found between baseline and T2. In conclusion, the preliminary data suggested no overall disease progression in a 2-year period.

This was followed by the Oxford experience in a large cohort of patients presented by Pedro Rodríguez Cruz. CMS is a very rare disease, with an estimated prevalence of 1:100.000 people [25], which means that Neurologists and Paediatric Neurologists are rarely exposed to CMS patients. The development of clinical services specific for rare diseases, such as the Oxford Referral Centre for CMS - funded through the UK National Specialist Commissioning (NSC) Department of Health - allows the centralization of clinical services and the development of large cohorts of patients for specific rare diseases. Integration of clinical and research activities with close cooperation from the clinical team, diagnostic and research laboratories allow for the improvement of quality of care and the development of clinical and translational research activities with direct impact on patients. The Oxford CMS service accepts referrals from across the UK. Furthermore, joint CMS clinics are held with other neuromuscular specialists in different locations of the UK to facilitate access to care. Remote advice to healthcare professionals and telemedicine appointments are also provided to facilitate follow-up and adherence. In this way, CMS patients can benefit from the expertise developed by the service over the long-term, which includes refined treatment strategies [9], functional assessment of genetic variants and gene discovery to provide a definitive diagnosis.

Sessions 6. Animal models.

1.11. New therapies in mouse models

Steve Burden presented his group's work on the development of MuSK agonist antibodies for treating *DOK7*-CMS. Mutations in *DOK7* are a common cause of CMS. *DOK7 c.1124_1127 dup* is the most frequent disease-causing *DOK7* allele for *DOK7* CMS and leads to truncation of DOK7 protein. We generated a mouse model of Dok7 *c.1124_1127 dup* CMS to better understand how this mutation causes disease and to develop therapies for treating *DOK7* CMS [26]. Dok7 *CMS* mice have severe deficits in NMJ formation, causing neonatal lethality. Truncated Dok7 is expressed poorly, leading to a severe reduction in MuSK tyrosine phosphorylation. The reduction in MuSK phosphorylation is central to the disease, as an agonist antibody to MuSK rescues neuromuscular synapse formation and lethality of Dok7 *CMS* mice as well as late onset disease in relapsing Dok7 CMS mice. These findings reveal an unexpected cause for disease and a potential therapy for treating Dok7 CMS and other neuromuscular diseases [26].

Hanns Lochmüller summarised the use of a muscle-specific GFPT1 knockout mouse to model *GFPT1*-deficient CMS. Patients with bi-allelic mutations in *GFPT1* present with fatigable weakness of the limb girdle muscles and tubular aggregates in muscle [27]. A complete, body-wide inactivation of the *GFPT1* gene is embryonically lethal in mice. Mice with muscle specific GFPT1 inactivation are born at lower rates than expected but survive well into adulthood. They show specific signs of defective neuromuscular transmission including fatigable weakness, pre- and postsynaptic abnormalities, and tubular aggregates, providing a model to elucidate the underlying glycosylation defect at the NMJ and enabling testing of novel therapies such as galactose supplementation and AAV gene therapy [28].

Yin Dong reviewed the different mouse models of *DOK7*-CMS that have been developed and how they have been used to develop new treatments for the disease. There have been four

different strategies for producing mouse models of *DOK7*-CMS published so far – a Dok7 knock out model that died immediately after birth; c.1124_1127dupTGCC (most common genetic variant found in patients) knock in mice that produce much fewer and smaller NMJs and die within a few weeks of birth [26,29]; post-natal shRNA knockdown of Dok7 expression that produced smaller NMJs and weaker animals; Y396F and Y406F knock in mutations to stop Dok7 C-terminal tyrosine phosphorylation that had few clinical physical abnormalities [26]. The differences between the disease phenotype in the mouse models and that of patients was discussed. Although the c.1124_1127dupTGCC knock in mouse model successfully models abnormal NMJ development and AChR clustering, the mice have a much more severe disease phenotype than in patients, with most animals failing to thrive within a few weeks of birth. Despite the differences, the c.1124_1127dupTGCC knock in models has still been useful for testing novel AAV-Dok7 gene replacement therapies [29] and MUSK agonist antibodies [26] for Dok7-CMS, demonstrating clear improvements in efficacy over the current best treatment salbutamol [30].

Patricio Sepulveda, the CEO of Amplo Biotechnology talked about Amplo's development of regenerative medicines to enhance NMJ function. Amplo's lead therapeutic candidate, AMP-101, was licensed from the University of Tokyo [29]. AMP-101 is an Adeno-associated virus that overexpresses Dok7 in skeletal muscle. AMP-101 has been developed to treat Dok7 CMS and other NMJ-affecting diseases characterised by AChR clustering instability. During the first half of the presentation, Amplo introduced the challenges and opportunities in the gene therapy space and contrasted those to the regulatory advice it has received. Then, the focused was set around summarising Amplo's progress with plasmid engineering, AAV manufacturing, pharmacology, and safety studies. The presentation finalised with Phase I/II clinical trial design and a discussion around further needs to improve understanding of CMS patient numbers and the burden of their disease.

Carsten Bönnemann reported on the PaVeGT (Platform Vector Gene Therapy) project of NCATS (National center for the Advancement of Translational Science), a joint effort with NINDS and NHGRI to develop platform-based gene therapy for ultra-rare disorders [31]. In this project, gene therapies are developed for related groups of ultra-rare disorders to take maximum advantage of building blocks that can be co-developed as a platform across the individual applications, including aspects of vector, cassette, and clinical trial designs. These building blocks, and their regulatory implications, including FDA filings, will be publicly available with the goal of maximum freedom to operate to facilitate more cost-effective gene therapy for ultra-rare disorders for which a commercial model might be difficult. The two groups of disorders selected to serve as a pilot are organic acidurias (*PCCA* and *MMAB*), developed at NHGRI (Chuck Venditti, PI) and CMS (*DOK7* and *COLQ*), developed at NINDS, (Carsten Bönnemann, PI).

This session concluded with Sally Spendiff who presented data summarising recent experiments testing a mini-agrin compound (NT1654, Neurotune) on three mouse models of CMS: 1. *Agrn*^{nmf380} mice harbouring a partial loss of function point mutation 2. Dok7 mice modelling the most common CMS mutation 3. COLQ$^{-/-}$ mice modelling another common form of CMS. NT1654 was designed to stimulate the agrin/LRP4/MuSK pathway to increase the clustering of AChRs at the endplate. The original paper demonstrated beneficial effects in a mouse model of ageing caused by NMJ dysfunction [32] and it recently rescued the phenotype of a zebrafish model of CMS caused by mutations to the MYO9A gene [33] which lies upstream of the site of agrin action. Due to the varying severity of the mouse models, different treatment protocols were employed. Data was presented demonstrating the effects of the compound on body weight, muscle strength, swallowing ability, myofibre characteristics, and NMJ structure. It was concluded that this compound was most effective when directly targeting the CMS causative mutation [34].

Session 7. Non-clinical biomarkers in CMS.

1.12. Electrophysiology as a biomarker

Margherita Milone reviewed the role of electrophysiology as a biomarker in CMS. CMS are characterised by a compromised safety margin of neuromuscular transmission (NMT), which arises from a variety of mechanisms and can be detected through electrophysiological tests. These play a crucial role in CMS diagnosis, and findings can sometimes suggest the underlying pathomechanism. Repetitive compound muscle action potential (R-CMAP) is a marker of cholinergic over reactivity and suggests slow-channel CMS (SC-CMS) or AChE deficiency. In SC-SCM, R-CMAP often correlates with extent of prolongation of the mutant AChR opening episodes, but lack of R-CMAP does not exclude SC-CMS. RNS at 2–3 Hz often shows >10% CMAP decrement with maximal decrement usually occurring at the 4th stimulus. Facilitation, an electrophysiological marker of autoimmune pre-synaptic defect of NMT, has been detected only in presynaptic CMS caused by mutations in *VAMP1, Munc13–1, SYT-2*, and some cases of *AGRN*-CMS. Facilitation was also reported in *LAMA5*-CMS. Mechanisms of compromised quantal release have been studied only in a few CMS with facilitation and low number of quanta available for immediate release demonstrated in *Munc-13*–1-CMS. In the absence of decrement, single-fibre EMG can unmask the defect of NMT. Conditioning with 10 Hz RNS for 5 min can trigger a decremental response at 2–3 Hz RNS and drop of CMAP in various CMS, while the following slow CMAP recovery over up to 20–30 min would suggest a defect in acetylcholine resynthesis, as seen in choline acetyltransferase-CMS (CHAT-CMS). Electrodiagnostic tests are helpful in detecting co-existing NMJ and muscle involvement, and can be used as biomarkers to assess CMS response to treatment [8,35].

1.13. Circulating biomarkers

Andreas Roos presented his group's work on finding circulating biomarkers for CMS. Biomarkers are defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological and pathogenic processes or pharmacological responses to a therapeutic intervention. An obvious benefit of blood biomarkers is the low invasiveness of sampling. Their potential to replace the invasive muscle and/ or nerve biopsies has previously been discussed in the context of neurological disorders including NMDs. Ideally, these biomarkers would link to pathophysiological processes leading to the clinical manifestation such as impaired NMT in CMS. Prompted by results of previous studies, we postulated that white blood cells express a variety of proteins crucial for proper functions along the neuromuscular axis and that dysregulation of these proteins in terms of the definition of novel biomarkers for CMS might provide insights into the underlying pathobiochemical processes. To test this hypothesis, a proteomic profiling was carried out on white blood cells collected from a patient with genetically confirmed *CHRNE*-related CMS (CHRNE-abundance in white blood cells was already demonstrated in the context of our previous studies [36]) as well as a patient with a syndromic phenotype dominated by myasthenia-like-features and responding to therapeutic intervention with pyridostigmine bromide. Intersection of proteomic data revealed the significant downregulation of a protein acting as a 'third messenger' substrate of protein kinase C-mediated molecular cascades during synaptic development and remodelling. This protein has already been

described as a biomarker for synaptic dysfunction in Alzheimer's disease. These preliminary data define the same protein as a biomarker candidate – with potential pathophysiological impact on perturbed dysfunction of the NMJ. Further functional studies on animal models and screening of this biomarker candidate in blood derived from further CMS-patients are needed to prove this hypothesis.

Session 8. Concluding session.

During this concluding session participants identified gaps in the current knowledge, discussed several action points and areas for collaboration. The importance of collaborations with patient organisations was stressed as this would increase the impact of research. It was agreed to engage further with organisations to get more patients into databases, understand the natural history of CMS, spread information, create lay friendly questionnaires, and prepare education programs. To aid novel gene discovery and diagnosis it was suggested to share laboratory protocols and submit unsolved CMS exomes for GPAP (16). The importance of submitting samples to biobanks was also highlighted. A large part of the meeting was devoted to outcome measures, and it was recommended that these should be tailored to underlying clinical patterns and genes. To further aid clinical trials it was suggested to share clinical protocols, along with preclinical and clinical data. Alongside using existing data infrastructures such as the EURO-NMD registry this would go a long way toward ensuring clinical trial readiness. Other suggestions included examining the MCID, outcome measures that can be applied in telehealth sessions, examining 'wearable digital devices', and looking at clinical trials in myasthenia gravis to see what has previously been successful, including PROMS. It was also suggested to think about patients going on or off current therapy to understand sensitivity to change for OMs. Finally, the desire for patients to be included in the research process and in the dissemination of information was re-emphasised.

Declaration of Competing Interest

DB: Funding from MRC Programme Grant MR/M006824/1 'Disease mechanisms and therapies for inherited disorders of the neuromuscular synapse'. SB: Argenx. PMRC receives the support of a Junior Leader fellowship from "la Caixa" Foundation (ID 100010434) and from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 847648. The fellowship code is LCF/BQ/PI21/11830012. HL: Ongoing projects with Argenx, Amplo Biotechnology, and Neurotune. LM: Received honoraria for speaking, advisory boards, and compensation for congress participation from Sanofi Genzyme, Roche, Amicus Therapeutics and Biogen, outside the submitted work. YD: Received funding from Amplo Biotechnology. MM: Received honorarium to serve as associate editor of Neurology Genetics. JP: Support for scientific meetings and honorariums for advisory work from Merck Serono, Novartis, Chugai, Alexion, Roche, Medimmune, Argenx, UCB, Mitsubishi, Amplo, Janssen, Sanofi. Grants from Alexion, Roche, Medimmune, UCB, Amplo biotechnology. Patent ref P37347WO and licence agreement Numares multimarker MS diagnostics. Shares in AstraZenica. Acknowledges Partial funding by Highly specialised services NHS England. SR: Served as investigator in clinical trials for Roche, Sarepta, Genetx, Sathera, Argenx and received honorarium for speaking and serving on advisory boards from Novartis, Roche and Argenx. DS:Funded by NINDS NS109491-01. PS: CEO of Amplo Biotechnology. SS: Ongoing projects with Argenx, Amplo Biotechnology, and Neurotune.

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References

[1] Thompson R, Abicht A, Beeson D, Engel AG, Eymard B, Maxime E, et al. A nomenclature and classification for the congenital myasthenic syndromes: preparing for FAIR data in the genomic era. Orphanet J Rare Dis 2018;13:211. doi:10.1186/s13023-018-0955-7.
[2] Durmus H, Shen XM, Serdaroglu-Oflazer P, Kara B, Parman-Gulsen Y, Ozdemir C, et al. Congenital myasthenic syndromes in Turkey: clinical clues and prognosis with long term follow-up. Neuromuscul Disord 2018;28:315–22. doi:10.1016/j.nmd.2017.11.013.
[3] Della Marina A, Wibbeler E, Abicht A, Kölbel H, Lochmüller H, Roos A, et al. Long term follow-up on pediatric cases with congenital myasthenic syndromes-a retrospective single centre cohort study. Front Hum Neurosci 2020;14:560860. doi:10.3389/fnhum.2020.560860.
[4] Natera-de Benito D, Töpf A, Vilchez JJ, González-Quereda L, Domínguez-Carral J, Díaz-Manera J, et al. Molecular characterization of congenital myasthenic syndromes in Spain. Neuromuscul Disord 2017;27:1087–98. doi:10.1016/j.nmd.2017.08.003.
[5] Abicht A, Dusl M, Gallenmüller C, Guergueltcheva V, Schara U, Della Marina A, et al. Congenital myasthenic syndromes: achievements and limitations of phenotype-guided gene-after-gene sequencing in diagnostic practice: a study of 680 patients. Hum Mutat 2012;33:1474–84. doi:10.1002/humu.22130.
[6] Rodríguez Cruz PM, Cossins J, Estephan E de P, Munell F, Selby K, Hirano M, et al. The clinical spectrum of the congenital myasthenic syndrome resulting from COL13A1 mutations. Brain 2019;142:1547–60. doi:10.1093/brain/awz107.
[7] Cossins J, Webster R, Maxwell S, Rodríguez Cruz PM, Knight R, Llewelyn JG, et al. Congenital myasthenic syndrome due to a TOR1AIP1 mutation: a new disease pathway for impaired synaptic transmission. Brain Commun 2020;2:fcaa174. doi:10.1093/braincomms/fcaa174.
[8] Engel AG, Shen XM, Selcen D, Sine SM. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. Lancet Neurol 2015;14:461. doi:10.1016/S1474-4422(15)00010-1.

[9] Rodríguez Cruz PM, Palace J, Beeson D. The Neuromuscular Junction and Wide Heterogeneity of Congenital Myasthenic Syndromes. Int J Mol Sci 2018;19:E1677. doi:10.3390/ijms19061677.
[10] Nicolau S, Kao JC, Liewluck T. Trouble at the junction: when myopathy and myasthenia overlap. Muscle Nerve 2019;60:648–57. doi:10.1002/mus.26676.
[11] Rodríguez Cruz PM, Sewry C, Beeson D, Jayawant S, Squier W, McWilliam R, et al. Congenital myopathies with secondary neuromuscular transmission defects; A case report and review of the literature. Neuromuscul Disord 2014;24:1103–10. doi:10.1016/j.nmd.2014.07.005.
[12] McQuillen MP. Familial limb-girdle myasthenia. Brain 1966;89:121–32. doi:10.1093/brain/89.1.121.
[13] Rodríguez Cruz PM, Belaya K, Basiri K, Sedghi M, Farrugia ME, Holton JL, et al. Clinical features of the myasthenic syndrome arising from mutations in GMPPB. J Neurol Neurosurg Psychiatry 2016;87:802–9. doi:10.1136/jnnp-2016-313163.
[14] Donkervoort S, Mohassel P, Laugwitz L, Zaki MS, Kamsteeg EJ, Maroofian R, et al. Biallelic loss of function variants in SYT2 cause a treatable congenital onset presynaptic myasthenic syndrome. Am J Med Genet A 2020;182:2272–83. doi:10.1002/ajmg.a.61765.
[15] Abicht A, Müller JS, Lochmüller H, et al. Congenital myasthenic syndromes overview GeneReviews®. Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJ, et al., editors editors. SeattleWA: University of Washington, Seattle; 1993.
[16] Barwick KES, Wright J, Al-Turki S, McEntagart MM, Nair A, Chioza B, et al. Defective presynaptic choline transport underlies hereditary motor neuropathy. Am J Hum Genet 2012;91:1103–7. doi:10.1016/j.ajhg.2012.09.019.
[17] Herrmann DN, Horvath R, Sowden JE, Gonzalez M, Gonzales M, Sanchez-Mejias A, et al. Synaptotagmin 2 mutations cause an autosomal-dominant form of lambert-eaton myasthenic syndrome and nonprogressive motor neuropathy. Am J Hum Genet 2014;95:332–9. doi:10.1016/j.ajhg.2014.08.007.
[18] Nota B, Struys EA, Pop A, Jansen EE, Fernandez Ojeda MR, Kanhai WA, et al. Deficiency in SLC25A1, encoding the mitochondrial citrate carrier, causes combined D-2- and L-2-hydroxyglutaric aciduria. Am J Hum Genet 2013;92:627–31. doi:10.1016/j.ajhg.2013.03.009.
[19] Laurie S, Piscia D, Matalonga L, Corvó A, Fernández-Callejo M, Garcia-Linares C, et al. The RD-Connect Genome-Phenome Analysis Platform: accelerating diagnosis, research, and gene discovery for rare diseases. Hum Mutat 2022;43:717–33. doi:10.1002/humu.24353.
[20] Philippakis AA, Azzariti DR, Beltran S, Brookes AJ, Brownstein CA, Brudno M, et al. The Matchmaker Exchange: a platform for rare disease gene discovery. Hum Mutat 2015;36:915–21. doi:10.1002/humu.22858.
[21] Evangelista T, van Engelen B, Bushby K. 200th ENMC international workshop “European reference networks: recommendations and criteria in the neuromuscular field”, 18-20 October 2013, Naarden, The Netherlands. Neuromuscul Disord 2014;24:537–45. doi:10.1016/j.nmd.2014.03.005.
[22] Hodgkinson V, Lounsberry J, M'Dahoma S, Russell A, Jewett G, Benstead T, et al. The Canadian neuromuscular disease registry 2010-2019: a decade of facilitating clinical research througha nationwide, pan-neuromuscular disease registry. J Neuromuscul Dis 2021;8:53–61. doi:10.3233/JND-200538.
[23] Muppidi S, Wolfe GI, Conaway M, Burns TM. MG Composite and MG-QOL15 Study Group. MG-ADL: still a relevant outcome measure. Muscle Nerve 2011;44:727–31. doi:10.1002/mus.22140.
[24] Burns TM, Conaway M, Sanders DB. MG Composite and MG-QOL15 Study Group. The MG Composite: a valid and reliable outcome measure for myasthenia gravis. Neurology 2010;74:1434–40. doi:10.1212/WNL.0b013e3181dc1b1e.
[25] Parr JR, Andrew MJ, Finnis M, Beeson D, Vincent A, Jayawant S. How common is childhood myasthenia? The UK incidence and prevalence of autoimmune and congenital myasthenia. Arch Dis Child 2014;99:539–42. doi:10.1136/archdischild-2013-304788.
[26] Oury J, Zhang W, Leloup N, Koide A, Corrado AD, Ketavarapu G, et al. Mechanism of disease and therapeutic rescue of Dok7 congenital myasthenia. Nature 2021;595:404–8. doi:10.1038/s41586-021-03672-3.
[27] Senderek J, Müller JS, Dusl M, Strom TM, Guergueltcheva V, Diepolder I, et al. Hexosamine biosynthetic pathway mutations cause neuromuscular transmission defect. Am J Hum Genet 2011;88:162–72. doi:10.1016/j.ajhg.2011.01.008.
[28] Issop Y, Hathazi D, Khan MM, Rudolf R, Weis J, Spendiff S, et al. GFPT1 deficiency in muscle leads to myasthenia and myopathy in mice. Hum Mol Genet 2018;27:3218–32. doi:10.1093/hmg/ddy225.
[29] Arimura S, Okada T, Tezuka T, Chiyo T, Kasahara Y, Yoshimura T, et al. Neuromuscular disease. DOK7 gene therapy benefits mouse models of diseases characterized by defects in the neuromuscular junction. Science 2014;345:1505–8. doi:10.1126/science.1250744.
[30] Webster RG, Vanhaesebrouck AE, Maxwell SE, Cossins JA, Liu W, Ueta R, et al. Effect of salbutamol on neuromuscular junction function and structure in a mouse model of DOK7 congenital myasthenia. Hum Mol Genet 2020;29:2325–36. doi:10.1093/hmg/ddaa116.
[31] Brooks PJ, Ottinger EA, Portero D, Lomash RM, Alimardanov A, Terse P, et al. The platform vector gene therapies project: increasing the efficiency of adeno-associated virus gene therapy clinical trial startup. Hum Gene Ther 2020;31:1034–42. doi:10.1089/hum.2020.259.
[32] Hettwer S, Lin S, Kucsera S, Haubitz M, Oliveri F, Fariello RG, et al. Injection of a soluble fragment of neural agrin (NT-1654) considerably improves the muscle pathology caused by the disassembly of the neuromuscular junction. PLoS ONE 2014;9:e88739. doi:10.1371/journal.pone.0088739.
[33] O'Connor E, Cairns G, Spendiff S, Burns D, Hettwer S, Mäder A, et al. Modulation of agrin and RhoA pathways ameliorates movement defects and synapse morphology in MYO9A-Depleted Zebrafish. Cells 2019;8:E848. doi:10.3390/cells8080848.
[34] Spendiff S, Howarth R, McMacken G, Davey T, Quinlan K, O'Connor E, et al. Modulation of the acetylcholine receptor clustering pathway improves neuromuscular junction structure and muscle strength in a mouse model of congenital myasthenic syndrome. Front Mol Neurosci 2020;13:594220. doi:10.3389/fnmol.2020.594220.
[35] Nicolau S, Milone M. The Electrophysiology of Presynaptic Congenital Myasthenic Syndromes With and Without Facilitation: from Electrodiagnostic Findings to Molecular Mechanisms. Front Neurol 2019;10:257. doi:10.3389/fneur.2019.00257.
[36] O'Connor E, Töpf A, Zahedi RP, Spendiff S, Cox D, Roos A, et al. Clinical and research strategies for limb-girdle congenital myasthenic syndromes: limb-girdle congenital myasthenic syndromes. Ann NY Acad Sci 2018;1412:102–12. doi:10.1111/nyas.13520.