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Workshop report

237th ENMC International Workshop: GNE myopathy – current and future research Hoofddorp, The Netherlands, 14–16 September 2018

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1. Introduction

Clinicians, researchers, industry and patient group representatives (in total 25 members of the study group from 12 countries) gathered in Hoofddorp in September 2018 to discuss current knowledge and perspective research in GNE myopathy (previously known as Nonaka disease, Quadriceps Sparing Myopathy, Distal Myopathy with Rimmed Vacuoles or Hereditary inclusion body myopathy type 2). GNE myopathy is an -rare autosomal recessive disease caused by bi-allelic mutations in the GNE gene (UDP-N-acetyl)−2-epimerase/N-acetylmannosamine kinase). The phenotype was described in 1980's under different names, and the disease-causing gene, together with the Middle Eastern founder mutation, was described over 10 years ago [1,2]. Since then knowledge about molecular mechanism of the disease, phenotypic variability and epidemiology has expanded significantly. A recent Phase 3 clinical trial conducted by Ultragenyx Pharmaceutical did not show a beneficial effect of sialic acid supplementation on muscle strength, which highlighted the need for a deeper understanding of the pathophysiology of the disease and exploring other therapeutic approaches.

The aims of this workshop were: to achieve a better understanding of GNE myopathy epidemiology, pathophysiology, phenotype and genetics; to discuss the strength and weakness of the current animal models; to discuss genotype-phenotype correlations; to agree on standards of care for GNE myopathy; to discuss clinical trial readiness and data collection; to agree on functional scale for GNE myopathy assessment; and to explore novel ways for a better understanding of the pathophysiology of GNE myopathy.

Zohar Argov, briefly described the results of the Phase 3 clinical trial of Sialic acid (SA) extended release (SA-ER) formulation for stabilisation of muscle strength in GNE myopathy. Despite previous studies, including a phase 2 trial [3], showing that SA-ER appeared to be effective; a large ($n = 89$) multicentre international, double blinded study did not show statistical difference between treatment and placebo groups in any of the studied muscle outcomes [4]. Potential reasons for this discrepancy may include the following: inefficiency of the compound, faults in study design or additional, unknown GNE pathways affecting the disease pathophysiology. This includes the possibility that, in addition to SA production, there are other, yet unknown, functions

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of the GNE protein (e.g. impaired autophagy leading to the rimmed vacuole formation), and other genetic or biochemical modifiers that may affect the disease phenotype.

Existing animal models do not accurately and consistently reflect a muscle phenotype in human GNE myopathy. Furthermore, the mouse models frequently show severe kidney pathology and less muscle involvement as compared to humans, who have no renal involvement. The transgenic mouse model developed in NCNP, Tokyo, Japan [5] has a phenotype that has not been reproduced by other GNE focused labs. Zohar Argov argued that there is a possibility for clinical trials to be designed and conducted without a robust animal model reflecting all aspects of human myopathy. Apart of biochemical pathways, design of future clinical trials would also have to take into account whether the proposed drug improves or stabilise muscle strength in patients.

There is a need to develop specific and sensitive disease markers and GNE antibodies. It is also important to better understand why, on a rare occasion, people with GNE myopathy develop thrombocytopenia and significance of this condition. It is also yet unclear whether pregnancy exacerbates the disease progression and what are other factors that can significantly influence the course of the disease such as diet and exercise. Finally, continuation of the translational research and clinical trials in particular, require significant resources.

2. Role of patient organisations and patient perspective in GNE myopathy translational research

Lale Welsh, executive director, introduced the Neuromuscular Disease Foundation (NDF) and its role in GNE myopathy research. NDF, located in USA, is well connected to GNE patient advocates and experts in many countries in Europe, Middle East and Asia. One of Its three primary Programs is to promote and support development of scientific research, in particular, gene therapy by fostering scientific collaboration, conducting fundraising events and investing in studies that will facilitate therapy development. Other programs include Patient Advocacy and Education, through an annual Symposium on GNEM at UCLA and with the support of its Certified Patient Advocates worldwide. Mona Patel, UK patient advocate, talked about awareness activities she leads in the UK, such as collaborating with Muscular Dystrophy UK to develop dedicated resources and running a UK-centric GNE myopathy support group on social media. She gave an overview of a patient day she had recently organised (August 2018, Manchester UK). She highlighted the importance of accessible communication between scientific and clinical community with patient groups. Creating effective communication for patients and carers is a practical and economical way of increasing understanding and knowledge. Accessible patient-friendly materials will help in relieving patients' fears and encourage engagement in their health and wellbeing. She recommended that the group consider investing in the services of a medical writer in order to produce content that explains complex scientific information in lay terms for non-scientific audiences, such as patients and carers. Maya Davidovich, patient representative from Israel, talked about daily struggles of people with GNE myopathy and practical issues. She emphasized the need for recommendations on how to improve performance of daily activities. She also requested information about general health advice, diet and exercise recommendations tailored to the people with GNE myopathy.

3. Lessons learned from cohort studies contribute to the development of standards of care

Anthony Béhin presented the French GNE myopathy cohort, which at present accounts for a total of 45 patients, 16 of which from the Reunion Island, a French overseas territory. Reunion Island potentially may have a higher than average prevalence of the disease. The French population is genetically and clinically very heterogeneous, recent publication reported 20 new GNE variants. Clinically mild, classical and severe phenotypes have been observed, with extreme cases reaching non- ambulatory status before the age of 30 years. [6]. Most molecular studies were centralized till recently in one single laboratory (Prof N. Lévy, Marseille Medical Genetics). However, a growing number of GNE myopathy cases are now diagnosed in other centres thanks to a wider use of gene panels. Currently there is an attempt to arrange centralised reporting in France. There is no designated GNE myopathy patient group in France yet, the main information source for patients being the AFM-Téléthon's website and treating clinicians. There is a clinical neuromuscular network in France, which keeps neuromuscular specialists updated about GNE myopathy scientific developments. Patients are seen on annual basis for a general follow up visit. Bjarne Udd commented that there are no GNE myopathy cases in Finland, only single heterozygous mutations have been spotted. Also, not every country has access to GNE genetic testing, therefore there is a need to support genetic testing in Europe, and this issue should be highlighted at the European Reference Network for neuromuscular diseases (ERN EURO-NMD).

Maya Davidovich presented the Israeli GNE cohort. This is one of the largest GNE myopathy cohorts comprising 171 patients from 71 families [7]. The vast majority of patients carry the typical Middle Eastern founder mutation (p.M743T) in a homozygous state. Social aspects, such as denial of the condition and community stigmatization, impair better dissemination, disease awareness, education (i.e. carrier status and pre marriage counselling) and management. The diagnosis is established based on the recognition of the characteristic pattern of muscle weakness followed by direct mutation testing. If clinical picture is atypical, GNE mutations may be picked up by other genetic tests including NGS. Clinically, the onset is seen between 17 and 48 years old, with the mean at 30 years, however severity can vary even between close relatives and siblings harbouring the same genotype. Maya leads the GNE myopathy patient advocacy and awareness effort in Israel. Social care facilities cover

mobility devices, disability allowance, and special services. The Ministry of Health provides financial support for ankle foot orthosis (AFO) and mobility devices. National Health Insurance supports hydrotherapy, physiotherapy and specialist consultations (but in limited form). Zohar Argov added a comment that there is medical insurance support for genetic testing in Israel, before family planning in relevant communities. The patient population appears to be genetically homogeneous in terms of the GNE myopathy disease causing mutations (only 5 families carry other mutations), in contrast with other GNE myopathy populations worldwide, where multiple mutations have been observed and most patients are compound heterozygotes.

Ivailo Tournev presented the GNE myopathy cohort among the Roma people in Bulgaria. By 2018, there are 60 patients from Roma ethnical background in Bulgaria, of them 58 homozygous for the p.I618T founder mutation in the kinase domain [8]. This mutation was reported to be the second most common mutation among GNE patients in Western India (Rajasthan). The disease was found in the specific Roma group of Jerlii. In some affected families, a pseudo-dominant pattern of inheritance has been observed. The clinical presentation is often severe, with a classical, distal muscle involvement. Occurrence of first symptoms was in second decade in 20.5% of the patients; third decade in 67% and in fourth decade 12.5% of the patients. Three patients reported weakness of the distal muscles of the upper limbs as the initial complaint. They have a more benign course of myopathy, being still ambulant 12–20 years after clinical onset. During a longitudinal observation period (approximately 20 years), 20 out of 39 patients became wheelchair users. Some mild to moderate cardiac structure and rhythm abnormalities were observed, however, this is not consistent. Specially trained health mediators help to bring health care to the Roma community.

Zohar Argov led the discussion on standards of care, measures to avoid misdiagnosis and genetic testing issues. Draft standards of care were reviewed and comments added by the members of the consortium (Addendum 1). Non- classical features may be present in GNE myopathy patients such as early hand involvement, axial muscle involvement, or very late onset. Respiratory muscles are clinically not involved until advanced stages of the disease when patients become wheel chair dependent.

4. Current clinical trial readiness and further steps in its development

Hank Mansbach presented an overview of a 4 year long multicentre GNE myopathy natural history study. In the 2-year analysis, a total of 74 participants were included. There was no validated tool tailored for GNE myopathy and a GNE myopathy functional activity scale (GNEM-FAS) had to be developed and validated [9,10]. It was envisaged that this tool will be used in clinical trials to detect functional changes in muscle activity and will meet regulatory requirements. GNEM-FAS has three domains: mobility, upper extremity, and self-care. It was included as an outcome measure in the GNE myopathy disease monitoring program, Phase 2 and 3 of the SA-ER clinical trials [3,11]. Overall, the scale showed good test/ re-test reliability and was able capture minimal, yet clinically important and meaningful differences, and was able to discriminate subgroups of patients according to their muscle strength. The scale was approved by FDA and CHMP as an adequate measure to evaluate outcome measure in GNE myopathy. An expanded version of the GNEM-FAS is also available to better capture functional capabilities for less functioning or non-ambulatory patients. The consortium recommended to include GNEM-FAS in upcoming studies and registries whenever possible.

Nuria Carrillo presented interim results of the Natural History study of GNE myopathy conducted at the NIH. This ongoing study has recruited 55 patients, age 21 to 65 at baseline visit (mean age 39 years), 60% of them were female. Patients undergo 5-day inpatient evaluations at the NIH Clinical, and are followed prospectively. Multiple outcome measures are tested as part of this study, including measures of strength (manual muscle testing (MMT), quantitative muscle assessment (QMA), grip and pinch strength), functional measures (6-minute walk test (6MWT), Adult Myopathy Assessment Tool (AMAT), muscle MRI, and patient-reported outcomes (Inclusion Body Myositis Functional Rating Scale (IBMFRS) and the Human Activity Profile (HAP).

Since GNE myopathy is a slowly progressive disease, the group developed a disease progression model based on longitudinal QMA muscle strength data collected as part of the Natural History study which has been recently published [12]. This has allowed greater understanding of disease progression, including the onset and rate of decline of different muscle groups in GNE myopathy. QMA has been chosen as the primary endpoint for the multi-center efficacy trial of ManNAc ($n = 50$ provides statistical power of 87%), as other endpoints are less sensitive and require significantly higher sample size to power trials for GNE myopathy [12].

Ichizo Nishino presented a description of one of the largest known GNE myopathy cohorts and the natural history study conducted in Japan. Currently, the cohort accounts for 318 patients of 297 unrelated Japanese families. Main GNE mutations distributed as follows: 45% p.V603L, 25% p.D207V, 4% p.C44S. Interestingly, very few patients are p.D207V homozygous, which may indicate that this mutation causes a mild, nearly asymptomatic form of the disease. A one year prospective natural history study recruited 24 patients. The most common mutation in the cohort was p.V603L. The study assessed MMT, 6MWT, GMFM and FVC. It was observed that individual muscles decline at a different pace [13] with MMT (especially hand grip, shoulder extension, shoulder abduction) significantly deteriorating in ambulant patients and FVC in non- ambulant. A further follow up showed a significant decline in 6MWT and GMFM over 5-year period. Based on the natural history studies conducted in Japan and USA it is becoming more evident that a longer period of observation may be required to detect

a substantial decline in muscle strength in GNE myopathy patients.

Oksana Pogoryelova presented a meta-analysis based on published GNE myopathy cohorts. Systematic literature review identified 11 publications reporting in total 759 patients. The GNE myopathy registry was used as an additional source of patients' data [14]. The analysis showed a wide variability in age of the disease onset. FVC decline was observed only in non-ambulant patients. Cardiac structure and rhythm abnormalities were reported, but no evidences of association between cardiomyopathy and GNE myopathy could be assured. Statistical analysis of age at onset predicts that up to 20% of variability is explained by the GNE mutation itself. Mutations also influence probability of the preserved ambulation by age. For example, individuals harbouring p.D207V have an expected age of onset 8.0 years later than those with different mutations and probability of continued ambulation at age 40. In contrast, p.L539S results in onset on average 7.2 years earlier than those with other mutations. Proven and measurable effect of GNE mutations on the disease severity should be factored in patient management and clinical research study for a better data interpretation.

Madoka Mori-Yoshimura presented an overview of the REMUDY registry–a Japanese national registry comprising records of over 200 GNE myopathy patients. Near 200 doctors representing 129 hospitals entered the data in this registry and continue to update the information with follow up visits. It is a very good source of high quality data on a large and variable GNE myopathy cohort in one country. Based on this data, p.V603L is considered to be the most severe mutation [15].

Jean Yves Hogrel presented preliminary results of a 3-year natural history in GNE myopathy, which recruited 10 patients. Among them, three patients were non-ambulatory. In this study, the following functional parameters were analysed: manual muscle testing (MMT) of 23 individual muscles bilaterally, plus neck flexors and extensors, also grip (MyoGrip), pinch (MyoPinch) and MoviPlate [16]. In addition, muscle MRI was conducted (cross-sectional area (CSA), fat fraction (FF), and contractile CSA).

The mapping of muscle weakness was in accordance with what is generally described in GNE myopathy: a global symmetry between sides, a relative sparing of knee extensors and a severe wasting of tibialis anterior and toe extensors. The obtained map of muscle strength did not show any significant changes at 12 months, which confirms the slow evolution of the muscle disease. Nonetheless, a high variability between patients was noted. For instance, grip strength ranged between some hundred grams and 60 kg. Contractile CSA strongly correlates with strength. Changes in contractile CSA over 1 year were statistically significant in most of the muscles groups. Importantly, muscle strength strongly correlates in a non-linear way with muscle fat fraction detected by MRI. The data suggested that MRI is a clinically meaningful outcome for clinical trials. Other muscle functions such as elbow flexion or grip strength could also be promising, because corresponding muscles are moderately affected and would be more prone to improve during a therapeutic trial.

The discussion on future clinical research and need for data sharing was led by Tahseen Mozaffar and Hanns Lochmüller. It is evident that the spectrum of GNE myopathy severity is wide, current molecular and cohort based studies suggest that factors other than GNE genotype may influence the phenotype. Therefore, there is a need to identify such disease modifying factors. This is particularly important for consulting patients and for planning clinical trials.

Lale Welsh stated that NDF has funding available to sponsor the research tools (such as WGS), that will help to identify the genetic disease modifying factors. This project will require collaboration of many sites, which can provide samples and phenotype data for testing. Also, to ensure consistency in reporting information in different registries and clinical studies we have to ensure that we are using the same clear definition of non-ambulatory status.

Marjan Huizing noted that coherent reporting of new GNE variants and mutations is important for the future collaboration and updating mutation library, therefore it is required that all newly reported mutations need to comply with the standards.

Rüdiger Horstkorte and Stella Mitrani-Rosenbaum suggested that biochemical studies need to be conducted to measure the enzyme activity of mutated GNE p.V603L and p.D207V to test hypothesis, based on the cohort studies, that p.V603L and p.D207V predispose to a severe and mild phenotype respectively. Another mechanism that can cause variability in the disease severity may be the variability in compensatory mechanisms. From biochemical perspective, sialic acid synthesis and GNE functions are not yet fully understood. Moreover, GNE tolerance is different among different tissues and organs, which also can add to the disease variability. Non-genetic factors may potentially help to alleviate the symptoms or speed up the progression, such as environmental factors, diet, exercise and pregnancy. Further research is needed in this area to identify correlation between those factors and severity of GNE myopathy. Finally, similarly to many neuromuscular diseases, there is a need to find reliable biomarkers that would help to assess the efficacy of potential therapies early.

5. Translational research session

5.1. Lessons learned from AC-ER clinical trials

Hank Mansbach presented results and comparison analysis of the Phase 2 and 3 clinical trials of SA-ER. Phase 2 clinical trial recruited 47 patients and randomized them to a placebo of one of two active treatment groups (3 and 6 g of SA-ER).The study showed significance difference for the upper limb composite score (measured by hand held dynamometry) in patients on 6 g either compared with placebo or with 3 g treatment group [3]. Higher functioning patient subgroup (able to walk > 200 m at 6MWT at baseline) responded better to the treatment. A further extension study

of 12 g of SA-ER did not show additional benefit on muscle strength, but increases GI side effects. Phase 2 trial data provided grounds for selecting primary and secondary endpoints, inclusion criteria and sample size for the phase 3 trial. Based on the Phase 2 clinical trial results, FDA gave advice to do a Phase 3 study prior considering the approval. EMA considered an approval based on Phase 2 data but the application was not approved.

Phase 3 was a multicentre, multinational study conducted in 7 countries and included 89 patients in total with 1:1 randomization stratified by gender. Upper limb composite score and GNEM-FAS were selected as a primary and secondary end points, respectively. At the end of the trial neither primary nor secondary endpoints reached statistical significance [4] Overall, patients showed a moderate decline in muscle strength over 48 weeks. Muscle strength levels varied significantly between patients and in the same patient between study visits, this included minimal to moderate decline and modest improvement of muscle strength. There was no statistical difference in response to the treatment between patients from different countries, also higher functioning patients did not show any better response compared to lower functioning patients.

Phase 3 clinical trial failed to confirm the positive results showed in the previous study, but it raised important questions to consider in the future trials. GNE myopathy is considered to be a sialic acid deficiency condition, which justifies SA supplementation therapy to muscle cells. The latest research suggested that GNE has also functions outside sialic acid pathway, which may contribute to the muscle phenotype and not respond to the treatment. Therefore SA substitution alone maybe not be sufficient to significantly improve muscle weakness. Secondly, intra and interfamilial phenotype variability suggests that GNE mutations, along with other modifiers, affect muscle weakness and disease progression velocity to a different extent. This implies that either study cohort needs to be stratified by mutations and/or data analysis should account for population heterogeneity. Finally, GNE myopathy, as a slow progressing disease, might require clinical trials that last for more than one year, to show the effect of the medication.

To conclude, clinical trials conducted by Ultragenyx Pharmaceutical introduced a new GNE myopathy specific scale (GNEM-FAS) that can be used for future trials; they proved that a large international study is feasible in such a rare condition, developed GNE specific infrastructure and supported the international GNE registry.

5.2. ManNAc clinical trial progress update

ManNAc, another potential therapeutic option, it is an intermediate of the sialic acid biosynthesis pathway and the substrate of GNE. Nuria Carrillo presented Phase 1 and 2 clinical trial results of ManNAc and Phase 3 study design, which is in development by NIH and NeuroNEXT.

A Phase 1 first-in-human study established that single-doses of 3 and 6 g of ManNAc are safe and well-tolerated. ManNAc was well-absorbed and quickly excreted through kidneys in a similar way to SA. Pharmacokinetics shows sustained elevation of sialic acid in plasma for up to 48 h. A dose of 10 g resulted in gastrointestinal side effects (e.g. loose stool) likely due to unabsorbed ManNAc [17].

A Phase 2 trial open-label single-centre study, which included 12 patients, has been recently completed. ManNAc was given in two daily doses of 6 g (12 g in total) for up to 30 months. The objectives of this study were to determine long-term safety, multiple-dose pharmacokinetics and biochemical efficacy of ManNAc. Two MRI-guided muscle biopsies were obtained pre-dose and after 3 months of ManNAc administration and stained with lectins to quantify changes in sialylation after 3 months of treatment. Results of this study are being prepared for publication. A multi-centre efficacy study will be conducted through the NeuroNext network (https://neuronext.org/projects/nn109-magine).

Lale Welsh commented that some patients take products containing SA and ManNAc off label and may require guidance on whether they will be eligible to join the study and what it is the washout period.

5.3. Phase 2/3 clinical trial of sialic acid conducted in Japan

Madoka Mori-Yoshimura presented Phase 2 and 3 clinical trial of SA in Japan and some of the preliminary results. Largely following the Phase 3 trial protocol by Ultragenyx, twenty patients were recruited in the study, one of them discontinued participation due to the pregnancy. Patients were randomized to treatment and placebo groups. The groups were matched on functional levels, demographics and height. Serum SA levels significantly increased in the treatment group, similar to the levels reported by Ultragenyx in Phase 2 trial. Overall, UEC and LEC scores improved and reached statistical significance, along with more specific knee extension and shoulder abduction. Sit to stand test remained unchanged. A closer look at individual patient's data showed a high variability among patients in their subjective response to the therapy. One patient improved particularly well and regained an ability to open plastic bottles. Further analysis is required to confirm the results.

5.4. Gene therapy progress update

Stella Mitrani–Rosenbaum presented a review of gene therapy research in GNE myopathy. She suggested that the GNE protein has additional functions outside SA pathway, which contributes to muscle weakness. This implies that SA substitution alone might not be enough to significantly improve muscle weakness and actual GNE protein itself is required in the muscle cells. Therefore, gene therapy directed to muscle would be an attractive therapeutic option.

Levels of *GNE* expression vary and are very low in muscle cells, so even a small amount of GNE protein might be sufficient to improve muscle cell performance. The study

rational is to design a construct for systemic or muscle based gene therapy using Adeno-associated virus). Single vector administration was performed on healthy and symptomatic mice [18]. hGNE expression was detected in muscle cells and other tissues in both cases. Vector is expressed in mice and provides a long-term expression (>1 year) of hGNE at therapeutic levels. It was immunologically well tolerated. Phenotypically, female mice gained weight while male mice maintained the same weight. Testing gene therapy in GNE myopathy mice is complicated as the only known animal model does not fully replicate muscle weakness observed in humans but instead shows an early and severe kidney impairment. Therefore, the next step in testing gene therapy might require to proceeding to trials in humans bypassing the animal model stage. This route is associated with the potential dilemma that patients can receive one gene-therapy application only, unless they are immunosuppressed. Collaborative work on clinical trial design is in discussion.

6. Regulatory interactions and post marketing surveillance

Hanns Lochmüller discussed with the participants the importance of data sharing, reusing and re analysing available data. In rare disease area, pool of information is often small and formed from data obtained through patient registries, natural history studies and clinical trials. The quality of the data and accessibility varies significantly, with the clinical trial providing the most robust data but least accessible to the scientific community outside the study team. On contrary, patient registry provides information on the largest number of participants, which (de-identified data only) can be accessed by wide group of scientists (upon receiving all relevant approvals). However, registry data sometimes can be incomplete or criticised for subjective patient self-reported assessments. Data sharing and appropriate linking in a GDPR and GCP compliant way (other regulations might be relevant for a specific research) will improve efficient use of the available data and help to move forward translational research.

Researchers should be encouraged to publish all their data including negative trials. In anticipation of a new compound reaching the market in the foreseeable future, it is important to plan post-marketing data collection in a single database, which will allow longitudinal data collection on the drug safety and efficacy in a disease specific fashion as per regulatory agency (EMA) recommendation.

A subgroup was formed to work on the standard data collection set which will be recommended to use to collect phenotype and genotype data from individual patients in anonymous way contributing to and facilitating patient registries and clinical trials.

6.1. Health economic aspects of GNE

Erik Landfeldt presented a concept of evaluating costs and health-related quality of life associated with a rare neuromuscular condition. He has previously conducted health economics and outcomes research across a wide range of indications, including Duchenne muscular dystrophy. Erik described key elements of an observational survey study designed to evaluate the burden of illness of GNE myopathy. In particular, he discussed the relevance and importance of including costs beyond direct healthcare resource use (e.g., costs associated with informal care) to derive estimates reflecting the true burden of illness from the perspective of society. Additionally, Erik also discussed strengths and weaknesses with instruments designed to measure health-related quality of life in diseases such as GNE myopathy, and the importance of recording clinical data of sufficient granularity to facilitate stratification across stages, or manifestations, of the disease (which in the case of GNE myopathy ranges from barely symptomatic to severely debilitating).

6.2. Prerequisites of a successful GNE myopathy clinical trial

The discussion was led by Bjarne Udd, it was agreed that future GNE workshops will be dedicated to discussions of standards of initial and follow up clinical patient management; in addition there will be discussions of validated outcome measures (i.e. physio, MRI and serum biomarker based). Traditional 6MWT is considered not sensitive enough to capture changes in a slow progressing disease, therefore the NIH-proposed QMA is preferred. It was agreed, that trial duration would have to be at least 2 years, and potentially as long as 5 years. Patient stratification by degree of muscle weakness and mutation may also be beneficial. Muscle group of EURO-NMD should participate in these discussions. New therapeutic targets might emerge in the future based on the latest research discovering new functions of GNE protein. Burden of illness study is necessary to guide regulatory authorities and commercial companies during the approval stage.

7. GNE myopathy molecular pathway and biomarkers of disease progression

7.1. Biomarkers of the disease progression and effective therapy

Rüdiger Horstkorte presented an overview of GNE pathway and its enzymatic activity in normal and mutated forms. UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) is the key enzyme in the biosynthesis of sialic acids. The sialylation of proteins varies during development, increase in aging, and also in some degenerative diseases or myopathies. It is not currently known whether this increase is a sign of aging or a compensatory mechanism. Simple GNE monomers do not show enzymatic activity as it requires more complex structure such as dimers and tetramers to exhibit kinase and epimerase function.

Mutated GNE protein is known to have less enzymatic activity compared to a wild type. In addition, mutated GNE has shorter half-life and less soluble in the cytosol compared to mutated one. In case of M743T it is due to appearance of additional phosphorylation site which leads to M743T having a significantly higher O-GlcNAcylation level in comparison to the wild-type GNE. After removal of O-GlcNAc, M743T increased activity to the comparable level of the wild-type GNE. In addition, the half-life time of the M743T variant doubled compared to the wild-type GNE [19]. There is also a possibility that hypoglycosilation affects autophagic process and this results in accumulation of protein in vacuoles.

Marjan Huizing expanded the discussion about causes of hyposyalylation. There are several muscle sialylation markers, e.g. o-linked glycan sialylation revealed by lectin staining. Lectin staining could therefore be a marker of sialylation in clinical trials [20]. Tissues of choice could be muscle or skin samples (most informative for lectin staining), plasma/serum, blood cells (white blood cells are an easy to collect and store cell type that does not require culturing), or skin samples (for cell culture).

Andreas Roos further elaborated on molecular pathways affected in GNE myopathy. Proteomic profiling is a powerful tool to unravel the molecular aetiology of disorders such as neuromuscular diseases [21] and was applied to identify the protein signature in fibroblasts as well as muscle biopsy specimen derived from GNE patients. Results showed that vulnerable proteins are belonging to different pathways and cellular function such as cytoskeleton, platelet signalling and protein synthesis (RNA processing and translation) as well as protein quality control. An additional targeted proteomic approach (multiple reaction monitoring) utilizing GNE-patient derived blood samples allowed the identification of potential biomarkers with key roles in muscle fibre regeneration and oxygen transport.

7.2. WGS of GNE myopathy cohort: design and goals

Monkol Lek presented an outline of the new project aiming to screen genetically diagnosed GNE patients for other genetic variants, using WGS, that may explain phenotypic differences, size of the haplotype, provide rigorous characterisation of *GNE* mutations and improve overall clinical readiness in GNE myopathy. The project will be sponsored by NDF and is hoping to improve collaboration, data and information sharing between GNE research groups. Patient samples will be obtained through collaboration with major GNE researchers. It was suggested to include in the study non-manifesting carriers and siblings of the index cases. Patient stratification and analysis of subsets such as: patients with homozygous mutation, extreme cases (severe vs mild) and pedigrees with multiple affected members- was advised. Minimal clinical data set should be collected along with the samples. Phenotype data set will be similar to the data set proposed earlier in the meeting. Results of the projects will be fed back to patient organisations and rare disease platforms (e.g. RD-Connect).

7.3. New insights in sugar metabolism in GNE deficient cell models

Anke Willems presented sialic acid metabolite profiles in several cell and patient models. This showed depleted sialic acid and ManNAc in patient muscle tissue, but not in patient fibroblasts, stressing the importance of tissue-specificity in improving diagnostic measures. However, glycopeptide profiling in patient plasma has shown to be specific for diagnosing other sialic acid related diseases and could potentially be used for diagnostics and monitoring of drug efficacy in GNE myopathy as well.

There are other genetic diseases associated with the sialic acid pathway such as NANS and NPL deficiency. Mutations in NANS lead to skeletal dysplasia, disruption in brain development, but no muscle defects [22]. In contrast, mutations in NPL, a protein that catalyses conversion of SA to ManNAc, lead to proximal myopathy and dilated cardiomyopathy [23]. An NPL knock down zebrafish model shows muscle weakness, which can be rescued with ManNAc and to a lower extent SA. The study of two siblings with mutations in NLP shows that disruptions in sialic acid catabolism causes depletion of ManNAc and phenotypic features similar to GNE myopathy, such as muscle weakness. This supports the rational for ManNAc replacement therapy in patients [24].

8. Animal models in GNE myopathy

Ichizo Nishino presented an update on the GNE mouse model development followed by the discussion of future steps. Complete GNE knock out is lethal at early embryonic stages. Knock-in hGNE M743T mouse (on a pure C57BL/6 background) does not survive beyond day 3 due to severe kidney phenotype which can partially be rescued by ManNAc oral solution. Knock-in GNE M743T model on a 129 v/ICR mouse background did show the same kidney phenotype, but a subcolony was generated with a normal lifespan and no pathology in either kidney or muscle. hGNE V603L knock-in mouse has a milder phenotype and leads to a longer survival (on a mixed 129/Ola and C57BL/6 background) [25]. Transgenic hGNED207V-Tg (overexpressing hGNED207V cDNA on a Gne knock-down C57BL/6 background) shows a good expression of hGNE and phenotype with reduced survival, lower body weight, muscle weakness, muscle fibre size reduces with age when compared with litter mates. CK was increased, they have rimmed vacuoles, autophagic vacuoles in EM as well as protein aggregation. The mice responded partially to sialic acid supplementation [26]. LC3 deposits is higher in the mutated mice than the litter mates. More recent mice, based on the previously described strain, have less severe phenotypes than the originally described and may be due to copy-number overexpression of the GNED207V containing cDNA). Zebrafish model shows a very severe muscle weakness and a wide range of other developmental abnormalities, which leads to conclusion that GNE mutation tolerance is different among species.

Stella Mitrani-Rosenbaum talked about analysis of primary muscle cells obtained from patients with the founder Middle East mutation (M743T). It showed no consistent change in glycan profile and in the sialylation level of individual glycoconjugates between controls and GNE M743T cells. Further study showed that GNE protein interacts with alpha actinin 1 and 2 and that different GNE mutations affect GNE- actinin affinity to a different extent [27]. Since GNE is expressed and integrated at Z-disk and M-line and binds to alpha actinin it is plausible to suggest that GNE is part of the filament system. Other genes may also contribute to the muscle phenotype in GNE myopathy: transcriptomic and proteomic studies in Gne M743T knock in mice suggest to look closer in significant overlap between genes involved in kidney and muscle development for GNE myopathy disease modifying candidates.

9. Patient perspective

Lale Welsh, Mona Patel, Maya Davidovich and Mireille Hek talked about GNE patient groups and patient organisations in Europe and in USA. In 2018, GNE patient meetings took place in Los Angeles, Manchester and Tel Aviv. Meetings were sponsored and supported by NDF and Ultragenyx Pharmaceutical. They highlighted the need for a medical and scientific information about GNE myopathy to be written in lay terms and translated in multiple languages. Patient groups are willing to support GNE research and networking and would like to be informed of any scientific developments in GNE myopathy area. With help of NDF, Mona has raised funds, which will go towards future patient meeting and disease awareness campaigns. NDF raised a substantial amount of funding which will be invested in projects helping to launch gene therapy in GNE myopathy.

10. Summary of the meeting and action plan

Teresinha Evangelista summarised clinical and research aspects of GNE myopathy discussed during the meeting. Oksana Pogoryelova, Nuria Carrillo, Maya Davidovich revised the data set for phenotype data collection, which is recommended to use for registries, natural history study, clinical trials and new case reports. The data set is included as an Addendum 2 to this article. Anonymised data should be available for sharing with researchers in accordance with GDPR, GCP and other relevant data protection recommendations to improve understanding of the disease, its versatile presentation and epidemiology, and also to facilitate faster progress of translational research.

Following the extensive discussion of the past and current clinical trials, participants agreed that a search for new therapeutic targets in GNE myopathy should continue. It is recommended to include in the late stage trials about $n = 100$ of patients with proven bi-allelic GNE mutations and stratify them by muscle strength and GNE mutation. GNE clinical trials should last for 2 years or more. QMA and HHD are considered more suitable and sensitive than 6MW and therefore are more preferred as an outcome measure. It is recommended to use GNEM-FAS, developed specifically for GNE myopathy. MRI guided muscle biopsy, followed by lectin staining shown to be a reliable marker of muscle sialylation and therefore can be used as a marker of a new therapy efficacy.

Zohar Argov, Oksana Pogoryelova, Teresinha Evangelista, Madoka Mori-Yoshimura, Tahseen Mozaffar and Mona Patel presented an outline of proposed standards of care for patients with GNE myopathy. The document will be further developed and will include brief recommendations, based on personal clinical experience (expert opinion) and scientific publications. It will be distributed through neuromuscular ERN (copy of the document in Addendum 1).

A set of general recommendations to facilitate translational research has been agreed. There is a need to increase disease awareness and encourage funding for research in GNE myopathy. It is recommended to improve anonymised data sharing through suitable platforms, in compliance with the current data protection rules. Multi-omics approaches will increase our understanding of the disease modifying factors, this requires a large number of biological samples such as serum, plasma, whole blood, muscle biopsy, fibroblast. This is possible if biological samples are shared between research laboratories cross border. Patient consent forms should clearly state that their samples might be use laboratories in other countries.

10.1. Molecular research and animal models

Majan Huizing and Monkol Lek proposed a recommended format for reporting new GNE variants and mutations. To ensure comprehensive information is given, a case report should include the following: a. both mutations (or note for homozygous case); b. detail of the bioinformatics/ predictive tools used along with the pathogenicity score for a new mutation according to ACMG Guidelines [28]; c. details of further functional work to evaluate pathogenicity of the mutation if available; d. in case of distal phenotype and one GNE mutation, include, details of any other tests used to find a second GNE mutation (e.g. CNV, MLPA or WGS); e. in case of a new variant or mutation, parents and/or siblings should be sequenced if available; f. variant or mutation should be uploaded to a relevant database (e.g. www.databases.lovd.nl/shared/genes/GNE; or www.ncbi.nlm.nih.gov/clinvar.

Andreas Roos, Stella Mitrani-Rosenbaum and Majan Huizing summarised the discussion of future development in biomarkers research. They are working to narrow down a list of potential perspective candidates by comparing results of their research to identify potential markers for clinical and clinical trial use. Interception of proteomics and glycoproteomics may lead to new promising results. To allow effective collaboration and data analysis, sharing of –omics data through suitable platforms is recommended.

Ichizo Nishino and Stella Mitrani-Rosenbaum summarised current progress and future development is creating GNE myopathy animal models. They suggested discussing a set

of minimal requirements/endpoints to achieve in an animal model to consider it suitable for drug testing. GNE myopathy zebra-fish model appeared to be a good option to screen for new therapies/compounds. Another potential option could be Xenopus, which expresses GNE. However, it is important to search further for other options of an animal model that will show a close phenotype to GNE myopathy in humans. There is a longstanding need for a good GNE antibody. It was suggested to write a summary of previous attempts to create antibodies along with a suggested way to move forward.

According to patient advocacy groups and patient representatives, stem cell research in GNE myopathy is one of the most discussed topics among people with GNE myopathy. It was agreed to release a statement in lay language explaining that currently there is no scientific evidence to suggest that this approach can be successful in treating GNE myopathy, similar to other neuromuscular diseases. Patient organisations should popularise the statement about stem cell research in NMDs and address patient's questions.

11. Conclusions

(1) GNE Patients' advocacy groups have an impact on furthering research and therapy trials.
(2) Standards of care of GNE myopathy patients are currently based on expert opinion only.
(3) Natural history of GNE myopathy may differ between various mutations. Overall, in many patients the deterioration rate is relatively slow.
(4) There are currently only two validated outcome measures for GNE myopathy: GNEM-FAS and QMA.
(5) Clinical trial duration of one year (especially in early phases) is minimally required, 2–5 years is recommended.
(6) The pathophysiology of GNE myopathy is not fully known and lack of sialic acid (hyposialylation) is not the sole factor in muscle weakness development
(7) Consistent phenotype of myopathy in GNE defective mice is lacking, other models i.e. zebrafish maybe beneficial for early stages screening of new therapies.

12. Recommendations

(1) More collaboration of all advocacy groups and establishing new ones.
(2) More controlled research toward certain issues in SOC (pregnancy, diet, exercise).
(3) Continue natural history studies both through medically supervised research and through patients' reported registries.
(4) Economic burden of GNE myopathy should be assessed.
(5) Validation of biochemical and histologic markers for disease progression should be sought.
(6) Disease modifiers (genetic and environmental) should be sought.
(7) Mutation reporting should be standardized and GNE mutation databases should be verified by clinical description and family testing if available.
(8) Therapy trials relying on currently validated measures should be at least 2 years long.
(9) Genetic therapy research toward human trials should be hastened.
(10) Developing new animal models that can serve as tools for therapy (genetic and metabolic) assessment are urgently needed.
(11) Invest in the services of a medical writer in order to produce content that simply explains complex scientific information for non-scientific audiences, such as patients and carers.

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ENMC 237th Workshop Study group

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Supplementary materials

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