



HAL
open science

**219th ENMC International Workshop Titinopathies
International database of titin mutations and
phenotypes, Heemskerk, The Netherlands, 29 April–1
May 2016**

Peter Hackman, Bjarne Udd, Carsten Bönnemann, Ana Ferreiro

► **To cite this version:**

Peter Hackman, Bjarne Udd, Carsten Bönnemann, Ana Ferreiro. 219th ENMC International Workshop Titinopathies International database of titin mutations and phenotypes, Heemskerk, The Netherlands, 29 April–1 May 2016. *Neuromuscular Disorders*, 2017, 27 (4), pp.396-407. 10.1016/j.nmd.2017.01.009 . hal-02333059

HAL Id: hal-02333059

<https://hal.science/hal-02333059>

Submitted on 15 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: 219th ENMC International Workshop: Titinopathies – international database of titin mutations and phenotypes

Author: Peter Hackman, Bjarne Udd, Carsten G. Bönnemann, Ana Ferreiro, Titinopathy Database Consortium

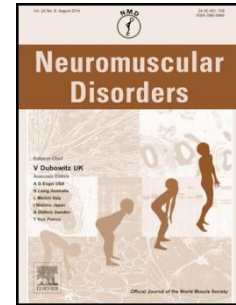
PII: S0960-8966(16)31166-X
DOI: <http://dx.doi.org/doi: 10.1016/j.nmd.2017.01.009>
Reference: NMD 3324

To appear in: *Neuromuscular Disorders*

Received date: 9-12-2016

Please cite this article as: Peter Hackman, Bjarne Udd, Carsten G. Bönnemann, Ana Ferreiro, Titinopathy Database Consortium, 219th ENMC International Workshop: Titinopathies – international database of titin mutations and phenotypes, *Neuromuscular Disorders* (2017), <http://dx.doi.org/doi: 10.1016/j.nmd.2017.01.009>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



**219th ENMC International Workshop: Titinopathies – international database
of *titin* mutations and phenotypes**

29 April – 1 May, 2016 Heemskerk, The Netherlands

Peter Hackman, Folkhälsan Institute of Genetics, University of Helsinki (Finland)

Bjarne Udd, Neuromuscular Research Center, Tampere University (Finland)

Carsten G. Bönnemann, NINDS/NIH (USA)

Ana Ferreira, Unité de Biologie Fonctionnelle et Adaptative, Université Paris Diderot/CNRS
(France) & Reference Center for Neuromuscular Disorders, Pitié-Salpêtrière Hospital, AP-HP
(France) on behalf of the Titinopathy database consortium*

* listed at the end of this report

Corresponding author:

Peter Hackman

Folkhälsan Institute of Genetics
Biomedicum Helsinki, Room C301b
Haartmaninkatu 8 (Pb 63)
00014 Helsinki
Finland

phone: +358-9-19125069

fax: +358-9-19125073

peter.hackman@helsinki.fi

Keywords: *TTN*, Titinopathies, International database

Highlights

- The workshop focused on reported and new emerging titinopathies.
- NGS have resulted in an exponential increase in identification of *TTN* variations.
- The aim was to establish an international database of *TTN* (*titin* gene) variations

1. Introduction

The 219th ENMC (European Neuromuscular Centre) workshop took place in Heemskerk, the Netherlands on the weekend 29 April – 1 May, 2016 and focused on titinopathies and the establishment of an international database of *TTN* variants and mutations with associated phenotypes. This ENMC workshop involved 16 clinicians and scientists from 6 countries as well as 2 patient and family representatives (<http://www.enmc.org/workshops/introduction/>). In this report, the state of the art and future directions related to the topics discussed during the meeting are summarized.

TTN mutations have to date been reported as the cause of various diseases collectively termed titinopathies, including a range of skeletal muscle and cardiac diseases, or a combination of both [1]. The following muscle diseases and phenotypes have been reported, with additional phenotypes emerging that are awaiting proper classification:

- Late-onset autosomal dominant tibial muscular dystrophy (TMD) (MIM #600334),
- Limb-girdle muscular dystrophy type 2J (LGMD2J; MIM #608807)
- Hereditary myopathy with early respiratory failure (HMERF; MIM #603689)
- Early-onset myopathy with fatal cardiomyopathy, EOMFC (MIM #611705)
- Congenital centronuclear myopathy (CNM) [2]
- Multi-minicore Disease with Heart Disease (MmD-HD) including clinical variations [3]

- Young adult onset recessive distal titinopathy [4]
- Early onset recessive Emery-Dreifus like without cardiomyopathy [5]
- Adult onset recessive proximal lower limb muscular dystrophy [6]

Due to its huge size, it has not been possible to routinely sequence *TTN* in research and diagnostic laboratories before the introduction of Next Generation Sequencing (NGS) methods, and thus, only a limited number of *TTN* mutations were identified. NGS approaches have enabled the rapid and thorough interrogation of genetic material and have resulted in an exponential increase in identification of both unequivocal mutations as well as, more frequently, variants of uncertain significance in the *TTN* gene. The last comprehensive review of *TTN* mutations associated with skeletal and cardiac muscle phenotypes included 127 *TTN* coding sequence variants [1, 7].

Numerous other *TTN* changes, particularly in patients with cardiomyopathies, have been published since then [8]. Published *TTN* mutations are recorded in the OMIM (<http://omim.org/entry/188840>), HGMD (<http://www.hgmd.cf.ac.uk/ac/index.php>), and/or LOVD (http://www.dmd.nl/nmdb/home.php?select_db=TTN) databases.

With its 364 coding exons (the first of *TTN* 365 exons being non-coding) and a full-length transcript of more than 100 kb, *TTN* encodes by far the longest known peptide in nature. The longest canonical isoform of *TTN* would produce a 3,960 kDa, 35,991 amino acids protein, although this isoform has not been confirmed experimentally. Titin protein functions as a molecular spring responsible for the structural integrity and passive elasticity of the muscle. Since *TTN* is a very central protein in the muscle sarcomere, it is expected that a much higher number of *TTN* mutations than is currently known will be involved in known and in so-far unreported human-striated muscle diseases, while the full extent of normal genetic variation in the gene will only become clear over time.

As titin emerges as a major cause of human disease, important questions are arising that need to be addressed. Virtually every individual in the general population carries rare *TTN* variants that are not necessarily associated with disease [1]. Not all rare *TTN* variants are pathogenic and a more profound understanding of phenotype-genotype correlations based on a comprehensive international titin specific database is needed. Along these lines, devising an experimental validation system for *TTN* variants is very important. Furthermore, understanding the molecular and pathophysiological basis of titinopathies is a mandatory first step to devise therapeutic approaches.

2. Aims of the meeting

The primary aim of this ENMC workshop was to establish an international database of *TTN* (*titin* gene) mutations, variations and associated clinical presentations, to be able to determine with more certainty if particular *TTN* variants are disease-causing.

The research community has responded to the challenges arising from the use of NGS through the formation of clinical and research consortia. These collaborations take advantage of the power of shared resources and expertise, and particularly the benefit of combining cohorts of patients into larger groups. This greatly increases the likelihood of success and also enhances the impact of these projects in terms of the clinically relevant data that is associated with them. There is, however, an urgent need to collect all reported, novel and rare *TTN* mutants and variants from patients all over the world and combine them into a single accessible database, for better comparison and more reliable genotype-phenotype correlations.

3. Session 1: Reported titinopathy phenotypes and genotypes

After welcoming remarks by Bjarne Udd and the ENMC Managing Director, the patient representative Sarah Foye opened the meeting by sharing several patient stories to convey patient perspectives.

Bjarne Udd then continued to describe already reported titinopathies. Tibial muscular dystrophy (TMD) was the first described human titinopathy. It is a mild adult-onset slowly progressive myopathy causing weakness and atrophy especially in the anterior compartment muscles of the lower leg, tibialis anterior, extensor hallucis longus, and extensor digitorum longus [9]. The disease is slowly progressive and clinical symptoms typically begin at age 35 to 55 years. TMD was first described in Finnish patients and the dominant founder mutation (FINmaj) was an 11bp insertion-deletion mutation exchanging 4 amino acids in the 364th and last exon MEX6 of titin (*TTN*), c.107780-107790 delAAGTAACATGG insTGAAAGAAAAA p.35927-35930 delinsValLysGluLys [10]. In addition to FINmaj several other mutations causing TMD in different European populations were reported in the last *TTN* exon, Mex6 of *TTN* [11-13]. Homozygosity of the FINmaj mutation causes a completely different limb-girdle muscular dystrophy phenotype, LGMD2J. It is a childhood onset disease causing proximal muscle weakness in the first or second decade and progresses over the next 20 years to wheelchair confinement [11, 14]. The prevalence of TMD in Finland is estimated to 1/2500 which means around 1000 patients due to the late onset, of which 500-600 have been diagnosed.

Some European patients proved to be compound heterozygotes with novel titin frameshift mutations, or novel missense mutation combined with previously reported mutations. Some showed an LGMD phenotype, some had earlier onset more severe distal myopathy, or a completely different adult proximal lower limb phenotype [6]. This expanded the complexity of muscular dystrophies caused by *TTN* mutations and suggested that the coexistence of second mutations explained the phenotype variability. [6, 11, 15, 16].

With targeted next-generation sequencing of myopathy related genes on seven families from Albania, Bosnia, Iran, Tunisia, Belgium and Spain with juvenile or early adult onset recessive distal myopathy, novel mutations in *TTN* Mex5, Mex6 and A-band exon 340 were identified. They were homozygous or in compound heterozygosity with frameshift or nonsense mutations in the *TTN* I- or A band region on the other allele. One nonsense p.Q35879ter in the second last exon Mex5 was common for Albanian, Bosnian and Iranian patients suggesting a Balkan-Middle East founder. Family members having only one of these *TTN* mutations were healthy. RT-PCR results suggested that the frameshift and nonsense mutations located upstream of the last exons of *TTN* caused degradation of mRNA through nonsense-mediated decay, and that the other mutated *TTN* allele was predominantly expressed. These results add yet another entity to the list of distal myopathies: juvenile or early adult onset recessive distal titinopathy [4].

Hereditary myopathy with early respiratory failure (HMERF) is a slowly progressive, proximal and distal, usually autosomal dominant myopathy that typically begins in the third to fifth decades of life. The histopathology is characterized by ‘necklace’ cytoplasmic bodies and myofibrillar changes. HMERF is associated with pathogenic mutations in exon344 encoding the 119th fibronectin-3 domain of titin [17-19], which establishes this exon as the primary target for molecular diagnosis of HMERF. The relatively large number of new families and mutations directly implies that HMERF is not extremely rare, not restricted to Northern Europe and should be considered in undetermined myogenic respiratory failure of undetermined origin, particularly in ambulant patients [20, 21].

Ana Ferreiro continued the theme, reporting on already described titinopathies. Nine homozygous or compound heterozygous *TTN* mutations have been identified in six families presenting with an autosomal recessive, congenital form of skeletal myopathy associated with

various forms of primary heart disease. The condition was first reported clinically in a consanguineous Sudanese family [22, 23] as a form of congenital muscular dystrophy with childhood-onset, dilated cardiomyopathy (DCM) termed Salih myopathy (MIM #611705) or early-onset myopathy with fatal cardiomyopathy, EOMFC [24]. After recent characterization of additional families, the range of phenotypical signs of the disease has been expanded to include antenatal defects and other forms of heart disease. The inclusive term of Multi-minicore Disease with heart disease (MmD-HD) has been proposed to span all these phenotypes including arthrogryposis multiplex congenital (AMC), an Emery–Dreifuss muscular dystrophy (EDMD)-like phenotype [3]. Most patients are ambulant, present with axial and proximal muscle weakness and develop spinal rigidity, joint contractures, scoliosis and respiratory insufficiency of variable severity, mild DCM, left-ventricular non-compaction (LVNC), or atrial and ventricular septal defects. Histologically, patient biopsies show minicores together with other changes in the fiber structure (star-shaped basophilic areas, internalized nuclei, mild endomysial fibrosis in some cases). Calpain 3 deficiency was identified in patients homozygous for truncating mutations in *TTN* exons encoding the M-line C-terminus. The study identified seven novel homozygous or compound heterozygous *TTN* mutations in five additional patients [3]. All the parents of this cohort (aged 38–55 years) were heterozygous and had no neuromuscular or cardiac abnormalities. Five of the mutations were truncating variants that are either homozygous or compound heterozygous with a second truncating or missense mutation in the affected patients. Interestingly, a new-born presented with multiple joint contractures (arthrogryposis multiplex congenital), LVNC, and heart failure requiring heart transplantation at the age of 5 years, which represents the most severe and the first antenatal titinopathy reported so far. She was found to be compound heterozygous for an I-band truncating mutation [c.9163+1G>C, p.Glu2989Glufs*4] and a missense mutation within the enzymatic site of the *TTN* kinase domain (TK); c.102214T>C, p.Trp34072Arg]. Additional ex vivo and in vitro studies showed that this represents the first

reported absence of a functional TK mutations in humans and suggested a role of TK in myocardial development and function [3].

Alan Beggs then reported on congenital centronuclear myopathy (CNM) titinopathies identified through a Boston, USA cohort. Centronuclear myopathies are a genetically heterogeneous group of congenital myopathies sharing prominent central myonuclei as a primary histopathological feature. Mutations in *MTM1*, *DNM2*, *BINI* or *RYR1* are the most well recognized and studied forms of CNM [15, 25-28]. *TTN*-associated CNM is a congenital myopathy that typically presents in infancy or childhood with generalized muscle weakness and wasting. Severe cases can have respiratory failure, while heart function is typically normal. While CNMs are characterized and were subsequently named after the presence of numerous muscle fibres with centrally localized nuclei, as opposed to their normal peripheral/subsarcolemmal position [29, 30], the *TTN*-associated forms are often distinct in having multiple internal nuclei throughout the myofibers, as opposed to a single line of centrally placed nuclei down the centre of the cells.

A recent study identified 12 autosomal-recessive *TTN* mutations of the *TTN* gene in five individuals presenting with infantile muscle weakness [2]. They had no overt cardiac involvement, although considering their young age (5–19 years), a risk of later-onset heart disease cannot be excluded. Their muscle biopsies showed several abnormalities in the internal muscle fibre architecture including multimimicres, target-like areas, and internal nuclei; because the latter were highly abundant and conspicuous, the patients were diagnosed as having CNM by clinical neuropathology services, although their histological pattern was virtually identical to that observed in patients with multimimicore disease with heart disease (MmD-HD), previously associated with *TTN* mutations as described above [2, 24]. The “CNM” *TTN* variants identified include 10 truncating and two in-frame insertions/deletions, all present in the compound heterozygous state in the affected patients; two patients carried three of these changes each. Biochemical analyses

suggested a reduction in the levels of calpain3/p94 and nebulin. One patient's mother, heterozygous for the (c.44816-1G>A, p.) mutation, was found to have a mild subclinical cardiac and skeletal myopathy at the age of 44 [2]. All the other heterozygous parents were currently healthy, including one who carried two in-frame indels in the same allele, confirming that not all *TTN* truncating mutations cause disease in the heterozygous state.

Beggs also described the results from a recent screen of 87 cases of genetically undiagnosed congenital myopathy using an NGS-based panel assay that queried 43 known neuromuscular disease genes including *TTN*. Six probands were identified to carry one or two heterozygous truncating variants in *TTN*. Most carried only one truncating variant together with multiple missense variants of uncertain significance. Although some of these missense changes may be disease causing, given the complexity of the gene and unknown sensitivity of the sequencing assay, it remains possible that many or all of these individuals carry second pathogenic truncating variants that have yet to be identified. Use of VisCap, a software program for inference and visualization of germ-line copy-number variants through analysis of relative NGS read depths [31], identified one additional proband with a large intragenic deletion encompassing at least exons 34-152. All seven probands were ambulant but presented with skeletal myopathy and were alive at ages ranging from 4 to 49 years. Three had histopathological features consistent with MmD, three were considered undefined myopathies, and one had a congenital fiber-type disproportion.

Isabelle Richard reported the identification of a recessive titinopathy phenotype presenting as a childhood-juvenile onset Emery-Dreifuss-like phenotype without cardiomyopathy caused by novel truncating mutations in the C-ter part of titin in 3 unrelated families [5]. Patient 1 was a man born from a consanguineous Algerian family with asymptomatic sibs and parents. Patient 2 is a woman from a non-consanguineous French Caucasian family with no other affected sibs. Patient 3 is the

only affected member of a non-consanguineous French Caucasian family. The patients shared common phenotypic features that include 1.) coexistence of both limb-girdle weakness and early-onset contractures, preceding or accompanying initial weakness; 2.) normal neonatal period, proximal weakness in infancy-childhood, and progressive course in adolescence and adulthood, with permanent loss of ambulation from age 13 to 36 years; 3.) unaffected facial, bulbar, and oculomotor muscles; 4.) high CK levels, decreasing in the late stages of the disease; and 5.) no identified cardiomyopathy to date. Finally, muscle biopsies showed rimmed vacuoles, increase of internal nuclei, cytoplasmic bodies, and dystrophic pattern. A key feature was the clear secondary reduction of calpain 3 on WB that was qualified as being likely secondary since no mutations in the calpain 3 gene exons were identified.

Targeted DNA capture focused on an 820 gene panel related to muscular function and high-throughput sequencing of DNA sample of patient 1 were performed and a novel homozygous nonsense mutation in the exon Mex3 of the *TTN* gene was identified. Interestingly, truncating mutations were identified in both alleles in the same region of the *TTN* gene in patients from 2 additional families. Sanger sequencing of the M-line titin identified 2 truncating mutations in patient 2: one single base deletion at the end of Mex1 (Chr2: 179395292; c.106275delT; p.E35351Nfs*54) and one nonsense mutation in Mex3 (Chr2: 179393500; c.107203C>T; p.Q35660*) and 2 truncating mutations in patient 3: a 5-base deletion at the end of Mex1 (Chr2: 179395428-432; c.106135_106139delACCTG; p.T35304Cfs*3). Segregation of the mutations with the disease were confirmed in all families. Molecular protein analyses confirmed loss of the C-ter part of titin.

Heinz Jungbluth, London, UK, presented on *TTN*-related myopathies in the context of the congenital myopathies. The congenital myopathies – Central Core Disease (CCD), Multi-Minicore Disease (MmD), Centronuclear Myopathy (CNM), Nemaline Myopathy (NM) and Congenital Fibre Type Disproportion (CFTD) - are a genetically heterogeneous group of conditions

characterized by defined histopathological features on muscle biopsy [32, 33]. Almost 20 different genes have been identified to date, encoding proteins with important roles in calcium homeostasis, excitation-contraction coupling, thin filament interaction and assembly, intracellular membrane trafficking, antioxidant defence and autophagy. Whilst some of these genes (for example the *MTMI* gene encoding myotubularin) have been implicated in distinct clinico-pathological phenotypes, others such as the *RYRI* gene have been associated with an extremely wide range of clinical and pathological features. *TTN* belongs firmly within the second group of genes.

Surprisingly considering the crucial role of titin for normal sarcomeric assembly and function, nemaline rods have so far not been reported as a prominent feature in *TTN*-related myopathies. Whilst there appear to be emerging genotype-phenotype correlations with regards to the degree of cardiac involvement, considerable overlap of clinical features and common occurrence of certain histopathological findings (for example, internal nuclei and cores) suggests that *TTN*-related congenital myopathies represent part of a continuous phenotypical spectrum rather than distinct entities. In contrast to other genetic backgrounds, extraocular muscle involvement has not been reported in *TTN*-related centronuclear and core myopathies. Vice versa primary cardiac involvement, common in the *TTN*-related forms, is not a feature in *RYRI*- and *SEPNI*-related CCD and MmD but may be prominent in association with *MYH7* mutations. Although scans from larger patient series will have to be analysed, as in *RYRI*-related congenital myopathies, muscle MRI findings appear to show greater consistency in titinopathies than clinical and histopathological features, characterized by early involvement of the posterior thigh (in particular semitendinosus) and predominant involvement of soleus and peroneal group in the lower leg, followed by (often patchy or “rimmed”) involvement of the rectus femoris and vasti in the anterior thigh in more advanced cases.

4.1 Session 2: Unreported titinopathies

Ana Ferreiro presented 7 unreported families with autosomal recessive MmD and probably pathogenic *TTN* variants, 5 of which had heart disease (MmD-HD). Patients were homozygous for missense variants (2 families) or compound heterozygous for two truncating for one truncating and one missense variant (3 families). All parents were healthy. Axial involvement was constant, with neck flexor weakness and rigid spine. Proximal weakness was sometimes more marked in upper than in lower limbs. Several patients had relative hypertrophy of the lower limbs calves compared with the upper limbs. Joint contractures developed in the first decade and coexisted with joint hyperlaxity (shoulders, fingers). This underlines the fact that congenital or infantile titinopathies should be considered in the differential diagnosis with collagenopathies, especially in young children without detectable heart disease. Some patients had ptosis, but none had ophthalmoplegia. Cardiomyopathy was identified by echocardiography from ages between 3 and 48 years, leading to premature death of 3 patients.

Carsten Bönnemann presented the NIH series of early onset recessive Titinopathy. This series currently encompasses 14 patients from 12 families with truncating/splice mutations on both alleles, 6 patients from 6 families with a truncating/splice mutation on one allele and putatively damaging missense mutation on the other allele, and 4 patients from 2 families with titin compatible disease and a truncating/splice mutation on one allele and a “missing second allele”. Clinical analysis of this series confirms the wide spectrum of possible clinical manifestations and severities, while there are certain elements in the clinical presentation that can be seen in various constellations in the patients and are elements of the “clinical Gestalt” of early onset titinopathy. There frequently is an axial presentation with neck weakness, rigid spine and scoliosis, as well as progressive contractures of elbows and Achilles tendons, in some aspects reminiscent of Emery Dreifuss and the collagen VI disorders. Weakness may be more pronounced in the upper than the lower extremity, while profound facial weakness would be unusual, and there is no external ophthalmoparesis. These features include presence of distal weakness and contractures (including

congenital arthrogryposis), as well as joint laxity in some patients. The degree of weakness may be such as to preclude independent ambulation, although ambulation was achieved in the majority of patients in the series. Cardiac involvement maybe early in the form of non-compaction cardiomyopathy of later and progressive as dilated cardiomyopathy, not correlating with the degree of extremity muscle weakness. The histological spectrum encompasses fibre type disproportion, internalized nuclei, core like lesions of various kinds, inclusions and a dystrophic appearance. Muscle MRI is variable, but relatively more severe involvement of the semitendinosus muscle in the hamstring group is seen in the majority of patients. Thus, even though there are many unanswered questions pertaining to how a given genotype relates to phenotype/histotype and physiological (physiotype) consequences, there is a titinopathy “Gestalt” emerging that can be helpful in the diagnostic assessment and putative weighing of in particular missense mutations that are classified as "uncertain significance" and that will be the starting point for further genotype driven sub classification if justified by the emerging data. Carsten Bonnemann commented that when diagnosing titinopathies clinicians need to consider genetic information (“genotype”), physical features (“phenotype”), microscopic lesions of the muscle cells (“histotype”) and functional defects (“physiotype”).

4.2 Italian and European NGS-titin patients,

Marco Savarese described a large Italian NGS-screening of more than 1000 myopathic patients which showed about 1% of patients with a putative titinopathy. However it also implicated the need for a careful approach in the interpretation of titin data, confirming the importance of mRNA and/or protein studies.

Using MotorPlex, a custom NGS platform, it was possible to investigate well known and putative disease genes responsible for skeletal muscle disorders [34]. MotorPlex identified probably causative mutations in about 43% of patients tested [35].

In one Italian patient, an already described HMERF variant (c.95134 T>C), was identified. In a Belgian patient, a mutation already described in another Belgian family with dominant tibial muscular dystrophy was identified. However, the patient also carried a second nonsense variant on the second allele, which explained the more severe phenotype observed, suggesting that a critical evaluation of the clinical phenotype and of the molecular findings is always needed, also in presence of a well-known causative mutation.

Several patients had putative protein truncating variants (PTVs) in the titin gene. However, the identification of heterozygous PTVs is not sufficient to make a diagnosis of titinopathy, and complete clinical characterization and a muscular biopsy for a WB analysis/mRNA analysis are always needed.

Finally, even in informative families, the interpretation of rare missense variants is very difficult. *In silico* predictions can be misleading and segregation analysis can only support or exclude the pathogenic effect. Further proofs can only be through functional tests (not easily available at this moment) or by other patients/families with the same mutations and a comparable phenotype.

In this same session **Teresinha Evangelista** presented genetic and clinical data from the John Walton Muscular Dystrophy Research Centre (JWMDRC), Newcastle cohort of patients with TTN mutations.

The autosomal dominant mutation p.C30071R in the 119th fibronectin domain of titin remains the most frequent in the JWMDRC cohort. Patients harbouring the p.C30071R mutation have a characteristic phenotype of hereditary myopathy with early respiratory failure (HMERF).

In a total of 178 undiagnosed patients studied by whole exome sequencing (WES) 183 rare *TTN* variants were found. 45/178 had another final diagnosis than titinopathy and none of these had

were truncating variants. Of the 116 missense variants found in this cohort, 33 were present in the group of patients diagnosed with other conditions (Table 1). 100 were very rare (frequency < 0.01%) and 42 (including 10 identified in cases diagnosed with conditions other than a titinopathy) predicted damaging by 3 *in silico* prediction tools, stressing the difficulties of interpreting TTN variants. 11 had *TTN* variants considered as disease causative (Table 2). In all cases either 2 truncated mutations or one truncated mutation associated with a missense one were found. Disease onset varied from the neonatal period to the 6th decade. Patients presented with both proximal and distal weakness and in 50% with rigid spine. The neonatal onset case developed a severe cardiomyopathy and respiratory insufficiency with the need for cardiac transplant and tracheostomy respectively. Two other patients presented with atrial fibrillation. Four patients studied had a reduction in calpain 3 on the muscle biopsy by western-blot. In these 4 patients the mutations were located in the A-band region of titin.

Three cases had a more detailed description. One with a phenotype corresponding to LGMD2J with tight Achilles tendons, but with mild cardiac involvement, and a high CK level of 5764 IU/L. The second patient had a Multicore Myopathy phenotype, with onset in childhood, respiratory insufficiency, generalised weakness and scoliosis and a muscle biopsy with cores. The third family presented an Emery-Dreifuss phenotype with generalised weakness, rigid spine and contractures (Achilles tendons, elbow flexion contractures, long finger flexors, hips and knees contractures) without cardiac involvement

4.3 Jelena Nikodinovic Glumac reported that through whole exome sequencing a novel stop gained mutation (c.107635N>T, p.Gln35879Ter) in the second last exon *Mex5* (the same as mentioned above), was identified in 14 patients with distal myopathy and Serbian ancestry. Three patients were homozygotes for this mutation and 11 were compound heterozygotes. They shared a common core haplotype indicating a founder allele. This variant was absent from a control group of Serbian extraction (n=103). In compound heterozygotes, nine other *TTN* mutations were

identified, including four stop gained, three frameshift, one missense and one splice donor. The data supported an autosomal recessive mode of inheritance. Age of onset varied from 14 to 40 years, and most of the patients remained ambulant throughout the observation period. The phenotype was fairly uniform, with predominant lower limb involvement and prominent weakness of distal muscles, especially the ankle and toe dorsiflexors. Distal arm muscles were not affected, but mild scapular winging and mild shoulder girdle weakness was present in half of the patients. There was no facial, bulbar weakness or respiratory involvement, and only one patient had a cardiomyopathy. CK levels were normal to mildly elevated. Muscle MRI (n=6) and muscle biopsy (n=6) findings were compatible with a recessive distal titinopathy. The patients with homozygous mutations did not show significant clinical differences compared to the compound heterozygous patients. The Serbian *TTN* founder mutation explains a sizable portion of distal myopathy patients in this region and may represent the most common single cause of distal myopathies in Serbian population. **Mark Davis** from Australia reported on a new congenital titinopathy collection on behalf of Team-titin. In a large international cohort of 27 individuals from 24 families recessively inherited nonsense, frameshift and/or ESS (Enhancer Splicing Site mutations) mutations were identified in *TTN*. Analysis of the clinical and pathological features of the patients confirmed marked prenatal, congenital or infantile onset weakness often associated with congenital contractures and fractures. Twenty nine core clinical features present in 50% or more cases were identified, the most clinically significant being that over 80% of patients had progressive weakness of axial and respiratory muscles resulting in scoliosis, chest deformities and/or respiratory insufficiency. Thirty seven percent of patients had congenital and/or early-onset cardiac abnormalities including cardiomyopathy. Despite significant muscle involvement, 67% of patients achieved and maintained independent ambulation. Ophthalmoplegia and focal muscle hypertrophy were universally absent. CK levels were normal or mildly elevated. Muscle biopsies typically showed increased internal nuclei (most commonly), cores (predominantly minicores), CFTD, and/or additional features such as caps, rods, ring fibres etc. Seven cohort members had

one mutation within an exon not encoded by the canonical skeletal muscle isoform. The clinical features in these individuals were not significantly different to other cohort members.

Ferreiro also discussed the potential therapeutic approaches for TTN-related myopathies.

Currently only supportive treatments are available, including assisted ventilation, scoliosis surgery and/or heart transplantation when necessary. Gene therapy approaches are complicated by the massive size of the gene and the scarce functional data concerning the different protein isoforms. Some of the pathways known to be implicated in the pathophysiology of titinopathies are drug-targetable. However, further studies are needed to differentiate pathogenic from compensatory alterations and to identify quantifiable and reliable biomechanical, biochemical, cellular and clinical parameters (readouts) to measure drug effects.

5.1 Session 3: NGS methods and bioinformatic tools for detection and prediction of TTN variants

In session 3 **Peter Hackman** reported on the targeted NGS MYOcap assay for detecting titin mutations in research and diagnostics. In patients with less distinct phenotype several genes may have to be sequenced in order to identify the correct diagnosis. The large number of possible candidate genes, overlapping phenotypes as well as an enormous size of some of the genes e.g. *TTN* and *NEB* have been challenging for molecular genetic diagnostics. Molecular characterization is nevertheless important for the final diagnosis and accurate management of the diseases. Targeted next-generation sequencing (TNGS) is an efficient and cost-effective method to sequence larger number of genes simultaneously.

A targeted NGS panel MYOcap for the coding exons and UTRs of initially 180 myopathy related genes was designed, with a total size of 1.3 Mb [36]. Sequencing was performed using Illumina

HiSeq1500 with a sequencing depth of 100X. In a validation study DNA samples of four controls with known mutations and 66 patients negative for previous candidate gene approaches were sequenced. A definite diagnosis based on disease-causing mutations was obtained directly in 10 patients and probable disease-causing mutations were found in another 10 patients. Ten patients had potential disease-causing *TTN* mutations with previously undescribed phenotypes. The MYOcap panel has since been updated and the version MYOcap3 contains the coding region of 265 genes and UTRs. By April 2016 more than 900 samples had been sequenced on Myocap. Putative truncating *TTN* variants (fs, nonsense, acceptor/donator splice site variants) gave 64 calls (59 variants) in 46 patients. There were 184 calls (40 variants) for missense mutations in M-band *TTN* in 151 patients and for *HMERF*: 14 calls (9 variants) in 14 patients in exon 344. It is still a challenge to detect some changes, like repeat expansion and copy number variations (CNV). However MYOcap is currently an effective tool for having a genetic diagnosis in neuromuscular disorders and can also be used to discover new phenotype-genotype correlations.

5.2 Session 3 Motorplex for detection of *TTN* mutations,

Marco Savarese reported that their group had included a core panel of 93 genes of non-syndromic muscle disorders in a custom enrichment kit for NGS (MotorPlex). MotorPlex captures at least 99.2% of 2,544 exons with a very accurate and uniform coverage. This quality is highlighted by the discovery of 20-30% more variations in comparison with whole exome sequencing [34]. The panel has been expanded to 199 muscle disease genes causing overlapping phenotypes. They studied nearly 1400 unresolved cases of myopathies for which the best candidate genes were previously excluded. 85% of the patients were Italian. Most patients were affected by either LGMD (51.2%) or Centronuclear myopathy (CM) (32.5%).

Focusing on 784 patients, 1554 variants in titin were identified, most of them rare and ultra-rare (MAF<0.00001) missense variants. Truncating variants were identified in an average of 1/18 patients. Duo and trio analysis is a straightforward approach that also can contribute to the identification of titin haplotypes as well as of probably harmless missense variants. Thus titin is a strong candidate gene in several unsolved cases of myopathy.

5.3 Session 3 Targeted NGS using Ion Torrent/Proton,

Mark Davis described the design of an all-of-neurogenetic disease gene capture panel in 2012, with a total of 336 genes across the range of phenotypes. Due to unexpected demand, the panel was reiterated in 2014 and expanded to 464 genes [37]. The probes were sourced from Life Technologies (TargetSeq), with the sequencing carried out on an Ion Proton. Aligning and variant calling were done via the supplied Torrent Suite software, with variant filtering done using commercial software (Cartagenia). Average coverage was around 250x, with 93-95% to 20x. The myopathy cohort consisted of 227 patients, with mutations being identified in 68 (30%). Mutations in TTN were the most common finding (13/68 – 19%). Two cases had known HMERF mutations, 11 were recessive congenital myopathies. Of the 24 alleles, 6 mutations were in exons not known to be included in the skeletal muscle isoform (5/13 patients). Variants in the triplicated repetitive region of titin may not be called, but may be detected by visual inspection of the BAM files. Interpretation of missense changes continues to be problematic, and in 3 cases only a single truncating mutation was identified in conjunction with multiple rare or private missense variants. Version 3 of the panel is currently under construction, with another ~100 recently described disease genes added, and via Illumina rather than Life Technologies.

5.4 Session 3 Targeted NGS (Myodiag), exome and genome (Myocapture) sequencing for detecting titin mutation

Jocelyn Laporte reported on two NGS approaches aiming to identify mutations and genes linked to myopathies and both based on Agilent enrichment kit and Illumina sequencing technologies [38, 39]. Myodiag aims for genetic diagnosis, consisted in the targeted sequencing of 142 genes (current version 220 genes) implicated in muscle disease, and tested a heterogeneous group of 130 novel patients not previously investigated [40]. Myocapture is a national network aiming to identify novel myopathy genes via exome and genome sequencing in homogeneous and well characterized cohorts of patients previously excluded for the main known genes (1045 exomes and genomes done from 565 families). Success rate for mutation identification ranged from 34 to 47% depending on the myopathy cohort for both NGS approaches. *TTN* was the most represented gene. Bioinformatic ranking tools (VaRank, CMRank) and an integrated diagnosis confronting genetic, clinical, histological and imaging data were performed. Comparison with an in-house exome database from non myopathic individuals suggested an enrichment in loss-of-function and number of variants in *TTN* in the myopathy cohort. Overall, looking for recessive inheritance, 4 families had compound heterozygous *TTN* truncations and 22 had one heterozygous truncation with another variant (generally missense). *TTN*-linked phenotypes ranged from muscular dystrophy, core or multi-minicore myopathy, centronuclear-like myopathy, arthrogryposis, or unclassified congenital myopathy, strongly suggesting *TTN*-related myopathies represent part of a continuous phenotypical spectrum rather than distinct entities.

6. Session 4 Titin molecular biology, functional aspects and mutational changes *in vivo*,

6.1 Titin: Structure, isoforms and relevance of animal models

Mathias Gautel described signalling in the sarcomeric Z-disc and the M-band region, which seems to be a hotspot for mutations leading to myopathies [41, 42]. He then discussed the high number of rare TTN variants found by NGS and the available prediction programs used to determine pathogenicity that seem to work poorly for titin mutations and are not really reliable in this setting. For example, polyPhen, SIFT and PROVEAN frequently mis-classify missense mutations. Known pathogenic mutations are found in 1000genomes, e.g. the recessive titin kinase (TK) domain variant Trp34072Arg. There are additionally more than 20 predicted disruptive TK variants in 1000genomes. Structural modelling is not trivial for many titin domains and e.g. the I-TASSER (Iterative Threading ASSEMBLY Refinement) structure prediction software often fails in accurately predicting even key side chain positions and whether an amino acid residue is buried or solvent exposed in the Ig/Fn3- beta-barrel-domains beta-strands. Similarly, polyPhen, SIFT and PROVEAN frequently mis-classify missense mutations. This was shown in experimental examples of recently solved titin fibronectin domain structures from his lab, where existing structure prediction programs like iTASSER and the variant classification programs polyPhen, SIFT and PROVEAN failed to accurately predict the mutation impact. While for many titin domains, measuring their mechanical stability would be an important measure of their biological function, single-molecule force spectroscopy is unlikely to become a tool available outside specialist laboratories. Other biophysical studies like DSF (Differential scanning Fluorimetry), ITC (isothermal titration calorimetry), and CD (circular dichroism spectroscopy) are more generally available and reliable, and thermal denaturation studies can be applied to mutations in titin domains [43]. These methods measure stability and structural changes caused by mutations and hence can predict the degree of pathogenicity, for which he gave some examples of measurable changes. Based on biophysical studies on validated pathogenic mutations from the Ferreiro and Jungbluth groups, a measurable change of thermal stability of >20 °C or total loss of folding is generally indicative of a pathogenic mutation, while a <10 °C change would not be a necessary indication of pathogenicity, unless it is significantly impacted on relevant protein-protein

interactions. Since these changes can lead to temperature sensitivity, it was discussed whether fever may trigger or exacerbate the cardiomyopathy phenotype. A new webtool database, TITINdb, was developed at King's College London that incorporates new experimental structures, biophysical evidence of pathogenicity and links to existing variant databases. It was discussed that TITINdb will be linked to the webtool database TitinViewer developed by I. Richard's team. Possible collaboration was discussed to test *TTN* variants found in other groups by these methods. However, changes in protein-protein interactions, in contrast to stability, cannot be determined unless the relevant interactor is known. The pathogenicity of variants in some regions is therefore easier to predict than in others, especially in regions with substantial fundamental data on protein interactions and supporting experimental structural information on protein-protein interfaces. This highlights the need to invest in fundamental research to understand key missing components of the titin interactome functionally and structurally.

He also discussed the value of animal models like zebrafish versus cell lines, primary cultured muscle cells and especially induced pluripotent stem-cell (iPSC) derived myocytes from patient material, allowing direct access to mutant myocytes. iPSC-derived cardiomyocytes are now a well-established tool, but deriving skeletal myotubes from iPSC has proven substantially more difficult. A caveat about these model systems is, however, the relative immaturity of the cardiomyocytes, their yet unknown titin isoform expression patterns, and the need for adequate control samples.

6.2 Titin: Functional domains and their diseases, Mex4-animal models,

Isabelle Richard presented the molecular and phenotypic characterization of several mouse models carrying various modifications in the M-line titin. One of the model reproduces the most frequent mutation (the so-called FINmaj mutation) present in the extreme C-terminus of titin, that

causes tibial muscular dystrophy (TMD) and limb girdle muscular dystrophy 2J (LGMD2J) when present on one or both alleles, respectively [5]. In heterozygous mice, dystrophic myopathology appears late at 9 months of age in few distal muscles. In homozygous (HO) mice, the first signs appear in the soleus at 1 month of age and extend to most muscles at 6 months of age.

Interestingly and in contrast to the human situation, the heart is also severely affected in HO mice. At the molecular level, as in human, the mutation leads to the loss of the very C-terminal end of titin and to a secondary deficiency of calpain 3, the protein deficient in limb-girdle muscular dystrophy 2A (LGMD2A). Several CAPN3 cleavage sites in C-terminal titin were defined by protein sequencing. The TMD/LGMD2J mutation FINmaj proved to alter this processing in vitro. However, the pathological loss of M-band titin due to TMD/LGMD2J mutations was found to be independent of CAPN3, whereas the involvement of ubiquitous calpains is likely. Nevertheless, by crossing the FINmaj model with a calpain 3-deficient model, the heterozygote TMD phenotype was corrected, demonstrating a participation of calpain 3 in the pathogenesis of this disease. The other models that were discussed include in particular a model where the penultimate exon of titin Mex5 coding for the is7 domain and part of the binding site for calpain 3 present in the M-line has been removed using the CRISPR/cas9 technology [44]. Interestingly, the suppression of the domain induces a phenotype mostly in tissues usually expressing the isoform that has been suppressed, indicating that it fulfills (a) specific function(s) in these tissues. Thus, the phenotypes present in these models confirm the crucial importance of the C-terminus of titin.

6.3 Titin disease defects on the protein level

The presentation of **Henk Granzier** and **John Smith** focused on biophysical studies that addressed the possible disease mechanisms by which a missense mutation in exon 37 (I10 domain) causes disease. Atomic force microscopy findings reveal a reduced folding stability and enhanced proteolysis susceptibility of mutated I10 domains. WB studies of an already reported DCM

mutation (exon 326) showed truncated protein. Additionally, WB studies revealed the existence of truncated protein in some samples from skeletal muscle myopathy patients with truncating variants and variants in canonical splice sites in titin. Finally, progress was reported on creating a mouse model with a compound heterozygous mutation in titin that mimics mutations found in a CNM patient. The mouse model currently exists and phenotyping has started.

7. Session V: Methods for the validation of titin mutations

Marco Savarese reported that more than 90% of rare variants identified in *TTN* are missense variants. More than 30% are ultra-rare variants ($MAF < 0,00001$) and many of them are private variants (never reported so far). In order to evaluate their effect, a MAF threshold should be set. However, to do this effectively the inheritance pattern (dominant or recessive) of the disease, its age of onset, prevalence and penetrance should be defined. Segregation analysis should always be performed, although it is insufficient to provide conclusive evidence of pathogenicity. As evidenced in the ACMG guidelines [45], co-segregation of a particular variant with a phenotype in a family only links the disorder to the locus. The variant may be in linkage disequilibrium with the real causative mutation. First data suggests that existing bioinformatic programs are unable to correctly predict the effect of missense variants.

7.1 Bioinformatics tools to assess pathogenicity of *TTN* mutations

Raphaël Schneider presented some bioinformatics tools to help interpret pathogenicity of *TTN* variants. To resolve coordinates problems in *TTN*, a simple tool was created to pass from one coordinate system to another (cDNA, protein, genomic) for each *TTN* transcript. Another tool was created to visualize the localisation of rare *TTN* variants in several individuals to highlight

similar/same variants in different patients/families. He also detected exon-size deletions in *TTN* in 3 patients by comparing and normalizing read depth in 1000 exomes (homogeneous data).

Comparison of variant types in HGMD vs ExAC showed an enrichment of loss-of-function mutations in HGMD (2/3 in HGMD vs <3% in ExAC). Most of the mutations in HGMD localized to the C-terminal part of *TTN* suggesting either an important function of this domain in heart and muscle or a diagnosis bias due to screening targeting mainly the last *TTN* exons.

To help interpreting the pathogenicity of *TTN* SNVs, on-going developments aim to gather information on *TTN* variants and exons into an integrated database: variant information like the number of transcripts impacted, splice site prediction, position conservation, pathogenicity prediction, exon information such as numbering, size, mutation already reported in this exon, number of variants and type in ExAC, domain information, structural data etc.

7.2 Titinviewer

Isabelle Richard discussed the initiation of a new titin interface tool linking gene-protein structural elements with variant data called Titin viewer. This interface is based on JBrowse Genome viewer and will at term integrate data on 1) alternative splice isoforms according to skeletal muscles and heart, protein interactors and related pathways (from literature and the interactome data based on a large-scale study using two-hybrid screens [46] known 3-D structure from PDB, known post-translational modifications, species conservation associated with filtering, scoring and ranking tools to discriminate mutations versus polymorphisms [46]. It will have a footbridge towards the database of sequence variants from patients and from unrelated individuals sequenced in unrelated pathologies. This database will be made available to the scientific community through a web portal.

7.3 False positive annotated dominant mutations based on newer population frequencies

Marco Savarese discussed the high frequency of TTN truncating variants observed in cohorts of patients with dilated cardiomyopathy as well as the comparatively lower prevalence of potentially disease-causing truncating variants in general population suggest a causative role of PTVs in such diseases [47, 48].

A positional effect has been also suggested. Truncating variants in the A-band domain of TTN seem to cause DCM while truncations in the I-band are less damaging, suggesting a positional effect due to an alternative exon splicing that may reduce the pathogenicity of I-band variants [49].

On the other hand, heterozygous PTVs are also present along the entire gene in asymptomatic individuals. This is consistent with the observation by all the participants' that the heterozygous TTN truncating variants identified so far do not produce a skeletal myopathy unless they are associated with a second recessive mutation. Truncating variants have been also found in myopathic patients without any clear evidence of cardiac involvement, suggesting that most truncating changes do not cause dominant cardiomyopathy.

7.4 What about mutations in exons with developmental or tissue specific expressions?

Peter Hackman discussed the fact that despite the expression of a mutated gene in all muscles, muscles are selectively involved in genetic muscular dystrophies. Different muscular dystrophies show characteristic patterns of fatty degenerative changes on muscle imaging, which is very useful in the diagnostic process. However, the underlying molecular mechanisms explaining the selective involvement of muscles are not known. With the hypothesis that different muscles may express variable amounts or different isoforms of muscle genes RNA-sequencing was performed to analyse the transcriptional profiles in a total of 42 samples collected from 12 different human adult

lower limb skeletal muscles [50]. The results of the first 20 samples indicated a highly variable expression of *TTN* and other selected genes in anatomically different lower limb skeletal muscles. Comparison of the known patterns of selective involvement of certain muscles in two autosomal dominant titinopathies (TMD and HMERF) and in autosomal dominant myosinopathy (Laing), with the isoform and gene expression results showed a correlation between the specific muscles involved and significant differences in the level of expression of the affected gene and exons in these same muscles compared with other muscles. This suggests that differential expression levels of muscle genes and isoforms are one determinant in the selectivity of muscle involvement in muscular dystrophies.

It can be challenging to get samples from anatomically different muscles from the same individual and from the same muscles at different developmental stages. However, data from public repositories like Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo/>) submitted by different research groups may be used as well.

8. Session 6 Creation of the TTN mutation database

Marco Savarese stated that a locus specific database for titin is needed. It should include genetic data (including quality parameters, such as depth, coverage, and altered/wild type allelic ratio) with a complete annotation from all the other publicly available databases (ExAC, dbSNP, 1000G, ESP). It should also list additional features including protein annotation, interaction data, etc.

For a correct interpretation of TTN findings, deep phenotype data should be included using existing tools like PhenoTips, that can be customised specifically for titinopathies

8.1 Does RD-CONNECT and Phentips provide the platform needed?

Teresinha Evangelista pointed out that rare disease (RD) research faces specific challenges due to the small number and fragmentation of patient groups, clinical expertise, and research communities. Next-generation sequencing (NGS) and genomics research have opened up new possibilities for gene discovery. However, many RD expert centres lack the bioinformatics expertise and computational support to take full advantage of the new genomic paradigm. RD-CONNECT developed a unique online platform that provides both a repository for RD research data and a user-friendly interface for NGS analysis. Research partners from any RD research project or clinical centre can submit data, analyse their own patients and compare with data submitted by other centres. Ethical and legal expertise is available to ensure data sharing and research meets appropriate consent and data protection provisions, and an active patient advisory council led by EURORDIS, the European alliance of rare disease patient organizations, ensures explicit engagement of patients at every level.

In the RD-CONNECT platform, genomic data is linked with phenotypic data at a per-patient level. Other types of omics data, in particular transcriptomic, metabolomic and proteomic profiles, are in the process of being incorporated where available. The corresponding clinical information from each individual is recorded in a connected PhenoTips instance, a software solution that simplifies the capture of clinical data using the Human Phenotype Ontology, and linked with OMIM and Orpha codes. Raw data is stored at the European Genome-phenome Archive (EGA). This raw data is reprocessed through a standard analysis pipeline before being made accessible in the RD-CONNECT platform. The standardization of variant calling and annotation helps output from different sequencing providers to become comparable. Within RD-CONNECT the sequencing data is combined with detailed phenotypic data standardized using the Human Phenotype Ontology (HPO) to allow combined genotype-phenotype analysis. Registered clinicians and researchers can analyse their own data and access data from other submitters. RD-Connect is not only a data repository but a full-featured genomic analysis platform with a particular focus on diagnosis and gene discovery. Validated RD researchers can upload their own data and perform

combined analyses of index cases with family members, filtering the many thousand variants in a single exome or genome using a range of filters and candidate gene lists. The underlying technologies are designed for big data, enabling real-time analysis at whole-genome scale. PhenoTips is an open source software tool for collecting and analysing phenotypic information for patients with genetic disorders. The user interface closely mirrors clinician workflows so as to facilitate the recording of observations made during the patient encounter.

8.2 The CMDIR and interface with the TTN database, Sarah Foye

The Congenital Muscle Disease International Registry (CMDIR: www.cmdir.org) is an international patient registry for congenital muscular dystrophy, congenital myopathy and congenital myasthenic syndrome, and registers through the late onset for all of these diseases. Titin patients who have muscle disease and/or heart disorders are invited to register. Data in the CMDIR is a hybrid of patient reported outcomes and curated medical records. Therefore, both phenotypic and genotypic data can be extracted from the CMDIR. At the time of the meeting, there were 34 cases of Titin related health conditions registered in the CMDIR. This registry is also connected to a tissue repository and a biobank and records specimen ID's within the donor's CMDIR profile so that a limited data set can be shared with researchers along with the specimen. The CMDIR also has the capacity to pursue additional survey and data collection to support future research queries. CMDIR data can also be connected with other databases using the NDAR globally unique identifier system. Patient education publications and videos available on the website. All data is maintained in accordance with U.S. federal healthcare privacy laws.

9. Session 7: Establishing a database for *TTN* mutations and their clinical phenotypes.

In the following group discussion moderated by Ana Ferreiro the Titin database consortium was established and the following issues related to the creation of a database for *TTN* variants discussed:

- what platform to be used and the physical location of the data
- the responsible institution and general requirements
- who uploads data and the need for curation
- ID for samples

The tailored clinical dataset to be included in PhenoTips was drafted. Final touches to these questions were scheduled within two months of the workshop. A pilot version of the database based on the RD-CONNECT platform at Centro Nacional de Análisis Genómico (CNAG-CRG) in Barcelona was decided to be tested and data from 100 samples generated in Helsinki, Finland were scheduled to be uploaded by July 2016 (Savarese, Hackman, Udd, Evangelista). An evaluation based on this pilot version of the database will be performed.

After the RD-CONNECT pilot has been evaluated an on-line meeting will be held to discuss the experiences and the execution of follow up-actions. If a decision is made based on the pilot study to use RD-CONNECT, the research groups which participated in the workshop will submit their data into the database. The “Titin Viewer” developed by Isabelle Richard, would be available for use and integrated with the database. A complementary tool TITINdb, was developed in London and will be available for validation.

10. Patients' point of view

Sarah Foye, Titin family representative, Congenital Muscle Disease International Registry (USA) and Alison Rockett Frase, patient representative, Joshua Frase Foundation (USA) participated in the meeting, which provided an opportunity for them and researchers working on titinopathies to meet and interact with each other. During her talk, Foye portrayed the personal experience of

living day to day with a Titin related disorder. She also discussed the importance of considering both muscle and heart disorders together, as well as the urgent need families have to find answers related to the disorders affecting them.

11. Future follow-up meetings:

Online meetings as needed to move the database forward will be held.

A new ENMC workshop will be applied for in one year, after the full report has been published in the journal of Neuromuscular Disorders and after the first experience with using the database in praxis has been accomplished, in order to address the wider access to upload samples with phenotype data and how to integrate the cardiology part of the titinopathies.

12. Participants

Participants in this workshop were:

Bjarne Udd, Neuromuscular Research Center, Tampere University (Finland);

Peter Hackman, Folkhälsan Research Center (Finland);

Ana Ferreiro Unité de Biologie Fonctionnelle et Adaptative (France);

Carsten Bonnemann, NIH/NINDS (USA);

Alan Beggs, Boston Children's Hospital/ Harvard Medical School (USA);

Mathias Gautel, King's College London (UK);

Mark Davis, PatheWest Laboratory (Australia);

Teresinha Evangelista, Newcastle University (UK);

Marco Savarese (via the ENMC Young Scientist Award), Folkhälsan Research Center (Finland);

Jelena Nikodinovic Glumac, Clinic for Neurology and Psychiatry for Children and Youth (Serbia);

Jocelyn Laporte, IGBMC (France);

John Edward Smith, University of Arizona (USA);

Isabelle Richard, Genethon (France);

Henk Granzier, University of Arizona (USA);

Raphäel Schneider, IGBMC (France);

Heinz Jungbluth, King's College (UK);

Sarah Foye, Titin family representative, Congenital Muscle Disease International Registry (USA);

Alison Rockett Frase, patient representative, Joshua Frase Foundation (USA).

Acknowledgements

This Workshop was made possible thanks to the financial support of the European Neuromuscular Centre (ENMC) and ENMC main sponsors: Association Française contre les Myopathies (France), Deutsche Gesellschaft für Muskelkranke (Germany), Muscular Dystrophy UK (UK), Prinses Beatrix Spierfonds (The Netherlands), Muskelsvindfonden (Denmark), Schweizerische Stiftung für die Erforschung der Muskelkrankheiten (Switzerland), Telethon Foundation (Italy), Spierziekten Nederland (The Netherlands) and Associated member: Finnish Neuromuscular Association (Finland). We are grateful to the CureCMD and the Joshua Frase Foundation, who helped fund the non-European travel expenses.

References

- [1] Savarese M, Sarparanta J, Vihola A, Udd B, Hackman P. Increasing Role of Titin Mutations in Neuromuscular Disorders. *J Neuromuscul Diseases* 2016;3(3):293-308.
- [2] Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, Schmitz-Abe K, DeChene ET, Swanson LC, et al. Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. *Neurology* 2013;81(14):1205-14.
- [3] Chauveau C, Bonnemann CG, Julien C, Kho AL, Marks H, Talim B, et al. Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. *Hum Mol Genet* 2014;23(4):980-91.
- [4] Evila A, Palmio J, Vihola A, Savarese M, Tasca G, Penttila S, et al. Targeted Next-Generation Sequencing Reveals Novel TTN Mutations Causing Recessive Distal Titinopathy. *Mol Neurobiol* 2016 Oct 29. [Epub ahead of print].
- [5] De Cid R, Ben Yaou R, Roudaut C, Charton K, Baulande S, Leturcq F, et al. A new titinopathy: Childhood-juvenile onset Emery-Dreifuss-like phenotype without cardiomyopathy. *Neurology* 2015.
- [6] Evila A, Vihola A, Sarparanta J, Raheem O, Palmio J, Sandell S, et al. Atypical phenotypes in titinopathies explained by second titin mutations. *Ann Neurol* 2014;75(2):230-40.
- [7] Chauveau C, Rowell J, Ferreira A. A rising titan: TTN review and mutation update. *Hum Mutat* 2014;35(9):1046-59.
- [8] Dalin MG, Engstrom PG, Ivarsson EG, Unneberg P, Light S, Schaufelberger M, et al. Massive parallel sequencing questions the pathogenic role of missense variants in dilated cardiomyopathy. *Int J Cardiol* 2016;228:742-48.
- [9] Udd B, Partanen J, Halonen P, Falck B, Hakamies L, Heikkila H, et al. Tibial muscular dystrophy. Late adult-onset distal myopathy in 66 Finnish patients. *Arch Neurol* 1993;50(6):604-08.
- [10] Hackman P, Vihola A, Haravuori H, Marchand S, Sarparanta J, De Seze J, et al. Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. *Am J Hum Genet* 2002;71(3):492-500.
- [11] Hackman P, Marchand S, Sarparanta J, Vihola A, Penisson-Besnier I, Eymard B, et al. Truncating mutations in C-terminal titin may cause more severe tibial muscular dystrophy (TMD). *Neuromuscul Disord* 2008;18(12):922-28.
- [12] Pollazzon M, Suominen T, Penttila S, Malandrini A, Carluccio MA, Mondelli M, et al. The first Italian family with tibial muscular dystrophy caused by a novel titin mutation. *Journal of Neurology*. 2010;257(4):575-79.
- [13] Van den Bergh PYK, Bouquiaux O, Verellen C, Marchand S, Richard I, Hackman P, et al. Tibial muscular dystrophy in a Belgian family. *Ann Neurol* 2003;54(2):248-51.
- [14] Udd B, Kaarianen H, Somer H. Muscular dystrophy with separate clinical phenotypes in a large family. *Muscle & nerve*. 1991;14(11):1050-58.
- [15] Penisson-Besnier I, Hackman P, Suominen T, Sarparanta J, Huovinen S, Richard-Cremieux I, et al. Myopathies caused by homozygous titin mutations: limb-girdle muscular dystrophy 2J and variations of phenotype. *J Neurol Neurosurg Psychiatry* 2010;81(11):1200-02.
- [16] Udd B, Vihola A, Sarparanta J, Richard I, Hackman P. Titinopathies and extension of the M-line mutation phenotype beyond distal myopathy and LGMD2J. *Neurology* 2005;64(4):636-42.
- [17] Ohlsson M, Hedberg C, Bradvik B, Lindberg C, Tajsharghi H, Danielsson O, et al. Hereditary myopathy with early respiratory failure associated with a mutation in A-band titin. *Brain* 2012;135(Pt 6):1682-94.

- [18] Palmio J, Evila A, Chapon F, Tasca G, Xiang F, Bradvik B, et al. Hereditary myopathy with early respiratory failure: occurrence in various populations. *J Neurol Neurosurg Psychiatry* 2014;85(3):345-53.
- [19] Pfeffer G, Elliott HR, Griffin H, Barresi R, Miller J, Marsh J, et al. Titin mutation segregates with hereditary myopathy with early respiratory failure. *Brain* 2012;135(Pt 6):1695-713.
- [20] Pfeffer G, Barresi R, Wilson IJ, Hardy SA, Griffin H, Hudson J, et al. Titin founder mutation is a common cause of myofibrillar myopathy with early respiratory failure. *J Neurol Neurosurg Psychiatry* 2014;85(3):331-38.
- [21] Uruha A, Nishino I. Think worldwide: hereditary myopathy with early respiratory failure (HMERF) may not be rare. *J Neurol Neurosurg Psychiatry* 2014;85(3):248-89
- [22] Salih MA, Al Rayess M, Cutshall S, Urtizberea JA, Al-Turaiki MH, Ozo CO, et al. A novel form of familial congenital muscular dystrophy in two adolescents. *Neuropediatrics* 1998;29(6):289-93.
- [23] Subahi SA. Distinguishing cardiac features of a novel form of congenital muscular dystrophy (Salih cmd). *Pediatr Cardiol* 2001;22(4):297-301.
- [24] Carmignac V, Salih MA, Quijano-Roy S, Marchand S, Al Rayess MM, Mukhtar MM, et al. C-terminal titin deletions cause a novel early-onset myopathy with fatal cardiomyopathy. *Ann Neurol* 2007;61(4):340-51.
- [25] Fischer D, Herasse M, Bitoun M, Barragan-Campos HM, Chiras J, Laforet P, et al. Characterization of the muscle involvement in dynamin 2-related centronuclear myopathy. *Brain* 2006;129(Pt 6):1463-9.
- [26] Sekulic-Jablanovic M, Palmowski-Wolfe A, Zorzato F, Treves S. Characterization of excitation-contraction coupling components in human extraocular muscles. *Biochem J* 2015;466(1):29-36.
- [27] Toussaint A, Cowling BS, Hnia K, Mohr M, Oldfors A, Schwab Y, et al. Defects in amphiphysin 2 (BIN1) and triads in several forms of centronuclear myopathies. *Acta Neuropathol* 2011;121(2):253-66.
- [28] Wilmschurst JM, Lillis S, Zhou H, Pillay K, Henderson H, Kress W, et al. RYR1 mutations are a common cause of congenital myopathies with central nuclei. *Ann Neurol* 2010;68(5):717-26.
- [29] Romero NB. Centronuclear myopathies: a widening concept. *Neuromuscul Disord* 2010;20(4):223-8.
- [30] Spiro AJ, Rorke LB. Skeletal muscle lesions in congenital rubella syndrome. *Am J Dis Child* 1966;112(5):427-8.
- [31] Pugh TJ, Amr SS, Bowser MJ, Gowrisankar S, Hynes E, Mahanta LM, et al. VisCap: inference and visualization of germ-line copy-number variants from targeted clinical sequencing data. *Genet Med* 2016;18(7):712-9.
- [32] Jungbluth H, Wallgren-Pettersson C, Laporte J. Centronuclear (myotubular) myopathy. *Orphanet J Rare Dis* 2008;3:26.
- [33] Jungbluth H, Ochala J, Treves S, Gautel M. Current and future therapeutic approaches to the congenital myopathies. *Semin Cell Dev Biol* 2016 Aug 8. pii: S1084-9521(16)30242-7.
- [34] Savarese M, Di Fruscio G, Mutarelli M, Torella A, Magri F, Santorelli FM, et al. MotorPlex provides accurate variant detection across large muscle genes both in single myopathic patients and in pools of DNA samples. *Acta Neuropathologica Communications* 2014;2:100-014-0100-3.
- [35] Savarese M, Di Fruscio G, Torella A, Fiorillo C, Magri F, Fanin M, et al. The genetic basis of undiagnosed muscular dystrophies and myopathies: Results from 504 patients. *Neurology* 2016;87(1):71-6.

- [36] Evila A, Arumilli M, Udd B, Hackman P. Targeted next-generation sequencing assay for detection of mutations in primary myopathies. *Neuromuscul Disord* 2016;26(1):7-15 [37] Ravenscroft G, Davis MR, Lamont P, Forrest A, Laing NG. New era in genetics of early-onset muscle disease: Breakthroughs and challenges. *Semin Cell Dev Biol* 2016.
- [38] Abath Neto O, Tassy O, Biancalana V, Zanuteli E, Pourquie O, Laporte J. Integrative data mining highlights candidate genes for monogenic myopathies. *PLoS ONE* 2014;9(10):e110888.
- [39] Bohm J, Vasli N, Malfatti E, Le Gras S, Feger C, Jost B, et al. An integrated diagnosis strategy for congenital myopathies. *PLoS ONE* 2013;8(6):e67527.
- [40] Gorokhova S, Biancalana V, Levy N, Laporte J, Bartoli M, Krahn M. Clinical massively parallel sequencing for the diagnosis of myopathies. *Rev Neurol (Paris)*. 2015;171(6-7):558-71.
- [41] Gautel M. Cytoskeletal protein kinases: titin and its relations in mechanosensing. *Pflugers Arch* 2011;462(1):119-34.
- [42] Gautel M, Djinovic-Carugo K. The sarcomeric cytoskeleton: from molecules to motion. *J Exp Biol* 2016;219(Pt 2):135-45.
- [43] Pernigo S, Fukuzawa A, Pandini A, Holt M, Kleinjung J, Gautel M, et al. The crystal structure of the human titin:obscurin complex reveals a conserved yet specific muscle M-band zipper module. *J Mol Biol* 2015;427(4):718-36.
- [44] Charton K, Suel L, Henriques SF, Moussu JP, Bovolenta M, Taillepiere M, et al. Exploiting the CRISPR/Cas9 system to study alternative splicing in vivo: application to titin. *Hum Mol Genet* 2016.
- [45] Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med* 2013;15(7):565-74.
- [46] Blandin G, Marchand S, Charton K, Daniele N, Gicquel E, Boucheteil JB, et al. A human skeletal muscle interactome centered on proteins involved in muscular dystrophies: LGMD interactome. *Skelet Muscle* 2013;3(1):3.
- [47] Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, et al. Truncations of titin causing dilated cardiomyopathy. *N Engl J Med* 2012;366(7):619-28.
- [48] Roberts AM, Ware JS, Herman DS, Schafer S, Baksi J, Bick AG, et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. *Sci Transl Med* 2015;7(270):270ra6.
- [49] Hinson JT, Chopra A, Nafissi N, Polacheck WJ, Benson CC, Swist S, et al. HEART DISEASE. Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. *Science* 2015;349(6251):982-6.
- [50] Huovinen S, Penttila S, Somervuo P, Keto J, Auvinen P, Vihola A, et al. Differential isoform expression and selective muscle involvement in muscular dystrophies. *Am J Pathol* 2015;185(10):2833-42.

Table 1 - Number of titin variants found in 178 patients studied by exome sequencing

	Number of TTN variants	Number of TTN variants in cases solved by the finding of other genes
Total	183	45
Frameshift variant	6	0
Missense variant	116	33
Splice region variant	8	1
Stop gained	5	0
Synonymous variant	48	11

Table 2 - Variants considered disease causative

ALLELE 1	Mutation1	ALLELE 2	Mutation2
chr2:179393000	c.107377+1G>A	chr2:179405030	c.97863G>A p.Trp32621*
chr2:179477082	c.50170C>T p.(Arg16724*)	chr2:179593674	c.19091G>A p.(Cys6364Tyr)
chr2:179477082	c.50170C>T p.(Arg16724*)	chr2:179593674	c.19091G>A p.(Cys6364Tyr)
chr2:179393000	c.107377+1G>A	chr2:179404188	c.98603delT p.(Phe32868Serfs*11)
chr2:179393000	c.107377+1G>A	chr2:179404188	c.98603delT p.(Phe32868Serfs*11)
chr2:179393000	c.107377+1G>A	chr2:179422552	c.87529A>T p.(Lys29177*)
chr2:179406036	c.97768_97768delinsTTCCA p.Lys32590PhefsTer23	chr2:179454637	c.61815G>A p.Ile20605Met
chr2:179396929	c.104413C>T p.(Arg34805*)	chr2:179393555	c.106923T>A p.(Asn35641Lys)
chr2:179531966	c.28226insA p.(Val9410Glyfs*6)	chr2:179575597	c.50714G>A p.(Arg16905His)
chr2:179481190	c.48312+2_48312+15del	chr2:179654710	c.1933G>T p.Glu645Ter
chr2:179398077	c.103260_103264del p.(Leu34421fs)	chr2:179531597	c.35828dupA p.(Glu11945fs)