anti-HMGCR autoantibodies both have predominantly skeletal muscle involvement. This is contrast to patients with other forms of myositis, who most often have multisystem disease including the skin, lungs, and/or joints.
Although anti-SRP and anti-HMGCR positive myositis patients share certain clinical features, these diseases are different. For example, only anti-HMGCR myositis can be triggered by statin exposure. Furthermore, most anti-HMGCR myositis patients have the class II HLA allele DRB1*11:01; this allele is not an immunogenetic risk factor for myositis patients with autoantibodies recognizing SRP or other myositis-specific autoantigens. It is also important to recognize that ~15% of anti-SRP and anti-HMGCR patients have significant inflammatory cell infiltrates on muscle biopsy but are otherwise indistinguishable from patients with the same autoantibody who have necrotizing muscle biopsies. Given these observations, we conclude that IMNM may have significant limitations as a disease category. Indeed, we propose that "anti-SRP myositis" and "anti-HMGCR myositis" be recognized as distinct diseases defined by the presence of one of these two autoantibodies.

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**GNE myopathy – mechanism and therapy**

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GNE myopathy, also called distal myopathy with rimmed vacuoles (DMRV) or hereditary inclusion body myopathy (hIBM), is an autosomal recessive muscle disease affecting adolescents and adults. The disease is characterized clinically by preferential involvement of tibialis anterior muscle and relative sparing of quadriceps, and pathologically by the presence of rimmed vacuoles, which are a pathological hallmark of aggrephagy, in addition to scattered atrophic fibers. Up to this time, no cure is available for this myopathy.

GNE myopathy is caused mostly by missense mutations in the *GNE* gene that encodes a protein with the activity of two enzymes in sialic acid biosynthesis, UDP-GlcNAC 2-epimerase and ManNAc kinase, resulting in the reduction of the sialic acid levels in serum and skeletal muscles. We generated a model mouse for GNE myopathy that expressed human *GNE* with the missense mutation p.D207V, but lacks the endogenous mouse *GNE*. This model mouse exhibited hyposialylation in serum and various organs which predated the skeletal muscle weakness, atrophy, rimmed vacuole formation, and deposition of amyloid and various proteins within the myofibers, supporting the concept that the hyposialylation causes the degenerative myopathy.

To see whether GNE metabolites ameliorate the phenotype, we treated our mice with ManNAc, NeuAc, and sialic acid conjugate, sialyllactose from around 15 weeks of age and continued to around 55 weeks. Interestingly, by any agent, clinicopathological manifestations were almost completely suppressed even at age 55 weeks when all mice are expected to show all clinicopathological features. Our results indicate that sialic acid deficiency is the cause of GNE myopathy and that the disease can be suppressed by sialic acid supplementation.

Following the animal study results, slow-release tablets of sialic acid up to 6000 mg/day were tested in phase 2 trial in the US and Israel. it seems efficacious with apparent dose-dependent effect especially in upper extremities, suggesting that less affected muscles may show better efficacy. Currently, phase 3 trial is being conducted in US, Canada, UK, and Israel, and sites in France, Italy and Bulgaria are scheduled to be added further, hopefully leading to the market release of the first fundamental therapeutic agent against GNE myopathy in a few years.

Symposium- Parallel Symposium FSHD
• *Sabrina Sacconi (FRANCE)* • *Silvère Van Der Maarel (THE NETHERLANDS)* • *Rossela Tupler (USA)*

Are FSHD1 and FSHD2 merging diseases?

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Facioscapulohumeral muscular dystrophies (FSHD) are characterized by typical pattern of muscle weakness, often asymmetric distinguishing them form other myopathies and including:

- Facial muscle weakness: Involving *orbicularis oculi* and *orbicularis oris* muscles
- Fixator scapulae weakness: (i.e., trapezius involvement later followed by serratus anterior, latissimus dorsi and pectoralis major, with sparing of spinati and subscapularis muscles (Tasca et al, 2014)
- Posterior leg and anterior foreleg muscle weakness: The most affected muscles are hamstrings followed by the tibialis anterior and the medial gastrocnemius. Psoas is frequently spared and vastus-, gluteal- and peroneal muscles are affected only late in the disease (Olsen et al, 2006)
- Abdominal muscles: giving rise to Beevor sign and lumbar hyperlordosis.

Nevertheless, FSHD shows a wide spectrum of clinical involvement ranging from very severe, progressive muscular weakness often associated with additional features (extra muscular involvement, dysphagia, respiratory muscle weakness..) to mild and slowly progressive forms and even asymptomatic cases.
Genetic/ epigenetic diagnosis of FSHD has been recently improved by the discovery of the genetic/epigenetic heterogeneity of this disease, which has been recently classified in two subtypes:

- FSHD type 1 (FSHD1) characterized by autosomal dominant inheritance of D4Z4 contracted permissive allele on chromosome 4 (≥1, <11 repeated units) that can be found in 90-to 95% of FSHD patients;
- FSHD type 2 (FSHD2) characterized by digenic inheritance of permissive non-contracted allele on chromosome 4 (>11 repeated units and pathogenic dominant mutation on *SMCHD1* (Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1) gene located on chromosome 18.

In some FSHD1 patient with quite severe clinical phenotype, FSHD1 contracted permissive allele has been found in combination with *SMCHD1* pathogenic mutation (FSHD1+FSHD2 patients). Interestingly these patients carry a borderline repeat of 8-10 RU. On the other hand, most of the FSHD2 patients identified up to now carry a relatively low number of repeat raising the possibility of a *continuum* between these two diseases that may represent a confounding issue in genetic diagnosis and counseling and need to be better clarified. This possibility may have important consequences in understanding FSHD physiopathology and in developing future therapeutic strategies.

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**Epigenetic derepression of DUX4 in FSHD**

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Facioscapulohumeral dystrophy (FSHD) is one of the most common types of muscular dystrophy typically involving the muscles of the face and upper extremities. With disease progression also other muscles may become affected. In most cases, this autosomal dominantly inherited muscle disorder can be molecularly recognised by discrete chromatin changes of the D4Z4 macrosatellite repeat array on chromosome 4 in somatic cells. The polymorphic D4Z4 repeat array normally varies between 8-100 units, and adopts a repressive chromatin structure in somatic cells. Because of repeat array contractions to a size of 1-10 units (FSHD1), or mutations in the chromatin repressor SMCHD1 (FSHD2), this epigenetic silencing is incomplete leading to aberrant expression of the *DUX4* retrogene in skeletal muscle of patients.

The DUX4 retrogene is encoded within each unit of the D4Z4 repeat array but lacks a stabilizing polyadenylation signal. Therefore, only in combination with a disease-permissive genetic background of chromosome 4, which contains a polymorphic *DUX4* polyadenylation signal, these chromatin changes lead to aberrant expression of DUX4 protein in skeletal muscle. DUX4 is a double homeobox transcription factor normally expressed in the germline and its ectopic expression results in activation of several muscle damaging pathways.

FSHD is characterized by a marked inter- and intra-familial variability in disease onset and progression. For a long time, the molecular mechanisms underlying this phenomenon were largely unknown, but recent studies indicate that a combination of genetic and epigenetic factors that act on the D4Z4 repeat array determine the probability of DUX4 expression in skeletal muscle. This possibly explains the extensive clinical variation in disease onset and progression, and the frequent presence of borderline FSHD repeat arrays in the control population. In this regard, FSHD1 and FSHD2 should not be considered separate disease entities, but rather opposite extremes of a disease continuum.

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**Phenotypic and molecular characterization of FSHD families: a systematic approach towards trial readiness**

*Rossella Tupler*

Facioscapulohumeral muscular dystrophy (FSHD) is characterized by vast clinical variability. The majority of FSHD patients, termed FSHD1, carry a reduced number of D4Z4 repetitive elements on chromosome 4q. There are also FSHD patients, termed FSHD2, who carry mutations in the SMCHD1 gene but have D4Z4 alleles of normal size. Both FSHD1 and FSHD2 patients carry D4Z4 alleles associated with the 4APAS haplotype, which is considered permissive for FSHD and present D4Z4 hypomethylation, suggesting a common pathogenic mechanism altering chromatin structure at D4Z4. However, 1.3% of healthy subjects carry a D4Z4 reduced allele (DRA), associated with the 1614APAS haplotype arguing for the role of additional elements in FSHD onset and progression.

This idea is supported by clinical data, in which we have used the power of very large patient cohorts from the Italian National Registry for FSHD, combining detailed phenotypic analyses with molecular characterization. For these analyses we designed a clinical evaluation form that measures the grade of motor impairment in FSHD and describes the diverse clinical phenotypes observed in FSHD families. I will discuss our analyses of 530 subjects from 176 families, all carriers of a DRA that showed that the genetic background influences the disease development and that additional elements influence disease progression. I will also discuss the study of 68 index cases carrying a DRA with 1-3 repeats that showed the size of D4Z4 allele is not always predictive of the clinical outcome, and that additional factors contribute to the phenotype complexity.
We also analyzed the D4Z4 methylation status on FSHD families, healthy controls, affected subjects by other neuromuscular diseases and tested for the presence of SMCHD1 variants. Our study revealed that D4Z4 methylation does not strictly correlate with the presence and severity of a FSHD phenotype as we detected both D4Z4 hypomethylation (≤25%) and normal levels of methylation (≥35%), in all analyzed subgroups. We also found FSHD patients showing D4Z4 hypomethylation and wild-type SMCHD1 sequences, suggesting the contribution of additional unidentified elements.

Overall, systematic studies of large cohorts of FSHD families suggest that a complex genetic and epigenetic network is altered in FSHD as highlighted by genotype-phenotype studies and inter- and intra-familial clinical variability. We propose standardized tools to study FSHD families on the basis of clinical phenotypes and follow diversified approaches for the interpretation of molecular results to be used in clinical practice. This approach will have important clinical implications with particular regard to genetic counseling and clinical trial readiness. It will also foster the dissection of genetics and epigenetic mechanisms involved in developing FSHD.

Plenary Session- Advances in Myology
• *Helge Amthor (FRANCE)* • *H. Lee SWEENEY (USA)* • *Ana Buj-Bello (FRANCE)*