Symposium- Myasthenia
• *Sonia Berrih-Aknin (FRANCE)* • *Hanns Lochmuller (UK)* • *Bruno Eymard (FRANCE)*

Classification and physiopathology of autoimmune Myasthenia Gravis diseases

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Autoimmune Myasthenia Gravis (MG) groups together several neuromuscular disorders due to autoantibodies directed against components of the neuromuscular junction. In most cases, the autoantibodies are against the acetylcholine receptor (AChR). Recently, other targets have been described such as the MuSK protein (muscle-specific kinase) or LRP4 (lipoprotein-related protein 4) that both contribute to the clustering of AChR at the neuromuscular junction. As new and already-well-known targets have been discovered, the number of seronegative MG patients decreased significantly. MG can be classified according to the age of onset of symptoms, the location of the affected muscles (ocular versus generalized), the profile of the autoantibodies, and thymic abnormalities.
According to their target, the autoantibodies differently alter the neuromuscular transmission in MG. In AChR-MG patients, the antibodies are essentially of IgG1 and IgG3 subclasses, they reduce the number of AChR, and the complement plays a significant role. In MuSK-MG patients, the antibodies are essentially IgG4, and the complement is not involved. It appears that several presynaptic and postsynaptic components are also affected. In LRP4-MG patients, the antibodies can interfere with AChR clustering. Interestingly, experimental models of MG can be obtained by active immunization with each of these antigens.
If the role of the autoantibodies in MG pathologies is well defined, why and how these antibodies develop are still unclear. The production of the autoantibodies is the consequence of immune deregulations in a genetically predisposed individual. The thymus is apparently involved in AChR-MG, but not in MuSK-MG. In the AChR-MG thymus, there is a chronic inflammation that could explain the escape of thymic T cells from regulation, and the development of germinal centers containing B cells able to produce the pathogenic antibodies. Functional defects of Treg cells and increased expression of TH17-related cytokines were demonstrated.
A better understanding of the pathogenic effects of the distinct MG autoantibodies may lead to new therapeutic adjustments according to the MG subtype. Future investigations on the immunoregulatory mechanisms will also result in therapeutic avenues able to restore the balance of the immune system and likely to long-term remissions.

Overview and update of congenital myasthenic syndromes

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Neuromuscular junction disorders, also called Myasthenic Syndromes (MS), are a rare heterogeneous group of acquired (Myasthenia Gravis, MG) and inherited (Congenital Myasthenic Syndromes, CMS) neuromuscular disorders associated with distinctive clinical, electrophysiological, laboratory and ultrastructural abnormalities. The genetic defects in CMS either impair neuromuscular transmission directly or result in secondary impairments, which eventually compromise the safety margin of neuromuscular transmission. CMS are clinically characterised by fatigable weakness with an onset at birth or in the first 3 years of life. Approximately 20 different genetic causes of CMS have now been identified, with the majority inherited in autosomal recessive traits. The most frequent genetic defects causing CMS are loss-of-function mutations of the *CHRNE* gene, encoding the epsilon subunit of the acetylcholine receptor, which leads to receptor deficiency at the endplate. We have identified two genes (*DOK7*, *GFPT1*) that cause fatigable weakness of muscles in a limb-girdle distribution, but rarely affecting facial or eye muscles, making the diagnosis of a CMS more difficult. Recent discoveries through next generation sequencing include new causative genes, but also new clinical phenotypes which challenge traditional classifications. While many patients with CMS respond favourably to pharmacological treatment with acetylcholine esterase inhibitors, some do not or even deteriorate. This can be largely attributed to the underlying genetic defect, and other drugs such as salbutamol and ephedrine have been used successfully I patients refractory to esterase inhibitors. We will cover the significant progress made in understanding the molecular pathogenesis of CMS, which is important for both patients and clinicians in terms of reaching a definite diagnosis and selecting the most appropriate treatment.

Congenital myasthenic syndromes (CMS): therapeutic strategy, general principles and he French experience.

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Non specific measures are essential: immediate treatment of respiratory distress, prevention of infections and of malnutrition resulting from swallowing disorders, and orthopedic care of spinal complications and limbs retractions. Nocturnal ventilation is indicated if persistent hypoventilation, despite a proper therapy.
Drug contraindications must be respected as for any other myasthenic syndromes. Pregnancy requires much attention due to the risk of CMS worsening in the mother and CMS onset in her baby. The choice of the specific medications will depend on the defective gene (see table). Cholinesterase inhibitors are efficient in most CMSs, except slow-channel syndrome, AChE deficiency, Dok-7 and b2-laminin CMS, which they can worsen.
3,4-Diaminopyridine (3,4-DAP), acting at presynaptic level by increasing the synaptic AChR vesicles delivery, is beneficial in post synaptic CMS, offering a significant synergy with cholinesterase inhibitors in several CMS due to AChR and rapsyn deficiency, fast-channel syndrome, *CHA*T, *MUSK*.

Patients suffering from slow channel syndrome get benefit from molecules reducing the AChR opening time: quinidine and fluoxetine. In our experience, the benefit of these two molecules is very variable, even in patients from the same family and may be disappointing.
Ephedrine or Albuterol, two b2 adrenergic molecules, the mechanism of action in CMS of which remaining unclear, were show to be very beneficial in CMS due to *COLQ* (ACHE), *DOK7*, *AGRN,* b2-laminin genes, even in severe cases. These medications require a careful survey due to cardiovascular risk (hypertension, tachycardia, arrhythmia, cardiac infarct…). The relative efficacy of Ephedrine or Albuterol may differ from one patient to the other. 3,4-Diaminopyridine may be useful in these 2 categories of CMS.
If the causative gene is unknown, the therapeutic strategy will be careful and sequential. If repetitive response to single stimulation, suggesting a Slow-channel or a *COLQ* CMS, cholinesterase inhibitors are totally contra-indicated. If not, cholinesterase inhibitors are proposed as first medication, but the survey is mandatory and if no improvement or worsening, the medication is to be interrupted. Then 3,4-DAP may be proposed and eventually b2 adrenergic.

Table

- AChE (Colq): Ephedrine or Albuterol ; **avoid AChE-inhibitors** ; if necessary add 3,4-DAP
- AChR deficiency: AChE-inhibitors; if necessary add 3,4-DAP
- AChR Fast-channel: AChE-inhibitors; if necessary add 3,4-DAP
- AChR Slow-channel: **avoid AChE-inhibitors,** Quinidine sulfate, Fluoxetine;
- b2-Laminin: Ephedrine; **avoid AChE-inhibitors**
- ChAT: AChE-inhibitors; if necessary, add 3,4-DAP
- Dok7: Ephedrine or Albuterol; **avoid AChE-inhibitors** ; if necessary add 3,4-DAP
- GFPT1, DAPGT1,ALG2,ALG14, GMPPB : AChE-inhibitors , if necessary add 3,4-DAP
- Rapsyn: AChE-inhibitors; if necessary add 3,4-DAP , Albuterol
- MUSK: 3,4-DAP ,Ephedrine or Albuterol
- Agrin : Ephedrine or Albuterol, 3,4-DAP
- Defect in sodium channels : AChE-inhibitors , acetazolamide

Plenary Session- Gene-Based Therapy and Muscular Dystrophies
• *Matthew Wood (UK)* • *Aurelie Goyenvalle (FRANCE)* • *Caroline Le Guiner (FRANCE)*

Advanced oligonucleotide therapeutics for neuromuscular disease
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Oligonucleotide-based therapies have potential for treating a range of inherited neuromuscular disorders via modulating gene expression e.g. via splice modulation or RNA silencing. The classical example is Duchenne muscular dystrophy (DMD), where modulation of pre-mRNA splicing of the DMD gene can restore a viable reading frame and the expression of functional protein. This approach is currently being evaluated in clinical trials. However, a major challenge in the application of such approaches to neuromuscular disease is poor delivery to affected tissues including skeletal muscle, heart and to the nervous system across the blood brain barrier. We have developed a range of peptide- and EV-based platform technologies to overcome this challenge. Peptide-oligonucleotide compounds provide greatly improved delivery and enhanced potency and are being developed for future clinical applications in both DMD and for other neuromuscular disorders, such as spinal muscular atrophy. Future prospects will be discussed.

Tricyclo-DNA: highly promising antisense oligonucleotides for splice switching therapeutic approaches
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Antisense oligonucleotides (AON) hold promise for therapeutic splice-switching correction in many genetic diseases; however, despite advances in chemistry and design, systemic use of AONs is still limited due to poor tissue/cellular uptake. This talk will describe a novel class of AONs made of tricyclo-DNA (tcDNA), which displays unique pharmacological properties and unprecedented uptake in many tissues after systemic administration. These outstanding properties have been demonstrated in different mouse models of genetic diseases such as Duchenne muscular dystrophy (DMD) and Spinal muscular atrophy (SMA). DMD is a neurogenetic disease typically caused by frame-shifting deletions or nonsense mutations in the gene encoding dystrophin and characterized by progressive muscle weakness, cardiomyopathy, respiratory failure and neurocognitive impairment. While current naked AONs do not significantly enter the heart or cross the blood brain barrier, systemic delivery of tcDNA-AONs allow high levels of dystrophin rescue in skeletal muscles as well as in heart and to a lower extent in the brain. Our results demonstrate for the first time physiological improvement of the cardio-respiratory functions and correction of behavioural features linked to the emotional/cognitive deficiency associated with the lack of dystrophin.
These properties, together with the safe toxicology profile of tcDNA make this chemistry particularly attractive for future therapies in DMD patients as well as in other neuromuscular disorders or diseases eligible for splice-switching approaches requiring whole-body treatment.

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rAAV vectors as potential therapeutics for Duchenne Muscular Dystrophy
Caroline Le Guiner, PhD Atlantic Gene Therapies, INSERM UMR 1089, Nantes, France & The "AFM-sponsored Duchenne consortium", which includes Atlantic Gene Therapies (Nantes, France)/Genethon (Evry, France)/Institut de Myologie (Paris, France)/Royal Holloway (Londres, UK)

Among vector systems that allow efficient *in vivo* gene transfer, recombinant Adeno Associated Virus vectors (rAAV) hold great promise and are currently evaluated in multiple clinical trials for the treatment of inherited diseases. In particular, gene-therapy of muscle diseases rapidly gained attention because delivery of rAAV vectors of several serotypes results in very