Helene Puccio
Abstract missing

Sudden death prevention in patients with muscle diseases: a great challenge
Karim Wahbi

Cardiac and respiratory involvements are associated with a great morbidity and mortality in patients with neuromuscular diseases. Sudden death represents one of the most frequent causes of death in this population, particularly in diseases associated with primary involvement of the cardiac electrical system leading to conduction defects or ventricular tachyarrhythmias, such as myotonic dystrophy type 1, laminopathies or mitochondrial diseases. Preventive strategies for sudden death, mainly based on prophylactic implantations of permanent pacemakers or cardiac defibrillators, should be applied in these patients to improve their long-term prognosis. Since the natural history of cardiac involvement and mechanisms underlying sudden death differ from a disease to another, these strategies have to be specific for each muscle disease. Regarding the low prevalence of muscle diseases, the identification of risk factors for sudden death is complex and, moreover, the benefit associated with preventive measures is particularly difficult to demonstrate and can hardly be based on randomized trials. We will discuss several disease-specific approaches for the prevention of sudden death.

Cardiac involvement in dystrophinopathies: best practice and current dilemmas in care.
John p. Bourke, MD- Consultant Cardiologist, Freeman Hospital, Newcastle upon Tyne, UK

Life expectancy has improved progressively for patients with Duchenne muscular dystrophy- mainly due to better integration of multi-disciplinary care teams. However, poor care during the early years after diagnosis cannot be compensated for by improved care later. This presentation will review why the *International Standards of DMD Care* require a cardiologist be fully integrated into every neuromuscular genetics team.
Progressive left ventricular dysfunction from about age 13 years is almost universal in patients with DMD. This cardiomyopathy- culminating ultimately in sudden arrhythmic death or heart failure, has become the latest barrier to prolonging high quality survival in DMD-adults. Timely information about cardiac involvement allows parents to decide whether to have their child start cardio-active medicines ahead of or with the onset of demonstrable left ventricular dysfunction. Drugs of established benefit include ACE-inhibitors (eg: perindopril, ramipril), angiotensin-receptor blockers (eg: losartan, irbesartan), beta-adrenergic blockers (ie: bisoprolol, carvedilol) and aldosterone antagonists (ie: eplerenone). Ivabridine can be used for sinus tachycardia when beta-blockers are poorly tolerated. The evidence that these readily available, well tolerated, inexpensive drugs slow the course of cardiomyopathy will be summarised.
The risk of arrhythmias increases as left ventricular function declines and fibrosis increases. In other forms of cardiomyopathy, implantable cardioverter defibrillators [ICD] are deployed routinely to prevent sudden death. To date these have rarely been offered to adults with advanced DMD because the effect on prognosis and quality of life is unknown. This presentation will make the case for ICD-therapy in DMD.
Because of its multi-system implications, DMD-patients are rarely considered for cardiac transplantation. However, a range of intra- or extra-vascular left ventricular assist devices (eg: *Heart Mate II* or similar or *Sunshine Heart*) are now used selectively in other contexts. These may also be acceptable to some DMD-patients with heart failure symptoms despite optimal therapies and prolong survival. Precise genetic characterisation of DMD-patients may allow 'disease modification' through exon-skipping, gene manipulation or 'read-through' therapies. Research to date is focused on the effects of these therapies on skeletal muscle. However, because of differences in tissue uptake, evaluation of whether they improve or aggravated cardiac involvement will be needed. The cardiologists role in the muscle team is to provide cardiac surveillance and deploy cardio-active therapies in a timely way to minimise the symptomatic and prognostic impact of DMD-cardiomyopathy. Increasingly in selected cases, this will involve adding ICD and left ventricular assist device therapies.

Symposium- Parallel Symposium SMA/ALS/STEM CELLS

• *Georg Haase (FRANCE)* • *Alexandre Henriques (FRANCE)* • *Cecile Martinat (FRANCE)*

ALS and SMA Disease Modeling and Therapy Development using pure FACS-isolated Motor Neurons.
Georg Haase
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Glucosylceramide and glycosphingolipids are part of the response to motor unit stress in ALS.
1. *Schmitt F, Hussain G, Dupuis L, Loeffler JP, Henriques A.*
A plural role for lipids in motor neuron diseases: energy, signaling and structure. *Front. Cell. Neurosc* 2014 Feb 20;8:25.
2. Henriques A, Croixmarie V, Priestman DA, Rosenbohm A, Dirrig-Grosch S, D'Ambra E, Huebecker M, Hussain G, Boursier-Neyret C, Echaniz-Laguna A, Ludolph AC, Platt FM, Walther B, Spedding M, Loeffler JP, Gonzalez De Aguilar JL. Amyotrophic lateral sclerosis and denervation alter sphingolipids and up-regulate glucosylceramide synthase. *Hum. Mol. Genet.* (2015) doi: 10.1093/hmg/ddv439

Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset disease characterized by upper and lower motor neuron degeneration, muscle wasting and paralysis. Growing evidence suggests a link between changes in lipid metabolism and ALS [1, 2]. We have recently studied the lipidome in SOD1 mice, an animal model of ALS [3]. We found that sphingolipid species, and particularly glucosylceramide, were drastically rearranged in SOD1 mice, before overt neuropathology. GM3,

GM2 and GM1a are glycosphingolipids of the a-serie and are downstream products of glucosylceramide. In SOD1 mice, levels of GM3 and GM2 were elevated in the skeletal muscles and levels of GM1a were increased in the spinal cord. In ALS patients, we found that the expression of UGCG, the enzyme responsible for the synthesis of glucosylceramide, was strongly increased in muscle biopsies and form aggregates in neurogenic muscle fibres. We hypothesized that deregulation of glycosphingolipid synthesis could underlies a physiological response to the disruption of motor units. Pharmacological inhibition of UGCG decreased glycosphingolipid synthesis, delayed axonal regeneration and impaired muscle response to denervation in an animal model of peripheral nerve injury. In all, we show that glycosphingolipid metabolism is stimulated during motor unit stress and represents a promising therapeutic target for ALS.

Human pluripotent stem cells for the study and treatment of neuromuscular diseases: myth or reality?
Cécile Martinat, INSERM/UEVE UMR 861, I-STEM, AFM, 2, Rue Henri Desbruères, 91100 Corbeil-Essonnes – France

Neuromuscular diseases correspond to a vast group of diseases that perturbs the function of the skeletal muscles by affecting motoneurons, muscles and/or NMJs. To date, no efficient curative treatments have been identified for NMD. Progresses towards identification of new treatment have been hampered by the incomprehension of disease pathogenesis, particularly in early phases, as well as the availability of relevant screening tools. Disease-specific human pluripotent stem cells, from embryonic origin or derived from reprogramming somatic cells, offer the unique opportunity to have access to a large spectrum of disease-specific cell models. Due to their ability of self-renewal and differentiation into various tissues affected in each pathological condition, the development of these human disease-specific pluripotent stem cells provide new insights in pathological mechanisms implicated in human diseases for which, accessing homogenous affected tissues is often challenging. Validating this concept, we previously demonstrated that human pluripotent stem cells and derivatives which, express the causal mutation implicated in the Myotonic Dystrophy type 1 (DM1), offer pertinent disease-cell models, applicable for a wide systemic analysis ranging from mechanistic studies to therapeutic screening. Thus, we identified, through a genome-wide analysis, two early developmental molecular involved both in myogenesis as well as in neurite formation and establishment of neuromuscular connections. These neuropathological mechanisms may bear clinical significance as related to the functional alteration of neuromuscular connections associated with DM1. In parallel to these functional pathological studies, we also demonstrated the pertinence of this new disease-specific cell model to identify new therapeutic strategies. Thus, our results identified the possibility to repurposing metformin, the most commonly prescribed drug for type 2 diabetes, for DM1 leading to a phase 2 clinical trial that is actually ongoing.
We are now extending our approach to another incurable neuromuscular disease, spinal muscular atrophy (SMA). This disease, considered as the leading genetic cause of infant death, is due to mutations or deletions in the "Survival of Motor Neuron" gene, SMN1, which results in low levels of the expressed SMN protein. Despite this ubiquitous SMN expression, the pathology is characterized by degeneration of spinal Motor neurons whereas other neuronal types are relatively preserved suggesting that spinal motor neurons specific features control this differential sensitivity. Based on our recent development allowing the efficient and robust conversion of human pluripotent stem cells into affected spinal motor neurons and non- affected cranial motor neurons, our objective is to deepen the mechanisms involved in the specific degeneration of spinal motor neurons in SMA as well as the mis communication of these neurons with their muscular target.

Closing Conference

• *Charles GERSBACH (Durham, USA)*

Genome Engineering for Gene Therapy and Controlling Cell Fate Decisions
Charles Gersbach (Durham, USA)

The advent of genome engineering technologies, including the RNA-guided CRISPR/Cas9 system, has enabled the precise editing and regulation of endogenous human genes and epigenetic states. We have applied these tools to the correction of mutations that cause genetic disease and also adapted them to manipulate the epigenome and control cell fate decisions. For example, we engineered CRISPR/Cas9-based nucleases to correct the human dystrophin gene that is mutated in Duchenne muscular dystrophy patients. When we delivered these nucleases to cells from patients with this disease, the correct gene reading frame and expression of the functional dystrophin protein were restored *in vitro* and following cell transplantation into mouse models *in vivo*. When delivered directly to a mouse model of this disease, gene editing by the CRISPR/Cas9 system led to gene restoration and improvement of biochemical and mechanical muscle function. In other studies, we have engineered CRISPR/Cas9-based tools to regulate the expression of endogenous genes and applied these tools to control genes relevant to medicine, science, and biotechnology. Genome-wide analysis of the DNA-binding, gene regulation, and chromatin remodeling by these targeted epigenome modifiers has demonstrated their exceptional specificity. We have recently applied these technologies to control the decisions of stem cells to become specific cell fates and reprogram cell types into other lineages that could be used for drug screening and disease modeling. Incorporating methods to dynamically control the activity of these proteins, such as optogenetic control of the proteins with light, has allowed us to pattern gene expression both temporally and spatially. Ongoing efforts include designing strategies to manipulate specific epigenetic marks that would enable deciphering the influence of epigenetics on gene regulation and disease states. Collectively, these studies demonstrate the potential of modern genome engineering technologies to capitalize on the products of the Genomic Revolution and transform medicine, science, and biotechnology.