Pompe disease, gene therapy, Intrathecal

Pompe disease- #3284

P12- 206- Lack of robust satellite cell activation and muscle regeneration during the progression of Pompe disease

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Pompe disease (PD), a recessively inherited metabolic myopathy caused by inactivating mutations in the acid alpha glucosidase (GAA) gene. GAA deficiency causes glycogen accumulation in several tissues that is particularly damaging in skeletal muscle. Classic infantile Pompe patients, which also develop a cardiac hypertrophy, are characterized by a rapidly progressing disease course and death within 18 months. Patients with a later onset of disease develop muscle weakness more slowly and may become wheelchair- and ventilator-dependent eventually. Skeletal muscle has a remarkable capacity to repair damage that is dependent on a rare population of muscle-resident stem cells, called satellite cells. It remains enigmatic why satellite cells fail to compensate the progressive muscle damage characterizing neuromuscular disorders. To address this, we have analyzed muscle fiber pathology, the satellite cell response and muscle regeneration activity in muscle biopsies from Pompe patients across all ages and stages of disease. Pathology included muscle fiber vacuolization, loss of cross striation, and immune cell infiltration. The total number of Pax7-positive satellite cells in muscle biopsies from infantile, childhood- and adult-onset patients was indistinguishable from age-matched controls, indicating that the satellite cell pool is not exhausted in Pompe disease. However, immunohistochemical analysis of PAX7/Ki67 and MyoD/Myogenin expression suggested that the levels of satellite cell activation and differentiation, respectively, were low. In line with this, the expression of embryonic Myosin Heavy Chain was weak in samples from the rapidly progressing classic infantile patients and undetectable in those from the childhood- and adult-onset Pompe disease patients. We conclude that satellite cells are not properly activated during Pompe disease progression and that this contributes to an impaired muscle regenerative response. The preservation of the satellite cell pool may offer a venue for the development of novel treatment strategies directed towards the activation of endogenous satellite cells.

Pompe disease, muscle regeneration, satellite cells, muscle stem cells

Pompe disease- #4498

P12- 207- Specific and redundant roles of the TEAD family of transcription factors in C2C12 cell and primary myoblast differentiation.

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The TEAD family of transcription factors recognise the MCAT element found in the promoters of muscle-specific genes. We previously used shRNA-mediated silencing to show that Tead4 plays an essential role in C2C12 cell differentiation with Tead4 silenced cells giving rise to shortened myotubes. Here, we have used siRNA-mediated silencing to address the role of the Tead factors in primary myoblast differentiation. In contrast to C2C12 cells where Tead4 plays a critical role, its silencing in primary myoblasts had little effect on their differentiation. Silencing of individual Tead factors had no significant effect on primary myoblast differentiation, whereas combinatorial silencing led to inhibition of their differentiation indicating redundancy amongst these factors. In C2C12 cells also, combinatorial Tead silencing had much more potent effects than silencing of Tead4 alone indicating a contribution of other Teads in this process. By integrating Tead1 and Tead4 ChIP-seq data with RNA-seq data following combinatorial Tead1/4 silencing, we identify distinct but overlapping sets of Tead regulated genes in both C2C12 cells and primary myoblasts. We also integrated the Tead1/4 ChIP-seq data with public data sets on Myog and Myod1 ChIP-seq and chromatin modifications to identify a series of active regulatory elements bound by Tead factors alone or together with Myog and Myod1. These data dissect the specific and combinatorial functions of these transcription factors in muscle differentiation regulatory networks.

P13- Hereditary neuropathies /- N° 208 to N° 211

Hereditary neuropathies- #2388

P13- 208- Motoneuron electrical activity, Na+/K+ pump and neuromuscular junction defects in Andermann syndrome

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Andermann syndrome is an autosomal recessive disease characterized by peripheral neuropathy with variable agenesis of the corpus callosum with most affected individuals being hypotonic and amyotrophic. This neurodevelopmental and

neurodegenerative disorder is caused by loss-of-function mutations within the cation-chloride cotransporter KCC3. The homozygous deletion of KCC3 in mice reproduces the peripheral neuropathy characterized by impaired locomotion, decreased peripheral nerve conduction as well as axonal swelling and neurodegeneration in the sciatic nerve. However, the contribution of KCC3 to motoneuron activity and synaptic maintenance has yet to be determined. Further, no molecular effector for the specific KCC3-dependent neuropathy has been identified.
In the present study, we show that KCC3 loss of function leads very early to abnormal intrinsic electrical properties of spinal motoneurons. No changes in the expression of other Slc12a family of cation-Cl cotransporters or in chloride handling were observed in KCC3-/- motoneurons. Instead, pharmacological inhibition of Na+/K+-ATPase (NKA) activity partially reproduces electrical abnormalities. Consistent with a role of the pump, we demonstrate that KCC3 co-immunoprecipitates with the ?1 subunit of NKA and the loss of KCC3 alters the sub-cellular localization of the NKA?1 subunit expressed in alpha-motoneurons. In addition, we uncovered, at the same developmental stage, pre- and post-synaptic neuromuscular junction abnormalities and marked muscular atrophy due to neuronal specific deletion of KCC3. In vivo treatment with carbamazepine partially restores membrane expression of NAK?1 and innervation of NMJ, confirming the role of electrical activity in synapse maintenance.

Our findings have two major implications. Firstly, they determine motoneuron-dependent defects as playing a central part in Andermann syndrome. Secondly, they demonstrate that decreased activity of Na+/K+-ATPase ?1, combined with the functional loss of KCC3, is an early contributor to Andermann syndrome, thus uncovering a novel and unexpected role for the Na+/K+-ATPase ?1 in neurological disorders.

KCC3, peripheral neuropathy, cation-chloride cotransporter, genetic, rare disease

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Hereditary neuropathies- #2462

P13- 209- Case report: ?EGR2 mutation enhance phenotype spectrum of Dejerine-Sottas syndrome?

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Bulbospinal atrophy is a rare neuromuscular disorder characterized by degeneration of nervous cells localized in the medulla and bulbar region of the spinal cord. It is clinically characterized by facial weakness, swallowing difficulties, drooling and tongue fasciculations. Bulbospinal atrophy has been reported in several syndromes with phenotypic overlap such as Fazio-Londe, Madras or Nathalie syndrome.
We report a 5-year old patient who presented with normal motor development until the age of 2, then with progressive proximal, axial and facial weakness leading to complete bulbar palsy and tongue fasciculations. After sequencing SLC52A2, SLC52A3 and SMN1 genes, we performed the whole exome sequencing for our patient and its parents and we found a de novo pathogenic mutation in EGR2 (early growth response 2) genec.1075C>T, p.Arg359Trp.
The knowledge of the progressive bulbar syndromes has significantly increased in recent years due to advances in next generation sequencing. This group of heterogeneous diseases can occur in children or adults and forms a spectrum of severity, based around the common phenotype of bulbar and motor deficit.

This report underlines EGR2 gene as a potential cause of progressive severe facial weakness with tongue fasciculations.

EGR2 mutation,Dejerine-Sottas syndrome

Hereditary neuropathies- #2523

P13- 210- Clinical and genetical study of patients with HMSN 1X from the Republic of Bashkortostan

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Hereditary motor-sensory neurophaty 1X (HMSN 1X) occurs in 13.3% of all HMSN cases in the Republic of Bashkortostan. Four GJB1 mutations were described in 25 families with HMSN 1X: p.Arg22Gln (c.65G>A), p.Thr86Ile (c.257C>T), p.Pro87Ala (c.259C>G), p.Arg220Stop (c.658C>T). The most common mutation was p.Pro87Ala (c.259C>G). The clinical manifestations in the families with GJB1 mutations included progressive distal muscle atrophy and weakness, reduced sensation of proprioception, areflexia, sensitive ataxia and bilateral pes equinovarus or pes cavus deformity. The postural tremor of hands was the most common additional symptom. The disease of male patients begins in their first or second decade of life and was characterized by more severe impairment of the peripheral nerves with mild clinical CNS involvement. The disease's onset of female patients ranged from their first to fourth decade of life. Their clinical picture was presented by milder impairment of the peripheral nerves. Some of female patients have not any complaints in their health, but their physical examination shows the absence of the Achilles reflexes. Median motor condution velocity (MCV) ranged from 21.0 to 49.3 m/s. The most of female patients had median MCV more than 38 m/s, what was considered as HMSN, type II. In these cases mutation analysis in the GJB1 gene helps to confirm the genetic diagnosis of HMSN 1X and provide genetic counseling.

Hereditary motor-sensory neuropathy 1X, Charcot-Marie-Tooth disease, GJB1 gene

Hereditary neuropathies- #2529

P13- 211- Rigid spine syndrome associated with sensory-motor axonal neuropathy resembling Charcot-Marie-Tooth (CMT) disease are characteristic of BAG3 gene mutations.

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BAG3 (Bcl-2 associated athanogene-3) mutations have been described in rare cases of rapidly progressive myofibrillar myopathies beginning in the first decade with axial involvement, contractures and associated with cardiac and respiratory impairment occurring in the second decade. Axonal neuropathy has been documented in some patients, but usually not as a key clinical feature.

We report here a 22 year old patient with rigid spine syndrome and sensory-motor axonal neuropathy resembling Charcot-Marie-Tooth disease, without cardiac involvement.

A 22 year-old female from Gabon born to non-consanguineous parents developed at 10 years of age lower limb weakness, with inability to run and maintain prolonged staining. She walked with a stick and was wheelchair bound respectively at 14 and 17 years of age. Meanwhile she developed rapidly progressive spine deformity with hyperlordosis, starting at 15 year old. Her clinical examination showed severe rigid spine syndrome, associated with lower limb contractures affecting neck, hip, knee and ankles. She also had varus foot deformity. There was a marked proximal and distal weakness of lower limbs. Upper limb muscle strength was rather preserved. There was bilateral scapular winging. Tendon reflexes were absent. Sensory testing was normal. She also had hypophonia. Nerve conduction showed a severe sensory-motor neuropathy predominant in the lower limbs. Lower limb muscle MRI showed severe fat infiltration without specific pattern. Spine and brain MRI were normal. Creatine kinase (CK) levels were normal. Deltoid muscle biopsy performed at 19 years of age showed neurogenic pattern, along with discrete myofibrillar abnormalities. Forced vital capacity (FVC) was 42% of the predicted value. At 22 years of age, her electrocardiogram and transthoracic echocardiography were normal. Genetic analysis performed on a large panel comprising 45 CMT genes showed no mutation. Since the rigid spine syndrome worsened over the years, BAG3 gene was screened and the previously reported c.626C>T, pPro209Leu, mutation was identified.

This report confirms that rigid spine syndrome and sensory-motor axonal neuropathy resembling Charcot-Marie-Tooth disease are key clinical features of BAG3 gene mutations, which should be screened even without cardiac involvement. This diagnosis is of great importance since patients with BAG3 mutations require a close monitoring of cardiac function, given that BAG3 is a risk factor of cardiomyopathy and heart failure.

Rigid spine, sensory-motor axonal neuropathy, Charcot-Marie-Tooth disease, BAG3

P14- Homeostasis in the adult muscle/- N° 212 to N° 218

Homeostasis in the adult muscle- #2506

P14- 212- Investigation of Telomeres and Associated Proteins (TRF2) in a post-mitotic model; Muscle.

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The current view of telomere function relies on their ability to prevent DDR activation at chromosome ends. Telomere dynamics have been well studied and trends nowadays use them as the mitotic clock of cells and tissues (Daniali et al., 2013). Hence, making telomere shortening a Hallmark of aging (López-Otín et al., 2013). Interestingly, evidences that telomere signaling can be uncoupled from DDR and linked to the ability of telomere capping factors to behave as genome-wide transcriptional regulators are emerging (e.g., extra-telomeric binding sites; Martínez et al., 2014, Biroccio et al., 2013; Ye et al., 2014). We here report findings of TRF2 modulation impact on skeletal muscle both in vivo and in vitro.